ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Determination of Hematological Parameters Reference Interval for Adults of Dire Dawa Population, Dire-Dawa, Ethiopia

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A research thesis submitted to the Department of Medical Laboratory Sciences, College of Health Science, Addis Ababa University, in partial fulfillment of Master of Science Degree in Clinical Laboratory Science (Hematology and Immunohematology).

June, 2019

Addis Ababa, Ethiopi

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Teklay Mengistu, entitled:

"Determination of Hematological Parameters Reference Interval for Adults of Dire Dawa Population, Dire-Dawa, Ethiopia'' and submitted in partial fulfilment 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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Acknowledgement

I would like to thank Addis Ababa University and department of Medical Laboratory for facilitating as well as financing the Master Program of Clinical Laboratory Sciences and planning of this work as part of MSc program.

I would like also to express my heartfelt gratitude to my advisors Dr. Aster Tsegaye and Ms. Melatwork Tibebu for their constructive guidance by giving valuable scientific advices to materialize this thesis.

My appreciation also extends to all the volunteers that participated in this study and to Dire Dawa Regional Health Bureau for providing preliminary data, which are necessary in developing this thesis.

Finally, I would like to thank Dil Chora Hospital CEO, laboratory head, laboratory quality officer and all laboratory staffs for kindly supporting in sample collection, processing and analysis. The hospital management is also sincerely acknowledged for sponsoring my study and for all the support given during this study. My special thanks also goes to my families for all the support during my study.

Table of Contents

List of Tables

List of Figures

Abbreviations

BMI	Body Mass Index
CBC	Complete Blood Count
CI	Confidence Interval
CLSI	Clinical and Laboratory Standards Institute
CSA	Central Statistics Agency
CRP	C - Reactive Protein
DBP	Diastolic Blood Pressure
EQA	External Quality Assessment
ETB	Ethiopia Birr
EPO	Erythropoietin
Gran#	Granulocyte
Gran%	Granulocyte percentage
Hgb	Hemoglobin
HIF-1	Hypoxia Inducable Factor-1
HCT	Hematocrit
IL	Interleukin
IFCC	International Federation of Clinical Chemistry
Lymph#	Lymphocyte
Lymph%	Lymphocyte percentage
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Corpuscular Volume
Mid#	Mid-sized cell
Mid%	Mid-sized cell percentage
MPV	Mean Platelet Volume
NCCLS	National Council of Clinical Laboratory Services
PCT	Platecrit
PDW	Platelet Distribution Width
PLT	Platelets
PPS	Probability Proportional to Size
RBC	Red Blood Cells

RDW	Red Cell Distribution Width
RDW-CV	Red Blood Cell Distribution Width - Coefficient of Variation
RDW-SD	Red Blood Cell Distribution Width - Standard Deviation
SD	Standard Deviation
SPSS	Statistical package for social sciences
SBP	Systolic Blood Pressure
TTI	Transfusion Transmittable infections
WBC	White Blood Cells
WHO	World Health Organization

Abstract

Background: Reference interval (RI) for hematological parameters are used for a comparative decision-making process by the treating physician, for diagnosis, management and monitoring of hematologic disorders. Manufacturers' package inserts RI, which is western population based, is the most frequently external source of hematologic parameters. However, many factors like gender, age, environmental, lifestyle, and ethnicity influence hematological parameters.

Objective: To establish hematological parameters reference interval for adults of Dire Dawa Population, Dire-Dawa.

***Methods*:** A cross sectional study design was employed for hematologic reference interval determination in Dire Dawa City Administration from January to May 2019. The study participants were from strata of adults (18-65 ages of both sexes) and pregnant women. After obtaining informed written consent, data was conveniently collected using a well-defined questionnaire that covers exclusion criteria. Fresh whole blood of 5ml was collected and 2ml poured into K_3EDTA and analyzed by Mindray BC-3000plus hematology analyzer and 3ml for serology tests. Data was analyzed using SPSS version 24, the 95% reference intervals for each hematological parameters were calculated. Statistical significance was determined at $p< 0.05$.

Result: A total of 472 individuals, aged 18 to 65 years for adult groups of both gender and 15 to 49 years for pregnant women were the final study participants in this study. Their mean ± SD age was 30.93±9.34 years. The respective 95% reference intervals for males, females and pregnant women were for WBC: 3.5-10.3x10^3/µl, 3.8-10.2x10^3/µl and 3.6-11.8 x10^3/µl; for Hgb: 12.40-17.50gm/dl, 10.78-15.25gm/dl and 9.5-13.50gm/dl; for RBC: 4.46-6.15x10^6/µl, 3.81-5.49 x10^6/µl and 3.67-5.07x10^6/µl; for HCT: 43.8-58.5%, 37.48-52.08% and 32.20-46.03%; for PLT: 164.0-447.00x10^3/µl, 177.0-442.27x10^3/µl and 157.5-421.5x10^3/µl. A statistically significant difference was observed between males and females for almost all hematologic parameters.

Conclusion: There was a significant difference for most hematologic parameters based on genders and between pregnant and non-pregnant women. The RI obtained in this study is different from the values currently practiced in Dire Dawa town underscoring the need for locally appropriate intervals.

Key words: *Reference Interval; Hematology; Adult men; Adult female; pregnant women*

1. Introduction

1.1. Background

Reference interval (RI) is used for the purpose of a comparative decision-making process by describing the typical distribution of results derived from healthy reference population which enable physicians for the interpretation of clinical laboratory results. Its interpretation provide critical information on patient care for medical diagnosis, therapeutic management decision and other physiological assessment (1–3). RIs for hematologic parameters are an important component used in clinical practice for the diagnosis, management and monitoring of hematologic disorders. This can be differentiated with a defined RI which defining the spread of results in health, and results outside a reference interval are assumed to indicate a high or relative risk of disease (4).

The complete blood count (CBC) is the most frequently requested clinical laboratory tests. Its evaluation is, usually, the first ancillary aid for a practitioner, following clinical examination, to establish a diagnosis and monitoring treatments for hematologic disorders and other medical problems. Determining whether a parameter or a profile is within normality requires a comparison with RI from a similar population of clinically healthy individuals (5,6). The increasing need for utilization of CBC analysis from all continents of the modern world in different clinical settings along with the availability of advanced diagnostic devices improve the quality of patient care. This can be achieved by providing rapid laboratory test results to clinicians or other healthcare workers by contribute to immediate patient management and decisions making (7).

Careful standardization of pre-analytical and analytical conditions are prerequisites for obtaining reliable reference interval (8). And this can be possible by selection of homogeneous groups of reference population, stratification according to age and gender and defining of health status by establishing clear criteria for inclusion and exclusion (9).

However, there are certain variations in laboratory test results that can be expected due to non-modifiable biological factors, such as age, biological rhythms and physiological changes during pregnancy. Similarly many factors influence haematological parameters and as a result, considerable differences exist in health. Some of those important healthy variations include:

gender, age (as in infants, children, puberty, young adults, menopausal women and elderly adults), environment, lifestyle, and ethnicity (10,11)**.** It is necessary to take into account all variables that can affect the hematologic parameter RI determination when selecting individuals, in order to investigate these influences (12–14).

The venous haemoglobin level correlates to a modest extent with the red cell mass, though the correlation is different for adult men and women. Lower haemoglobin level in females cannot be ascribed to a lack of bone marrow or renal erythropoietic capability that adult females maintain their venous haemoglobin levels at a lower level than adult males as a physiological steady state; they do not try under physiological conditions to maintain the same levels as adult males. These factors may also indicate that males set their physiological haemoglobin levels higher than females, or rather that both sexes set their mean optimum level separately and to some degree independently (15).

Although age and sex are the two most common used as RI partitioning criteria, there are others for example during pregnancy the variation indicated from the non-pregnant (16,17). Since it is a state characterized by many physiological hematological changes, which may appear to be pathological in the non-pregnant state (18). The pregnant mother undergoes significant anatomical and physiological changes in order to nurture and accommodate the developing foetus (19). There is disproportionate increase in plasma volume up to 50%, RBC 33% and Hgb 18- 20% mass. In addition there is marked demand of extra iron during pregnancy especially in the second half of pregnancy. Since iron and folic acid in amounts necessary to the foetus are preferentially transported to the foetus, the mother is likely to develop iron deficiency anemia and folic acid deficiency anemia (20). Conversely, the hemodilution factor tend to increase in WBCs count during pregnancy (21).

The regional factors including ethnicity, genetics, and environment could affect the complete blood count. Standardizing local or regional hematology reference interval should be mandatory (22). For example, healthy Africans tend to have lower white blood cell counts than Caucasians, but there is no evidence that they suffer any additional risk of developing severe infection or other sequelae. Also, African American populations, with environmental exposures more like their white American counterparts, tend to have lower hematologic parameters than Caucasian Americans, suggesting a genetic basis for these population differences (23). African and Asian women have lower Hgb and HCT than Caucasian. Racial

and population specific references may have significant clinical and public health implication for more accurate disease diagnosis and appropriate treatment (24). The existence of regional variations in hematologic parameter RI between the African and Western population needs to provide region-specific reference values which can be used to guide patient management and interpretation of clinical research findings and which may potentially improve the quality of clinical care provided to patients (25).

Moreover, the body reacts in differing ways at varying levels of altitude. When one goes to altitude, there is a depletion of oxygen percentage in air which affects the delivery of oxygen to tissues (26). Hemoglobin is involved in the regulation of O_2 transport in two ways: a long-term adjustment in red cell mass is mediated by Erythropoietin (EPO), a response to renal oxygenation. Short-term, rapid-response adjustments are mediated by ventilation, cardiac output, Hgb oxygen affinity (P_{50}), barriers to O_2 diffusion, and the control of local microvascular tissue perfusion (27). As altitude rises, the air becomes thinner, and oxygen content gradually reduces. In response to the lack of oxygen, the human body undergoes a number of physiological changes by increasing concentration of circulating Hgb (28). And it is mandatory to consider variation in reference values for erythrocyte differs in altitude of even with the same county of different regions (29).

Several subsequent studies reported from Africa supports the premise that the difference in haematological parameters RI was attributed by multifactorial reasons. Decreasing pattern of the RBC count on increased parasitaemia prevalence state, ethnic diversity, genetic diversity, and variation in altitude were suggested as an attributing factor for district wise significant difference for all hematologic parameter, except for neutrophil (30). Effect of high frequency alpha thalassemia and the existence of iron deficiency anaemia suggested as factor for RBC parameters differ from African countries (31). Lower Hgb, HCT, RBC#, MCV and Neut# with high Eos# Compared with U.S- derived RI and age-related variation was indicated (32). Different RI from it had been used and not represent the population they are serving for values of RBC#, Hgb, HCT, MCV, MCH, and RDW (33).

A few attempts in Ethiopia had been made for the determination of hematological reference interval. A study conducted from healthy adults in Gojjam showed variations with reference interval used in the study community, reported in Africa, Western countries and books. Difference in altitude and gender highlighted significantly affects the reference intervals. (34)

A study conducted in the capital city of the country on adult blood donors had also indicated haematological RI was statistically significantly different from clinical practice that are currently utilized (35). Similar cross-sectional study from factory workers in Akaki had indicated significant gender differences for the RBC parameters (RBC, Hgb, and HCT) had higher values in males than females. During interpretation of hematologic results special insights needs to be consider for lowlands origin dwellers and other ethnic origins was suggested (36).

1.2. Haematological parameters

1.2.1. White Blood Cells Count (WBC)

The white blood cell count used to evaluate for any patient with signs, symptoms, or conditions associated with infections, inflammatory processes, bone marrow alterations, and immune disorders. WBC is classified into granulocytes, lymphocytes, and monocytes. Granulocytes owe their name to the presence of distinct cytoplasmic granulation. Three varieties are recognized: neutrophils (polymorphonuclear granulocytes), eosinophil, and basophils. Each type of cell plays a different role in protecting the body. The numbers of each one of these types of white blood cells give important information about the immune system (37).

1.2.2. Red Blood Cells Count (RBC)

The RBC count measures the number of circulating erythrocytes. It carries oxygen from the lungs to the rest of the body. They also carry carbon dioxide back to the lungs so it can be exhaled. If the RBC count is low (the condition is called anaemia), the body may not be getting the oxygen it needs. If the count is too high (the condition is called polycythaemia vera) (38).

1.2.3. Hemoglobin (Hgb)

Hemoglobin is the protein contained in red blood cells that is responsible primarily to carry oxygen to the cells and remove carbon dioxide from the cells. Hgb is a complex protein made up of heme and globin. It is produced in the immature RBC. There are approximately 300 million molecules of Hgb in one RBC. Hgb is measured in grams per decilitre. The heme portion contains iron atoms and the red pigment, porphyrin. The heme portion is responsible for the red colour of blood. When the RBC is saturated with oxygen, the red colour is brightest. The globin portion is made up of 4 amino acid chains.

One heme molecule attaches to each of the 4 amino acid chains. Therefore, each Hgb molecule has 4 heme sites that can bind with 4 oxygen molecules (39).

1.2.4. Hematocrit (HCT) or Packed Cell Volume (PCV)

"Hematocrit" means "to separate blood" The Haematocrit or Packed Cell Volume (PCV) represents the percentage of red blood cells as compared to the total blood volume whole blood in a sample. The test is usually ordered as part of the CBC and is used to diagnose and monitor anaemia, dehydration and to check the severity of ongoing bleeding (39).

1.2.5. RBC indices (MCV, MCH, and MCHC)

RBC indices are calculated mean values that are used to define the size, weight, and Hgb content of the RBC. They are mainly used to classify anemias. RBC indices consist of MCV, MCH, and MCHC (39).

- **Mean Cell Volume (MCV)**

  MCV describes the RBC by size or volume. This measure uses the size of the RBC to identify possible causes of anemia as well as other disorders. The MCV classifies RBCs as microcytic, normocytic, and macrocytic. *Microcytic cells* are small or undersized. They are seen with iron deficiency anemia and thalassemia. In hemorrhagic or hemolytic anemias, the decrease in oxygen carrying capacity is caused by a decrease in the number of RBCs; the cells that remain are normal in size, thus the RBCs are normocytic. RBCs that are macrocytic are large or oversized. Traditionally the mean red cell volume was determined by the equation: MCV = (PCV/RBC)*10. Modern, automated hematology analysers measure the MCV directly and is expressed as femtoliters (10^{-15} fl) or as cubic microns (μm^3) (39).

- **Mean Corpuscular Hemoglobin (MCH)**

  It quantifies the amount of hemoglobin per red blood cell. An alteration in MCH tends to track along with the MCV. For example, a small sized cell has less Hgb within it compared with a large-sized cell, therefore its weight would be lower. Decreases are related to microcytic anemias, and elevations are related to macrocytic anemias. Therefore, the MCH adds little information independent of the MCV. The MCH is expressed as picograms (pg) of hemoglobin (per average red cell) (39).

- **Mean Corpuscular Hemoglobin Concentration (MCHC)**

  It is the ratio of the weight of hemoglobin to the volume of the erythrocyte, and is expressed either as a percentage or in grams per decilitre of red cells (g/dL). The MCHC is classically determined by the equation: MCHC = (Hgb/HCT) X 100. In

automated equipment, however, the HCT is a calculated value based on the RBC and the MCV values, which are directly measured parameters (39).

1.2.6. Red Blood Cell Distribution Width (RDW)

Measures the variability of the red blood cell volume, which represents the coefficient of variation of the RBC volume distribution (size) and expressed as a percentage (39).

1.2.7. Platelet count (PLT)

Platelets (thrombocytes) are the smallest type of blood cell. They play a major role in blood clotting. When bleeding occurs, the platelets swell, clump together, and form a sticky plug that helps stop the bleeding. If there are too few platelets, uncontrolled bleeding may be a problem. If there are too many platelets, there is a risk of a blood clot forming in a blood vessel (39).

Mean platelet volume (MPV)

The mean platelet volume is analogous to the mean red cell volume (MCV) and is the average size of a single platelet expressed in femtoliters (fL = 10^{-15} L) (39).

Platelet Distribution Width (PDW)

The platelet distribution width is the coefficient of variation of platelet size multiplied by 100 and expressed as a percentage value. The PDW is an objective measure of variation of platelet size, similar to the RDW), which is an objective measure of variation (anisocytosis) of red blood cells (39).

1.3. Statement of the Problem

Currently, developing counties are affected by numerous public health problems with the pre-existed communicable disease, emerging and re-emerging infectious agents and life style related disease, which needs early diagnosis. Rapid, accurate, and relevant laboratory testing is essential in an era of cost-effective medicine; in resource poor countries inaccurate diagnosis may lead to economic suffering (40).

Laboratories throughout the world are realizing that their RIs are either not accurate or inappropriate for the population they are serving. Reference intervals currently in-use, were generated mainly for the Caucasian population, and are often inappropriate for the diverse population that many laboratories serve. Only minority of clinical laboratories are sufficiently resourced in terms of time, finance and expertise to establish reference intervals for all, or indeed any of the tests they routinely perform (8,41).

The most frequent external source of clinical laboratory tests RI was manufacturers' recommendations/package inserts, which many laboratories adopt without on-site testing of healthy individuals (42,43). Similarly, practices in resource-limited settings especially, developing countries are using RIs obtained from information provided by reagent/analyzer manufacturers for evaluating patients (44). However, this is applicable when the method should only be applied to biologically homogenous populations. Text books/ medical journals, publications, multicentre studies and non-laboratory medical staff recommendation were the source for some laboratories (45,46).

Adopting these RI that has been derived from a population dissimilar sources without consideration of local differences on which the RIs are based leads to misdiagnosis of disease, increase number of incorrect decisions, increase cost by the unnecessary investigations and risk in patient safety (47,48). Moreover, many laboratories do not even report RI for 'females, much less for pregnant women (49).

Studies illustrated these discrepancies from one study to another related to different factors such as age, sex, geographic origin, altitude, and ethnic origin had indicated the variability of key haematological indices associated with the heterogeneity of individuals' characteristics (50,51). Monitoring the health status relies in part on the availability of exact reference

intervals for key haematological parameters. And it is crucial to have reference interval oriented to special defined populations which must derive from a part of the population which represents the study participants to avoid misinterpretation of findings and miss conclusion in disease diagnosis (52,53). The available studies in Ethiopia, also underscore the need for locally established values (34–36).

Evidences indicated that differences observed in the RBC components between African and Caucasian populations may be attributed to lower dietary iron intake, genetic polymorphisms such as thalassemia and sickle cell trait or chronic exposure to endemic parasites including helminths, malaria and schistosomiasis (23). The interpretation of the laboratory results in Dire Dawa town is currently using manufacturer source derived mainly from western reference interval, which they are distributing their instruments and reagents to developing countries. Moreover, there is no previous study for hematologic reference interval in Dire Dawa town, which is characterized by low land altitude and different environmental conditions, which are an important component in the climate system, and plays a key role in RBCs parameters. Therefore, this study had been carried out to determine hematological parameters reference interval in healthy adults of Dire Dawa population by applying the classical and advanced statistical methods recommended by Clinical Laboratory standard Institute (CLSI) (1).

1.4. Significance of the Study

The aim of the present study is to establish reference intervals for hematology parameters of Dire Dawa adult population. Hence, this study will aid clinicians for reliable interpretation for the diagnosis, monitoring and therapeutic management of disease; promote early diagnosis of haematological disorders, provide more accurate laboratory test result interpretation to improve patient care and reduces long-term complications. The finding of the study will also be used as baseline data for haematology parameters of adults for future research the region. Moreover, it will benefit policy planners and decision makers in planning and utilizing the data for appropriate planning and management of hematological disorders.

2. Literature Review

A complete blood count is a blood panel requested by a physician or other medical professional that gives information about the cells in a patient's blood, such as the cell count for each cell type and the concentrations of various proteins and minerals. Reference interval**s** used by many healthcare facilities in developing countries are typically obtained from information provided by reagent or analyzer manufacturers. Interpretation of the CBC can be broken down into three sections: evaluation of the erythrocytes, leukocytes, and platelets. Each of these parameters can be interpreted individually; however, integration of the data is important for the highest diagnostic yield (1–3).

Many studies all over the world showed variability of hematological parameters reference interval. Justifications given for this variation were due to the difference in sex, age, altitude, geographic location, and ethnic origin. Physiological factors are amongst the factors to influence hematologic RI. Age and sex are well described in adults. The dominant form of partitioning applied in clinical laboratory medicine is by social consensus: adulthood begins at age 18 (or 21) and can be divided into decades and old age is the age of retirement, which is about 65 to 70 years. The gestational age in pregnancy is divided into three trimesters (52). However, there are conflicting data and studies indicating variation in hematologic RI from different continents of the world, countries and regions and locality as well.

2.1. Adult Reference Interval

A comprehensive study conducted in North American revealed that there were age-dependent trends for many of the tests, mainly in RDW, MCV, PLT count, granulocyte and lymphocyte percentages; sex-dependent change was observed in Hgb values, and race-related trends cantered around mononuclear and lymphocyte percentages, HCT, MCHC, MCH, and Hgb value (53). Similar comprehensive study, conducted in Middle East Oman population, found mainly sex dependent trend of difference for hematologic parameters across all age group. The mean values of RBC count, Hgb and HCT higher in males across all age group than females while PLT were higher in females. The authors put forward the necessity to establish hematologic reference interval among the healthy population in each country or at least in each region (54)**.**

A cross sectional study conducted in Bangkok, Thailand in 2015, recruited participants on voluntary basis from a health check-up program among adults of both genders found that statistically significant difference based on gender for many hematologic parameters, particularly for Hgb= 12.7gm/dl-16.9gm/dl for males and 12.0gm/dl -14.9gm/dl for females; HCT= 40.3%-51.9% for males and 37.0%-45.7% for females; RBC=4.2-6.1*10^6/ul for males and 4.0-5.5*10^6/ul for females; MCHC= 30.8gm/dl-34.6gm/dl for males and 30.2gm/dl -34.2gm/dl for females, and PLT= 160-356*10^3/ul for males and 179-435*10^3/ul for females had clinically importance difference was observed. The findings of this study highlighted on the importance of establishing hematologic reference intervals representing to the population to be served (55).

A cross study was conducted in Zimbabwe among 769 adult of 18–55years of age found significantly higher in males for most red cell parameters (RBC#: males=5.5 x10^6 cells/μL and females=5.1 x10^6 cells/μL, Hgb: males=13.2–18.3g/dL and females=10.2–15.9g/dL, PCV: males=48.5% and females=45.4%, and MCH: males=29.4pg and females=28.9pg) than females, while WBC# (males=4.6 ×10^3cells/μL and females=5.2 × 10^3cells/μL), PLT# (males= 229.0× 10^3 cells/μL and females= 268.5× 10^3cell/μL), neutrophil#, and lymphocyte# were higher in females than males (56).

A cross sectional study conducted in Mali on healthy volunteers to establishing reference ranges of hematological parameters showed significantly higher in male for the median value of RBC (males=5.14×10^6/μL; females=4.67×10^6/μL) and Hgb (males=14.5g/dL; females =12.8 g/dL) than females; while MPV were higher in females. The study also indicated currently used RI in the routine laboratory is different from the western derived, whom they are genetically and environmentally different from African population (57).

A study conducted in Israel found significant neutropenia among Ethiopian heritage born in Ethiopia (mean±SD=3080±1570*10^3cells/μL) than Ethiopian heritage born in Israel (mean±SD=3470±1710*10^3cells/μL), whose parents were born in Ethiopia and a control group who were not of Ethiopian heritage while significant eosinophilia among Ethiopian heritage born in Ethiopia than the two groups. It was suggested that for eosinophilia was attributed by environmental influence while neutropenia was familial-genetic nature (58).

A community based cross-sectional study conducted in southwest Ethiopia found that most of the hematological parameters significantly varied across all age groups and significantly higher median value in adult male than females for: RBCs (male=5.32*10^6/μL and females= 5.02*10^6/μL, Hgb (males=155 g/dl and females=146 g/dl), HCT (males=45.2% and females= 43.1%), RDW-CV (males=13.7% and females=13.6%) and Eos# (males = 0.28*10^3/μL and females=0.22*10^3/μL), while the PLT count (males=275*10^3/μL and females=288*10^3/μL and MCV (males=84.3fl and females=86.15fl) was higher in female. According to the author, as compared to other studies conducted in Ethiopia, effect of altitude difference was indicated owing to lower median values for Hgb and HCT value to the contrary higher for RBC count, Hgb and HCT but lower MCV than in Caucasians and other African countries. The study also indicated that the hematologic has been different from the reports from other countries and the standards described in western literature (59).

A cross sectional study conducted for Hematologic RI determination in 240 blood donors of 18-50 years of age at Gondar indicated that majority of hematologic parameter RIs was different and lower from Caucasian population, African countries and also studies conducted other part of Ethiopia. A significance difference based on gender for majority of RBC parameters (RBC: males= 5.01*10^6/μL and females=4.8*10^6/μL, Hgb: males= 14.2gm/dl and females= 12.9gm/dl, PCV: males=46.9% and females 45.2%, MCH: males=29.0pg and females=28.6pg and MCHC: males=31.3gm/dl and 30.8gm/dl) in which males were higher than females. Adapting local reference interval was highlighted by the author (60).

A cross-sectional study conducted in Bahir Dar Town, which is located at an altitude of 1,830 meters above sea level, on 405 adults of 18-60 years found that a statistical significance difference based on gender for mean values of RBC count (4.9±0.4*10^6/ul for female and 5.4±0.5*10^6/ul for male); PCV (44±4% for females and 49±4.5% for males) and Hgb (14.7±2gm/dl for females and 16.5±1.8gm/dl for males) in which the values were higher in males than females. The differences in RBC counts, Hgb and HCT levels RI was higher than other African countries and the effect of altitude induced erythropoiesis highlighted by the author (61).

2.2. Pregnant Reference interval

A Study conducted in Bankura aimed to determine the effect of pregnancy on haematological parameters and compare the haematological parameters at different stages of pregnancy indicated no significant difference in the value of all haematological parameters analyzed when compared at different trimesters of pregnancy. However, the finding showed that significantly lower in pregnant for Hgb (9±1.5gm/dl Vs 12±0.6gm/dl), PCV (30.6±4.2% Vs 37.7±3.6%), Mono# (1.4±0.8*10^3/ul Vs 4.1±1.9*10^3/ul) and Lymph# (34.6±14.5*10^3/ul Vs 43.8±12.5*10^3/ul) than non-pregnant while WBC (7.2±3.02*10^3/ul Vs 4.9±0.88*10^3/ul) and Eos# (10.3±4.2*10^3/ul Vs 6.3±3.3*10^3/ul) were significantly higher in pregnant than the non-pregnant women (62).

Ethnic based difference among pregnant women of Caucasian and non-Caucasian were observed for various hematologic parameter in a retrospective review of a large maternal database, study conducted in Pennsylvania. The study illustrated significantly higher for Hgb concentration among Caucasian than non-Caucasian from the 27 weeks gestation until delivery. At different gestational age, significantly lower for parameters for HCT, MCV, MCH, and MCHC among non-Caucasians than among Caucasians but only RDW was higher among non-Caucasians than Caucasians were also indicated in the study (63).

The changes in hematologic parameters during pregnancy were observed from a study conducted in Beijing among productive age groups of pregnant women. During 1^{st} and 2^{nd} trimester RBC (RI: 1^{st}=3.7-5.07*10^6/ul; 2^{nd}=2.85-4.59*10^6/ul; 3^{rd}= 2.75-4.64*10^6/ul), Hgb (RI: 1^{st}=11.0-14.7gm/dl; 2^{nd}= 8.8-13.6gm/dl; 3^{rd}=8.4-14.1gm/dl) and HCT (RI: 1^{st}= 33.0-43.0%; 2^{nd}= 27.0-40.0%; 3^{rd}= 26-42% declined, and began to rise again in 3^{rd} trimester. WBC (RI: 1^{st}=4.68-12.87*10^3/ul; 2^{nd}=5.97-16.78*10^3/ul ; 3^{rd}=5.53-19.56*10^3/ul), neutrophil# (RI: 1^{st}=2.27-9.92*10^3/ul; 2^{nd}= 4.16-14.1*10^3/ul ; 3^{rd}=3.73-17.24*10^3/ul), monocyte# (RI: 1^{st}=0.2-0.66*10^3/ul; 2^{nd}=0.22-0.98*10^3/ul; 3^{rd}=0.26-1.1*10^3/ul), RDW (RI: 11.9-16.8%; 2^{nd}=12.3-17.2%;3^{rd}=12.3-19.8%), and PDW (RI: 1^{st}=9.0-16.4fl; 2^{nd}=9.1-18.1fl; 3^{rd}=10.2-19.1fl) had progressively increased from 1^{st} to 2^{nd} and then 3^{rd} trimester. On the other hand; MCHC, lymphocyte#, PLT, and MPV gradually decreased during pregnancy(64).

Similarly, a cross sectional study conducted in Nigeria, among productive age group of pregnant and non-pregnant study participants found that the normal haematological parameters

of pregnant women by gestational age for Hgb concentration decreases when the gestational age of study participants increase from 11.68-12.25gm/dl to 10.89-11.14gm/dl then 10.80-11.07gm/dl during 1st, 2nd then 3nd trimesters, respectively. The PLT# decreased from 241.27-285.49*10^3/ul; 229.91-251.38*10^3/ul and 182.33-202.13*10^3/ul during the 1st, 2nd and 3rd trimester of pregnancy, respectively. According to the study, the WBC count increases from 5.82-6.73*10^6/ul to 6.30-6.85*10^6/ul then 6.73-7.22*10^6/ul as the gestational age increases from 1st to 2nd and 3rd trimester respectively but the neutrophil# were largely increased as the gestational age increases (65). Altered haematological parameter for RBC indices (RBC#, PCV, MCV, MCH and MCHC) and WBC# were seen during 3rd trimester of normal pregnancy in comparison to non-pregnant women in a cross sectional study conducted in Surat among 25 pregnant women in 3rd trimester of gestation age (66).

A cross-sectional study conducted in Gondar, Northwest Ethiopia in 2015 among pregnant women found statistically significant difference between different trimesters for HCT, which showed progressive increment as the trimester of study participants increased from 1st to 2nd and then to 3rd. The mean ±SD and 95% range for HCT of all study subjects were 40.8±4.7% (RI= 40.19–41.49%), 39.18±6.70% with RI=37.17–41.19%, of 45 pregnant, 40.53±3.77% with RI= 39.63–41.44% of 69 pregnant and 41.96±3.70% with RI=41.17–42.75% of 86 pregnant women of 1st, 2nd and 3rd trimester respectively. Although there was no statistically significant difference based on trimester for PLT#, a decreasing trend with gestational age was observed from 1st to 2nd and then to 3rd trimester. The author suggested the finding may not be applicable for other ethnic groups who dwell in the lowland areas (67).

Recently, a cross sectional study undertaken at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia, to establish immunohematological reference range among HIV sero-negative pregnant women found mild increase for WBC and MID% (mononuclear, Eosinophil and Basophil cells). Based on trimester, an increase in the mean±SD for WBC count (1st=7.02±2.61*10^3/ul, 2nd =7.83±2.62*10^3/ul and 3rd trimester=8.22±2.68*10^3/ul) while a decrease in RBC, Hgb, PLT and Neut% count from first to third trimester and during 2nd trimester for HCT was indicated. It was also shown that as compared studies conducted in Ethiopia; higher than for RBC, Hgb and HCT. This was illustrated by the author that altitude difference which induced for erythropoiesis but when compared with Europe and United States, low values of WBC and granulocytes were found in each trimesters in the study (68).

3. Objectives

3.1. General Objective

To determine hematological parameters reference interval for adults of Dire Dawa Population, Dire-Dawa.

3.2. Specific Objectives

- To analyze hematological parameters reference interval for adults of Dire Dawa population, Dire-Dawa
- To determine hematological parameters reference interval for pregnant women of Dire Dawa population, Dire-Dawa

4. Material and Methods

4.1. Study area

This study was conducted in Dire Dawa City administration (DDCA), which is located 515 km away from the capital city Addis Ababa. It is estimated population size of 384,834, of whom 171,461 are men and 170,461 are women. Based on residence, 74% (284,160) live in urban Dire Dawa while the remaining 26% live in rural area. It has a land coverage of approximately 1,977 square kilometres, of which 187 square kilometres is urbanized while the remaining 1,790 square kilometres is rural. DDCA is geographically located in between 9º49 North latitude and 42º19 East longitude and it lies with an altitude ranging from 950-2260 (An average of 1160) meter above sea level. Its eastern, low land position generates a hot climate with average temperature and rainfall of 34.6 °C and 637 mm, respectively. The weather is characterized by warm and dry climate and relatively low level of precipitation (69,70).

4.2. Study design and Period

A cross-sectional study design had been employed to collect data from January to March 2019.

4.2.1. Source population

All adults living in Dire-Dawa City Administration and surrounding area have been the source population

Partitioning

Partition-1: All men with the age groups of 18-65 years
Partition-2: All women with the age group of 18-65 years
Partition-3: All pregnant women with the reproductive age group (15-49 years)

4.2.2. Study population

Subjects who have been satisfied the inclusion criteria (in each partition), given written consent explaining the detail and exact nature of the study including any risks involved in taking part after understanding with information sheet (Annex II) and volunteer to donate whole blood samples (Annex IV) have been included in the study. Classifying reference individuals into different subclasses favours in making clinical differences to homogenously categorized study

subjects. Therefore, in this study non-parametric partitioning, which is based on the clinical differences, had been employed for partitioning of study participants.

- Age: ranging eighteen to sixty-five years for both genders and between fifteen and forty nine years of age for pregnant women.
- Gender: male and females
- Pregnancy status: pregnant and non-pregnant with reproductive age group (15-49) and the pregnant further sub-partitioned into gestational age: first, second and third trimester.

Partition-1: men with the age groups of 18-65 years old

Partition-2: women with the age group of 18-65 years old

Partition-3: pregnant women with the reproductive age group (15-49 years

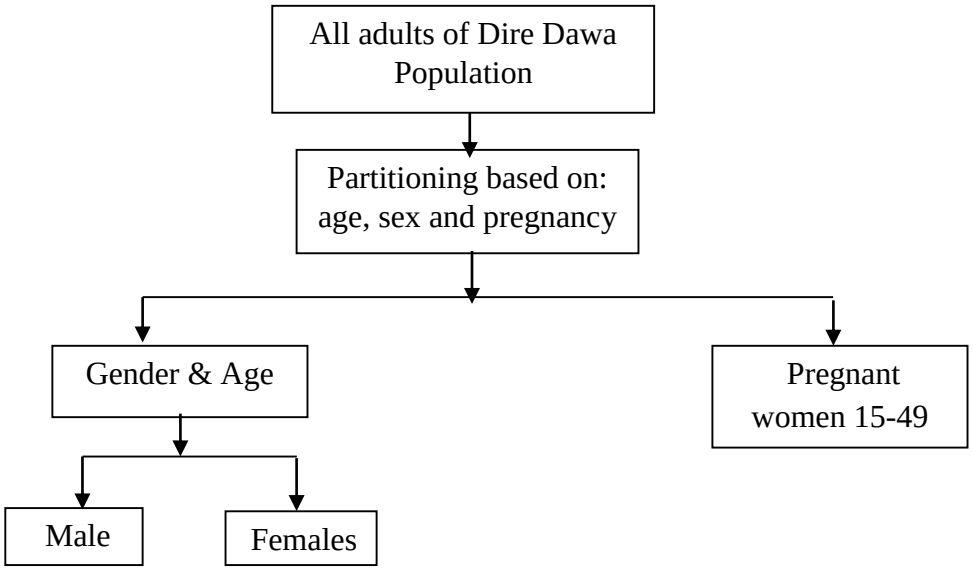

Figure 1: Flow diagram of study population partitioning of Dire Dawa population, Dire Dawa, Ethiopia from January-May, 2019

4.3. Eligibility

4.3.1. Inclusion criteria

- Individuals who were 18-65 years and 15-49 years (for pregnant) whom they reside in Dire Dawa town at least for five years.
- Individuals who were volunteer to answer the questionnaire prepared for this study; had given a written consent and sign to participate in the study; and volunteer to provide whole blood and urine sample (for females).

4.3.2. Exclusion criteria

- Individuals with: unusual or strenuous exercise during the previous 3 days; skin rashes; take over-the-counter for iron tablets or vitamins., and individuals with history of: hypertension; blood transfused in the last 1 year; blood donation in the last 6 months; hospital admission for the last 1yr; surgical procedure for the last 3yr; chronic gastritis; malaria for the last 6 month; TB for the last two years; cancer; cardiac illness; bleeding disorders; allergy; wheezing; on any medication, exhibiting febrile symptoms, observable mental illness; anemic, having chronic diseases, with recent immunization (last 6 months), acutely ill patients, administration of pharmacologically active agents exposed drug abuse; exposed in hazardous chemicals in their job were not invited to take part in the study. Regular (Once/day) and more than occasional alcohol drinker; more than occasional *Khat* chewer; and smokers. Individuals with high blood pressure as the standard of WHO were excluded. Similarly, individuals positive for HIV; HBsAg; HCV-Ab; Syphilis; CRP; and blood film had been excluded during the final statistical analysis.

  For pregnant women: with active bleeding during the data collection period, encountered with pregnancy and obstetrics complication were excluded from the study. For non-pregnant women: who were menstruating during the data collection period; average menstruation stay >7days; taken over the counter of oral contraceptive; and breast feeder have also been excluded from the study.

4.4. Study Variables

4.4.1. Dependent variables

- Reference interval for hematological parameters

4.4.2. Independent variables

- Socio-demographic characteristics (age, sex, altitude, residence, monthly income, educational status, etc.) and nutritional parameters

4.5. Measurement and Data collection

4.5.1. Sample size determination

The CLSI recommends that studies to establish reference intervals should have a minimum of 120 individuals in each group of study participants. Therefore a minimum of 120 reference individuals were selected for determination of non-parametric RI and 90% confidence intervals (CI) of the reference limits with enough extra individuals by assuming screening (HIV, HBsAg, HCV-Ab and Syphilis) related rejection rate of 30% .

According to previous studies conducted at seven sites of adult Africans to describe African laboratory reference intervals applicable to potential volunteers in HIV vaccine clinical trials for recruiting participants found nearly one-third (883/2990, 29.5%) of all persons were not eligible. (71) Therefore, based on this study, 30% was taken to exclude study subjects during data analysis.

Partitioning reference intervals is required when there are significant physiological changes that need to be recognized. Therefore, in this study 120 study participants have been partitioned based on gender (male & female) whom 18-65 years of age to each category and pregnant women with the reproductive age group (15-49 years).

$$120*(1/1\text{-exclusion})$$

$$120*(1/1\text{-}0.30) = \underline{171}; \quad \text{Total sample size: } 171*3 = 513$$

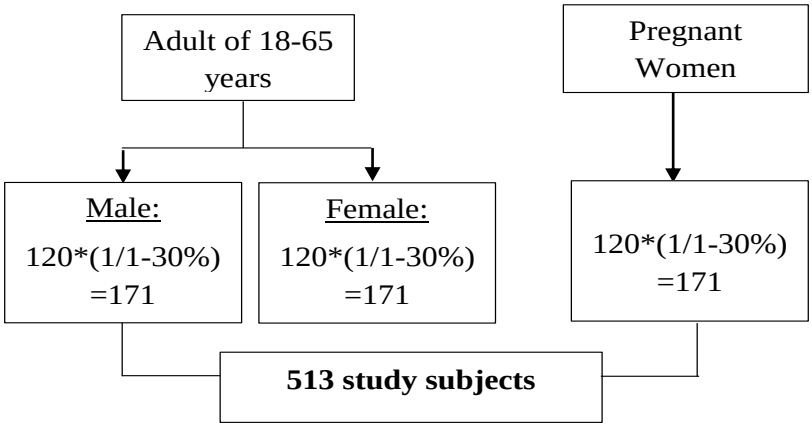

Figure 2: Flow chart of study subjects sample size of Dire Dawa population, Dire Dawa, Ethiopia from January-May, 2019

4.5.2. Sampling technique

A Convenient sampling technique had been employed to collect whole blood samples from study participants recruited by health extension workers from the community and from ANC clinic by the clinical nurses worked at DCH hospital, from individuals whom they fulfilled the inclusion criteria, during the data collection period.

4.5.3. Data collection procedure

Following screening of physical examination, written informed consent was obtained from all eligible volunteers. The consent process included an explanation and discussion of the study procedures. Assessment of the potential volunteer's was performed using a pre-tested and structured questionnaire, which was prepared by the principal investigator, for the assessment information from study participant on socio-demographic factors, clinical history and health condition, risk factors assessment, personal life styles and gestational (for pregnant women) was used via face to face interview. The questionnaire for all partition of participants is similar, but for those pregnant women the questionnaire incorporated pregnancy related questions. In order to ensure the consistency of questionnaire, first it was translated to the local languages; Amharic (Annex VI), Oromiffa (Annex VIII) and Somalifa (Annex VII**)** then retranslated to English (Annex V).

The vital sign assessment was measured by HEW, which include: body temperature, body weight, and blood pressure and pulse rate by digital thermometer, balance and digital device respectively. For all study participants the vital sign result was recorded on vital sign and physical examination sheet, which was prepared for this study purpose.

For the determination of hematologic parameters, all subjects were required to maintain their normal lifestyle, avoid strenuous physical exercise within 3 days of the physical examination and laboratory testing, and refrain from drinking alcoholic beverages for at least 1 day prior to the testing. The study subjects were fasting overnight (fasting time was for at least 8 hrs) and sit for at least 15 min before specimen collection. Whole blood samples were collected from the study participants, who fulfil the inclusion criteria, between 08:00 A.M. and 11:00 A.M. by veni puncture, under aseptic conditions from the antecubital vein. The collected blood (about 2ml) was poured into evacuated tube and immediately anticoagulated vacutainer tubes, which contained 0.5 ml Ethylene diamine tetra acetate (E_3DTA) tubes (Becton Dickinson), and

mixed with 5 to 7 times. About 3 ml blood was collected in the same way for serological testing that can be stylized for exclusion during data analysis step.

The whole blood then was transported at ambient temperature to Dil-chora Hospital laboratory within 1 hours of drawing. All the whole blood samples were subjected to Mindray BC-300Plus hematology analyser within one hour of post arrival at Dil-chora Hospital laboratory for CBC analysis. Anlyzer print out paper for hematologic parameter result was attached with the respective study participant questionnaire. After clotting, serum was harvested and stored at -20 ^{0}C by centrifugation for transfusion transmittable infections (TTI) screening using rapid tests according to manufacturers' instructions.

4.5.4. Hematologic analysis

Complete blood count (CBC) analysis was performed immediately following arrival of the sample by Mindray BC - 3000 plus, which is a quantitative and automated hematology analyzer for *in vitro* diagnosis use in clinical laboratories. BC-3000plus hematology analyzer operates for closed vial whole blood mode, whole blood mode for venous blood, and pre-diluted mode for capillary blood. It has a counting speed of 60 samples per hour. The analyzer produces 3-part differentiation of WBC; 19 parameters (WBC, Lymph, Mid, Gran, Lymph%, Mid%, Gran%, RBC, Hgb, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW, PC and 3 histogram (WBC, RBC, PLT). It also automatically cleaning sampling probe, dilute, lyse, mix, rinse and clog-clear.

4.5.4.1. Principle of CBC by Mindray BC-3000plus

There were two independent measurement methods used in Mindray BC-300Plus hematology analyser:

1. The Impedance method for determining the WBC, RBC, and PLT data
2. The colorimetric method for determining the Hgb

WBC, RBC and PLT count

WBC, RBC and PLT were counted and sorted by the electrical impedance method, which were based on the measurement of changes in electrical impedance produced by a particle passing through an aperture. During each analysis cycle, the sample is aspirated, diluted and mixed before the determination for each parameters were performed. After automatic dilution of the whole-blood sample, lysis, and count the device gives a printout result of WBC count, platelets,

neutrophils, eosinophil, monocytes, Bands (all expressed as number of cells*10^3]/ lit), lymphocytes (expressed as number of cells *[10^9]/lit and percentages).

In addition, for RBCs and PLTs, volumetric metering was used. An accurate cell count cannot be obtained unless the precise volume of diluted sample that passes through the aperture during the count cycle is known. The analyzer uses a volumetric metering unit to control the count cycle and to ensure that a precise volume of sample was analyzed for the measurement.

Haemoglobin measurement

The HGB is determined by the colorimetric method in which the lyse reagent releases HGB when RBC is broken down and react with Hgb to generate a mixture for Hgb measurement. The WBC/Hgb dilution was delivered to the WBC bath where it is bubble mixed with a certain amount of lyse, which converts haemoglobin to a hemoglobin complex that is measurable at 525 nm. An LED was mounted on one side of the bath and emits a beam of light, which passes through the sample and a 525nm filter, and then is measured by a photo-sensor that is mounted on the opposite side. The signal was then amplified and the voltage is measured and compared to the blank reference reading (readings taken when there is only diluent in the bath). The HGB was calculated per the following equation and expressed in g/L.

$$\text{Hgb (g/L)} = \text{Constant} \times \text{Log}_{10} (\text{Blank Photocurrent/Sample Photocurrent})$$

4.5.4.2.Serological testing

Tranfusable Transmittable Infectious (TTI) screening test had been used for screening HIV, Syphilis, Hepatitis B and Hepatitis C using commercially available rapid tests. Serum samples, which was obtained by centrifugation of plane tube blood was used for screening each infectious agents; ABO typing (direct typing method); C-reactive protein for inflammatory infection; blood film (BF) examination for hemo-parasite detection and identification using both thin and thick film; and HCG testing for non-pregnant female study subjects, not visibly pregnant, were tested for the presence of β-HCG hormone by qualitative one step pregnancy test using a freshly voided urine sample were employed at Dil chora Hospital laboratory.

HIV1/2 STAT-PAK employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles, and HIV1/2 antigens, which are bound to the membrane solid phase. The sample is applied to the sample well followed by the addition

of running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigen.

One step HBsAg, which was wondfo cassette, kit was employed for the screening of HBV using a rapid immunochromagraphic test principle, which when the specimen is added into test device the specimen is absorbed into the device by the capillary action, mixes with the antibody conjugate and flow across the pre-coated membrane. When the antigen levels are at or above the detection limit of the test, HBsAg in the sample combines to the antibody conjugate in the pad then are captured by the antibody immobilized in the test region.

One step HCV strip test was employed with a principle of the antibody-capture immuno-chromatographic assay, detecting the presence of HCV antibodies in the serum/plasma samples. Specific HCV antigens are conjugated with colloidal gold and deposited on the conjugate pad and immobilised on the test line of nitrocellulose membrane. When serum or plasma added, it rehydrates the gold antigen conjugate and the HCV antibodies, if any in the samples, will interact with the gold conjugate antigen.

One step ANTI-TP rapid screening test was employed for detecting the presence of antibodies of *trepanomal pallidum* (TP) in the sample of study subjects using chromatographic immunoassay. The test relied on the purified recombinant antigens of TP are used in test band and capture materials and gold conjugates. If the antibody anti-TP is present in the sample in the concentration above the labelled, complex will be formed. This complex is then captured by the antigens immobilised in the test zone of the membrane.

4.5.4.3.Quality control

Before each series of blood sample tests, quality control was performed using commercial specimens and recorded on the QC log sheet. Three level (Low, Normal and High) of control, which are commercially prepared whole-blood products purchased from the instrument manufacturer and containing known value for CBC parameter, run each day before analysis begin to verify the analyzer is functioning properly and to ensure reliable results had been obtained. During study period in case IQC failure encountered, the remedial actions was taken in accordance with Dil chora Hospital laboratory procedure and the manufacturer's instruction. The analyzer had been be calibrated as per standards recommended by the manufacturer. External quality assessment (EQA), which is one of the quality assurance tools, performed at

Dil chora Hospital for one scheme achieved during sample analysis of this study and its result verified for its acceptance. Moreover, between-run precision was assessed using the three level of IQC materials at least 20 consecutive days. The mean, SD and CV was calculated for each hematologic parameters and compared to those quoted in the analyser manuals and reagent inserts.

4.6. Data Quality Assurance:

4.6.1. Data collection tool QA

The questionnaire had been pre-tested and translated to the local language: Amharic, Oromifa and Somalifa; and retranslated to English in order to assure its consistency. In addition, the data collectors were trained on the purpose of the study, data collection and handling procedure by the principal investigator prior the data collection period. The principal investigator was supervising daily for the completeness of data collection tools. The data was entered at the end of each day to the statistical software.

4.6.2. Pre-analytic

Blood samples were collected after 15 minutes of rest study subjects in comfortably sitting position on a portable chair. After ensuring all the necessary materials and equipment's (Annex XII) for blood drawing procedure was available, and the rubber-type with closure tape tourniquet put on the arm of a study participant and make skin preparation by using 70% ethyl alcohol rub the surrounding skin area near to the suitable vein. Using K_3EDTA, sterile needle of sized 19-23 gauge and 5ml sterile syringe the blood samples had been collected from antecubital vein of study subjects. The collected whole blood in EDTA and plane tubes were transported to Dil chora hospital within 1hrs of collection by sample transporting device of maintaining 20-25^{o}C according to SOP. Serum was separated and stored at -20^{O}C until analysis. On arrival, each E_3DTA sample was inspected visually by gently mixing the tube and observe for clots. And each sample was also be checked prior to analysis for sample acceptable criteria. The rejection criteria were:

1. Sample Quality: hemolyzed, leaked tube, clotted
2. Sample Volume: Inadequate or overfilled sample
3. Sample Labelling: Improperly labelled, unlabelled, mislabelled, miss matched with the questionnaire code and collection of specimen in wrong tube

4. Sample Transport: delay sample and inappropriate transport material
5. Questionnaire: incomplete questionnaire

   However, none were rejected due to these criteria.

4.6.3. Analytic

- Prior to analysis, IQC and whole blood samples was homogenized by inverting 8-10 times.
- Running daily the three level of commercial controls by Mindray BC-3000 hematology cell controls (Low, Normal and High).
- Processing IQC and whole blood at room temperature (27.5±0.5°C).

4.6.4. Post Analytic

- Inspect histogram prior to documenting reference value results.
- Inspect and ensure for each CBC result of the study subject with the corresponding health assessment tools prepared by the PI for the study purpose.

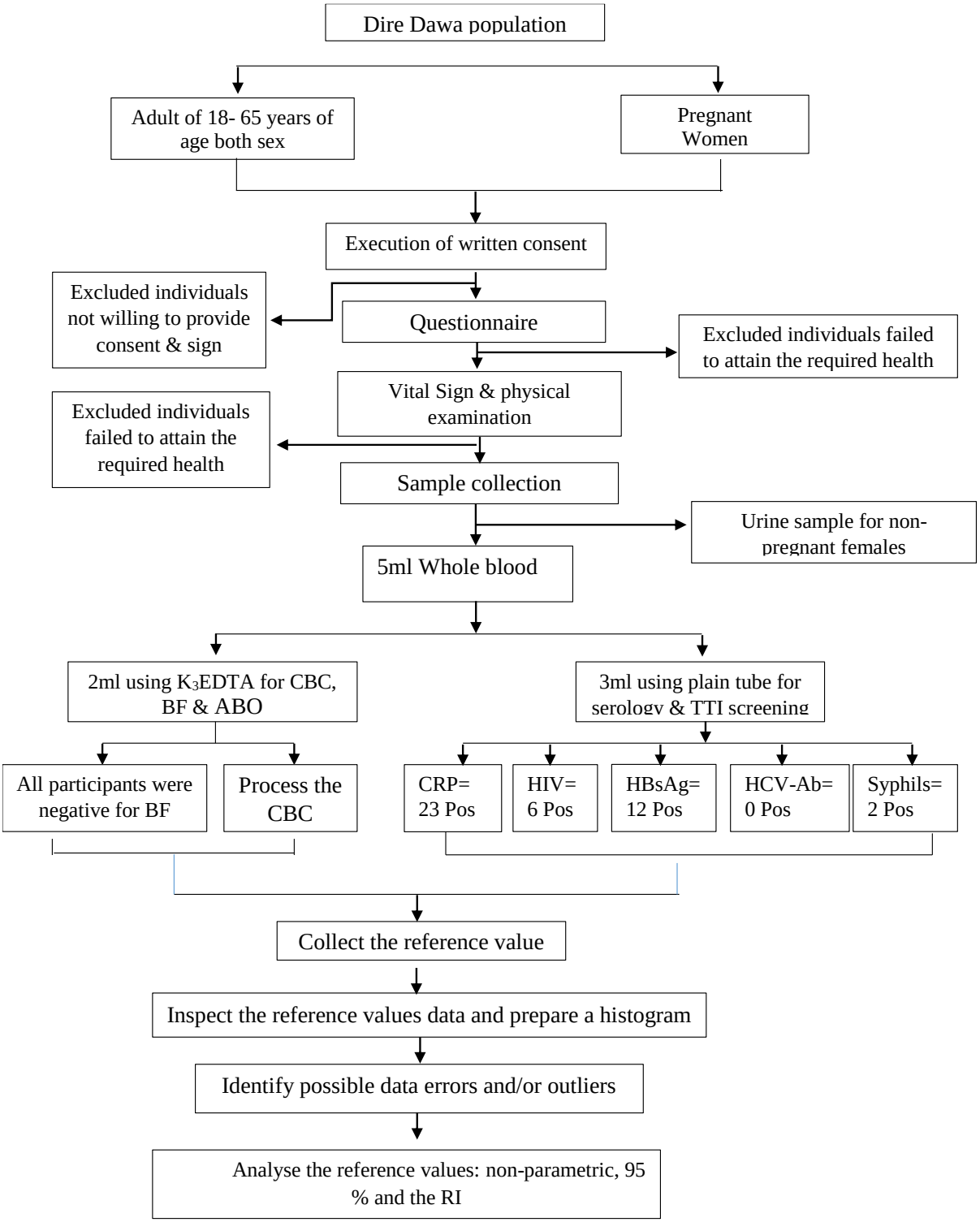

Figure 3: Workflow of study subjects recruitment and reference interval determination of Dire Dawa population, Dire Dawa, Ethiopia from January-May, 2019

4.7. Data analysis and interpretation

Data was cleaned, edited and checked for completeness and categorized based on their partition. Statistical analyses were carried out using NCSS software (Version 12.1 free trial) for reference interval determination and SPSS Version 24 (SPSS version 24.0, SPSS Inc. Chicago, IL, USA) for the rest of statistical computation. All calculations for determining reference interval was performed based on the guidelines found in the Clinical and Laboratory Standards Institute (CLSI: formerly National Council of Clinical Laboratory Services) guideline document C28-A2. The Dixon outlier range statistic had been used to identify and determine outliers. Nonparametric method was employed for reference interval determination for all hematological parameters which is according to 2.5% and 97.5% in a ranked list of reference value data, as the IFCC and CLSI recommended on formulas: lower limit has the rank number 0.025*(n+1) and the upper limit the rank number 0.975*(n+1) (1,72–74). The normality of the data distribution was tested with the K-S test. Effect of gender of study subjects were compared using the Mann Whitney U test for normally distributed parameters while independent-sample t-tests test for non-normal distributed hematologic parameters. Trimester specific variations of haematological parameters were evaluated by one-way analysis of variance (ANOVA) for normally distributed while Kruskal Wallis test for non-normal distributed hematologic parameters. A two-sided $p\ value < 0.05$ was considered statistically significant.

4.8. Ethical considerations

Prior to starting the study, ethical clearance for conducting the study had been obtained from Addis Ababa University, Department of Medical laboratory Science. After the official letter obtained from the Department of Medical Laboratory Sciences, it was submitted to Dire Dawa Administration Health bureau to obtain letters of permission by explaining the objective of the study. Subsequently, the official letter of permission had been given to Dil Chora Hospital. The study population had been informed that all the necessary information for the study such as; purposes and procedures, potential risk and benefits of conducting this study briefly elaborated to the study participants and the interviews will be conducted with strict privacy, and with assurance of their data will be handled and kept confidentially. They were also informed about their right to withdraw from the study at any step.

The samples were collected after the participant signed on the consent form prepared for conducting this study. Data collectors were trained for half-day training on; purpose of the study, data collection, and ethical issues, which aimed confidentiality and privacy; by principal investigator to maintain the privacy of individual and appropriate measures had been taken to assure confidentiality of the information both during and after data collection period.

Moreover, after interviewed using questionnaire for medical and clinical history, study participants were informed that for any abnormal physical examination and vital sign finding detected which require follow up by a nearby health facility. Study participants who had fulfilled physical examination and vital sign assessment had been provided a counselling service by HEW for notifying them for any abnormal CBC finding and infectious screening.

4.9. Dissemination of Result

The finding of the study will be submitted to Addis Ababa University, Department of Medical laboratory Science, to Dire Dawa Administration Health bureau and Dil chora Hospital. Findings will be communicated to the medical community during annual conferences and through publications on peer-reviewed journals.

4.10. Operational Definitions

Reference interval: The interval between, and including two reference values defined by a specific percentage (usually 95%) for hematologic parameters of healthy individuals.

Hematological parameter: Are the complete blood count which embrace the eighteen parameters result value of an individual (WBC, Lymph, Mid, Gran, Lymph%, Mid%, Gran%, RBC, Hgb, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW and PCT)

Pregnancy: The state of carrying a developing embryo or fetus within female body, which is confirmed by urine HCG positive result.

Adult: A human being with the age 18-65 years old for both gender.

Healthy: Study subjects who fulfilled the inclusion criteria for health related assessments designed for this study.

5. Result

5.1. Study Population

A total of 513 individuals, from ages 18 to 65 years for adult groups of both gender and 15-49 years for pregnant groups, participated in this study. Of them, 41(8%) were excluded due to positive serology results following pathogen screening. Among the excluded, 6(14.6%) were HIV positive, 12(29.2%) were HBsAg positive, 2(4.9%) were Syphilis positive and 23(51.1%) were CRP positive. The final study population comprised 472 healthy study subjects and thus data were included for final analysis. Of the total adult study participants, 160 were males, 155 females and 157 pregnant women. With regard to pregnant women, consecutive pregnant women whom they were with normal pregnancy and attended at different health facilities of Dire Dawa for ANC follow up was included in this study. Of the total 157 pregnant women, 42(26.8%); 50(31.8%); and 65(41.4%) were first (1-12 weeks of gestation); second (13-27 weeks of gestation); and third trimester (28-40 weeks of gestation), respectively. All the volunteers participated in the study were healthy and having no sign of any disease or disorder.

5.2. Socio demographic characteristics

The mean ± SD age of study participants was 30.93±9.34 years (95% CI=30.10-31.79). The mean ± SD age for adult males, adult females and pregnant women were 34.19±10.09 years, 32.36±9.98 years and 26.2±5.1 years respectively. No significant age differences (p=0.058) were found between male and female, but there was significant differences (p=0.00) between pregnant and non-pregnant women. In this study, most (90.9%) of study subjects were urban dwellers. With respect to the educational level, almost half, 255(54%) of study subjects were college diploma/degree and above and 133 (28.2%) were secondary school (9-12 grade). Regarding ethnicity, 164(34.7%); 135(28.6%); 25(5.3); 69(14.6%) and 79(16.7%) of the study participants were Amhara, Oromo, Somali, Mixed and Others respectively. Almost half, 249(52.8%) of study participants were government employee. In terms of marital status, 341(72.2%) were married and 119(25.2%) were single. In regard to religion, 283(60%); 138(29.2%); 39(8.3%); 5(1.1%); and 7(1.5%) were Orthodox Christians, Muslims, Protestant, Catholic and Others respectively. Table 1 shows socio demographic characteristics of the study population.

Table 1: Socio demographic characteristics of study participants of Dire Dawa, Ethiopia from January-May, 2019

Variables	Category	Frequency (n=472)	Percentage (%)
Sex			
Male		160	33.9
Female	Pregnant	157	33.3
	Non-pregnant	155	32.8
Age group (years)			
18-30		280	59.3
31-45		157	33.3
46-65		35	7.4
Place of residence			
Urban		429	90.9
Rural		43	9.1
Marital status			
Single		119	25.2
Married		341	72.2
Widowed		5	1.1
Divorced		7	1.5
Education			
Illiterate		20	4.2
Read and write		9	1.9
Primary (1-8)		55	11.7
Secondary school(9-12)		133	28.2
College or more		255	54
Religion			
Orthodox Christian		283	60
Muslim		138	29.2
Protestant		39	8.3
Catholic		5	1.1
Others		7	1.5
Occupation			
Student		11	2.3
House wife		107	22.7
Government employee		249	52.8
Private employee		79	16.7
Farmer		5	1.1
Unemployed		7	1.5
Others		14	3
Ethnicity			
Amhara		164	34.7
Oromo		135	28.6
Somali		25	5.3
Mixed		69	14.6
Others		79	16.7

5.3. Anthropometric data of study participants

The mean ± SD height of study subjects were 167.88±7.7 centimetres ranging between 148-186 centimetres and the body weight was 66.3±7.79kg with a range of 45-91kg; The body mass index (BMI) showed mean ± SD value of 23.51±2.14 Kg/m^2 varying between 18.5-29.8 Kg/m^2. Most of them (n=360; 76.3%) had a BMI 18.50-24.90 Kg/m^2 and 112(23.7%) had a BMI ranging 25-29.9 Kg/m^2 with no obese (BMI > 30.0 Kg/m^2) participant. The mean ± SD value for the Systolic Blood Pressure (SBP) was 118.06 ± 9.17 mmHg ranging between 87.0 to 161.0 mmHg, while Diastolic Blood Pressure (DBP) was 79.6±7.2 mmHg with a range of 63.0 to 128 mmHg. The pulse rate was 88.69±8.8 beats/ minute ranged from 68 to 115 beats/minute. The anthropometric data of each study partition is illustrated in table 2.

Table 2: Mean ± SD and median values of anthropometric data of study participants of Dire Dawa population, Dire Dawa, Ethiopia from January-May, 2019

Variables	Category		Mean ± SD(range)	Median
Age (years)	Adult male		34.19±10.09(18-65)	32
	Adult female		32.36±9.98(18-61)	31
	Pregnant		26.2±5.1(16-45)	26.05
Height (cm)	Adult male		172.34±7.44(155-186)	172
	Adult female		166.07±7.06(148-186)	166
	Pregnant		165.1±6.5(150-181)	166.0
Weight (kg)	Adult male		68.68±8.04(50-91)	69
	Adult female		63.53±7.08(45-84)	166.0
	Pregnant		66.6±6.5(46-89)	66.0
BMI (kg/m^2)	Adult male		23.07±1.7(18.7-29.8)	22.9
	Adult female		23.07±2.12±(18.5-29.3)	22.6
	Pregnant		24.39±2.3(18.9-29.8)	24.5
BP (mmHg)	SBP	Adult male	116.86±7.12(99-161)	118
		Adult female	116.3 ±7.25(101-142)	116
		Pregnant	119.1±12.17(87-156)	118.0
	DBP	Adult male	80.1±7.8(70-128)	79
		Adult female	78.7±6.17(70-97)	78
		Pregnant	79.95±7.53(63-101)	79.0
Pulse (beats/min)	Adult male		85.28±8.2(68-101)	86
	Adult female		87.45±8.39(72-102)	90
	Pregnant		93.39±7.7(72-115)	93.0

5.4. Life Style and Nutritional Habit

Seventy-two participants (15.3%) stated to be ‘‘occasional’’ *Kha*t users on which they used it only during holiday and special ceremony. In addition, forty-six (9.7%) were participants reported ‘‘occasional’’ alcohol users whom they used it only during holiday and special ceremony. Table 3 shows dietary style of study participants.

Table 3: The dietary style of study participants of Dire Dawa population, Dire Dawa, Ethiopia from January-May, 2019

Diet type	Dietary frequency				
	Once/day	> Once/day	2-3times/week	Occasionally	Never
Roots and Tuber	213(45.1%)	18(3.8%)	178(37.7%)	60(12.7%)	3(0.6%)
Legumes	123(26.1%)	20(4.2%)	271(57.4%)	57(12.1%)	1(0.2%)
Cereals	57(12.1%)	402(85.2%)	10(2.1%)	3(0.6%)	-
Vegetables	263(55.7%)	21(4.4%)	134(28.4)	54(11.4%)	-
Fruits	161(34.1%)	21(4.4%)	199(42.2%)	91(19.3%)	-
Meat	24(5.1%)	18(3.8%)	242(51.3%)	186(39.4%)	2(0.4%)
Milk and its products	40(8.5%)	14(3.0%)	181(38.3%)	222(47%)	15(3.2%)
Egg	32(6.8%)	15(3.2%)	176(37.3%)	221(46.8)	28(5.9%)
Tea and/or coffee	126(26.7%)	107(22.7%)	44(9.3%)	38(8.1%)	157(33.3%)

5.5. Distribution ABO Blood group and Rh phenotype

The most prevalent blood group was blood group O, but the least was blood group AB. Regarding Rh (D) positivity, most of study participants were indicated the presence of Rh antigen in their blood cells. Figure-4 shows distribution ABO and Rh phenotypes

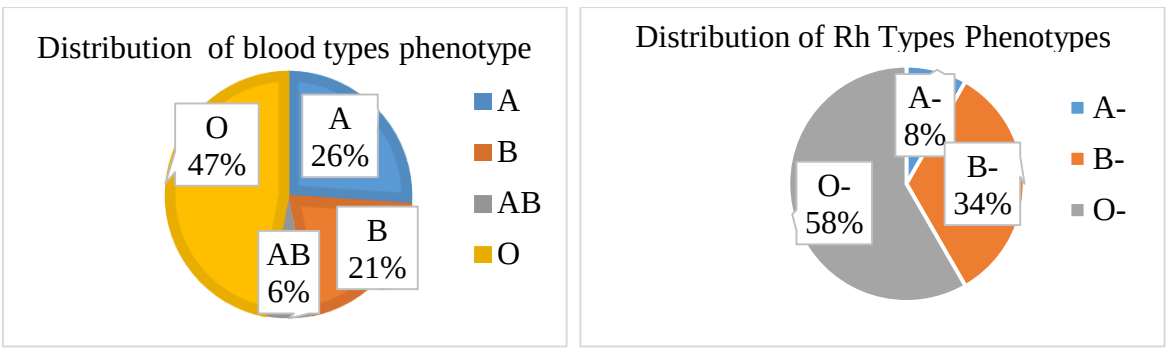

Figure 4: Distribution ABO and Rh blood phenotypes of study participants of Dire Dawa population, Dire Dawa, Ethiopia from January-May, 2019

5.6. Reference intervals of Hematologic Parameters

The 95% (2.5^{th}-97.5^{th} limits) reference intervals for hematologic parameter of for three partitioned study participants are illustrated in tables 4-6.

5.6.1. White Blood Cells parameters reference interval

Table 4 shows significant difference based on gender for most of white blood cells parameter and for majority of white blood cells parameter between pregnant and non-pregnant women.

Table 4: Median and 95% RI values of white blood cells parameters of adult males, adult females and pregnant women of Dire Dawa population, Ethiopia from January-May, 2019

Parameter	Category	N	Median	RI (2.5^{th}-97.5^{th})	90% CI		p-value	
					LLC	ULC	M & F	NP & P
WBC ($x10^3/\mu l$)	Male	158	5.90	3.5-10.31	3.5-3.8	9.6-10.5	[a]0.006*	[b]0.000*
	Female	150	6.85	3.8-10.2	3.7-4.2	9.9-10.5		
	Pregnant	151	7.80	3.68-11.8	3.5-4.6	11.0-11.8		
Lymph# ($x10^3/\mu l$)	Male	157	2.1	1.2-3.81	1.0-1.4	3.30-4.10	[a]0.029*	
	Female	149	2.3	1.38-4.03	1.2-1.4	3.5-4.2		
	Pregnant	151	1.9	1.1-2.62	1.0-1.2	2.4-2.8		[a]0.000*
MID# ($x10^3/\mu l$)	Male	155	0.6	0.2-1.0	0.1-0.3	1.0-1.1	[a]0.878	
	Female	152	0.5	0.3-1.0	0.3-0.3	0.9-1.1		
	Pregnant	156	0.6	0.29-1.2	0.20-0.3	1.0-1.2		[a]0.104
GRAN# ($x10^3/\mu l$)	Male	159	3.3	1.4-6.8	1.2-1.5	6.30-7.1	[a]0.003*	
	Female	151	3.9	1.4-7.0	1.1-1.8	6.50-7.10		
	Pregnant	151	5.4	2.36-9.12	2.10-2.8	8.30-9.60		[b]0.000*
LYMPH %	Male	157	36.60	18.2-54.8	17.9-20.8	52.5-56.9	[a]0.098	
	Female	152	34.35	18.16-55.16	17.7-20.8	55.1-57.5		
	Pregnant	151	23.50	14.06-39.6	13.3-15.4	36.6-40.5		[a]0.000*
MID %	Male	157	9.5	5.18-13.92	4.5-5.6	13.5-15.6	[b]0.001*	
	Female	149	8.3	4.7-13.13	4.6-5.5	12.3-12.7		
	Pregnant	152	8.1	4.57-12.34	4.4-5.2	11.4-12.7		[b]0.095
GRAN %	Male	156	52.6	33.16-72.8	28.5-36.7	69.0-75.4	[a]0.042*	
	Female	147	57.1	39.94-71.25	33.5-39.9	69.7-72.5		
	Pregnant	152	67.7	49.3-79.83	48.7-52.6	78.8-80.7		[a]0.000*

LLC, Lower Limit Confidence interval; ULC, Upper Limit Confidence interval; M, Male; F, Female; NP, Non-Pregnant; P, Pregnant

[a] independent-Samples Mann-Whitney U Test **[b]** independent sample t-test

***** *p values* < 0.05: statistically significant different

5.6.2. Red cells parameters Reference interval

Significantly higher median in males for RBC count, Hgb, HCT, MCH and MCHC than females, while higher median value for RDW-SD in females than males was indicated in this study (table 5).

Table 5: Median and 95% RI values of red blood cells parameters of adult males, adult females and pregnant women of Dire Dawa population, Ethiopia from January-May, 2019

Parameter	Category	N	Median	RI (2.5^{th} -97.5^{th})	90% CI		p-value	
					LCL	UCL	M & F	NP & P
Hgb (gm/dl)	Male	158	15.2	12.4-17.5	11.4-13.3	17.1-17.60	[a]0.000*	[a]0.000*
	Female	152	12.8	10.7-15.2	10.2-11.1	14.9-15.6		
	Pregnant	145	11.7	9.5-13.50	9.4-9.90	13.2-13.8		
RBC# ($x10^6$/µl)	Male	154	5.39	4.46-6.15	4.05-4.69	6.07-6.22	[b]0.000*	[b]0.000*
	Female	149	4.73	3.81-5.49	3.75-4.06	5.43-5.65		
	Pregnant	149	4.19	3.67-5.07	3.33-3.49	54.98-5.19		
HCT (%)	Male	159	51.3	43.8-58.5	42.6-44.8	57.70-60.6	[a]0.000*	[b]0.000*
	Female	151	44.8	37.4-52.0	35.5-38.8	50.8-52.7		
	Pregnant	148	39.2	32.2-46.0	30.5-34.2	45.3-46.8		
MCV (fl)	Male	150	94.2	86.3-104.4	85.1-88.8	102-105.4	[b] 0.854	[b]0.479
	Female	141	94.7	83.4-104.2	81.3-86.5	102.7-105.7		
	Pregnant	147	94.4	84.7-103.4	82.7-86.0	101.7-104.7		
MCH (pg)	Male	153	27.8	24.6-31.1	24.1-25.7	30.6-31.6	[b]0.000*	[a]0.001*
	Female	143	27.3	23.0-30.1	22.1-24.1	29.3-32.2		
	Pregnant	147	27.6	23.7-32.6	23.1-24.2	31.7-32.8		
MCHC (gm/dl)	Male	155	29.5	27.6-30.8	27.2-28.0	30.7-31.2	[a]0.000*	[b]0.008*
	Female	150	28.7	26.7-29.9	26.1-27.2	29.6-30.3		
	Pregnant	148	29.1	27.4-33.2	27.0-27.8	32.7-33.5		
RDW-CV	Male	153	13.6	12.3-15.3	12.3-12.5	15.1-15.9	[a]0.883	[b] 0.000
	Female	143	13.6	12.4-15.7	12.1-12.6	15.0-15.8		
	Pregnant	148	14.0	12.5-17.6	1.40-12.7	16.7-18.2		
RDW-SD	Male	153	49.8	42.1-61.3	41.5-43.1	58.1-63.1	[b]0.003*	[a]0.037*
	Female	146	51.4	43.1-60.9	41.50-44.8	58.4-62.3		
	Pregnant	150	50.6	40.6-60.6	39.00-43.1	58.1-60.6		

LLC, Lower Limit Confidence interval; ULC, Upper Limit Confidence interval; M, Male; F, Female; NP, Non-Pregnant; P, Pregnant

[a] independent-Samples Mann-Whitney U test; **[b]** independent sample t-test;

*****p *values* < 0.05: statistically significant different

5.6.3. Platelet Parameters Reference interval

Significantly higher median in non-pregnant females for PLT# and PCT than males and pregnant women, while significantly higher median in males for PDW than females was observed in this study (table 6).

Table 6: Median and 95% RI values of Platelet parameters of adult males, adult females and pregnant women of Dire Dawa population, Dire Dawa, Ethiopia from January-May, 2019

Parameter	Category	N	Median	RI (2.5^{th}– 97.5^{th})	90% CI		p-value	
					LCL	UCL	M & F	NP & P
Platelet ($x10^3/\mu l$)	Male	155	279	164-447	137-189	410-466	[a]0.004*	
	Female	148	310	177-442	144-213	405-482		
	Pregnant	149	276	157-421	140-178	387-445		[a]0.000*
MPV (fl)	Male	158	9.1	7.7-10.9	7.40-7.8	10.6-11.1	[a]0.476	
	Female	151	8.9	7.3-10.6	77.1-7.6	10.4-10.8		
	Pregnant	152	8.9	7.6-10.30	7.5-8.0	10.0-10.5		[b] 0.833
PDW	Male	152	15.6	15.0-16.3	15.0-15.1	16.1-16.5	[a]0.003*	
	Female	153	15.5	15.0-16.2	14.9-15.1	16.0-16.2		
	Pregnant	153	15.9	15.1-16.6	14.9-15.3	16.5-16.6		[a]0.000*
PCT	Male	155	0.254	0.15-0.36	0.13-0.17	0.35-0.37	[a]0.000*	
	Female	147	0.280	0.18-0.38	0.14-0.20	0.37-0.39		
	Pregnant	149	0.252	0.15-0.35	0.14-0.17	0.33-0.39		[b]0.000*

LLC, Lower Limit Confidence interval; ULC, Upper Limit Confidence interval; M, Male; F, Female; NP, Non-Pregnant; P, Pregnant

[a] independent-Samples Mann-Whitney U test; **[b]** independent sample t-test;

*****p *values* < 0.05: statistically significant different

5.7. Comparison of hematological parameters based on age group

The findings of this study were divided into three groups on the bases of age: 18-30 years, 31-45 years and 46-65 years. Although, there was no statistically significant difference for all hematologic parameters across all groups of both male and female participants, the mean ± SD showed slightly variation for majority of hematologic parameters. For males: the WBC count, Hgb and HCT was highest between the age group of 31-45 years (WBC=6.74±1.82x10^3/µl; Hgb 15.26±1.25gm/dl; HCT=51.65±3.98%) with p=0.088, p=0.054 and p=0.191 for WBC count, Hgb and HCT respectively among the age groups. The RBCs count decline progressively from 18-30 years (15.19±1.28x10^6/µL) to 31-45 years (15.26±1.25x10^6/µL) and

then to 46-65 years (14.52±1.21x10^6/µL) with p= 0.694 among the age groups. The PLT count increased at the age group of 46-65 years than the age groups of 18-30 years and 31-45 years. In adult females, the WBC count, Hgb concentration, and HCT percentage increased progressively as the age group advanced from 18-30 years to 31-45 years and then to 46-65 years of age. However; the platelet count decreased as the age group advanced from 18-30 years to 31-45 years and then 46-65 years (p=0.060).

5.8. Hematologic parameter in pregnant women

Among the pregnant women, whom conceived for the first time were 63(40.1%); conceived for the second times and had one child were 57(36.33%); conceived for the third times and had two children were 22(14.0%); conceived for the fourth times and had three children were 9(5.7%); and conceived for the fifth and above times, and had four and above children were 6(3.2%) participants. Comparison of RBC parameters between primigravida and multigravida indicated that significantly higher mean±SD (28.25±2.19pg) in primigravida for MCH (p=0.016) than multigravida (27.41±1.95pg), while significantly higher RBC count (p=0.034) in multigravida (4.26±0.45*10^6/µl) than primigravida (4.10±0.43*10^6/µl)

5.8.1. Comparison of hematologic parameters between pregnant and non-pregnant women

Comparison of pregnant and non-pregnant women for hematologic parameters indicated significantly higher mean value for RBC, HCT, Hgb, HCT, MCHC RDW-SD, Lymph# Lymph%, PLT# and PCT in non-pregnant as compared to the pregnant women. Whereas, significantly higher mean value was observed for WBC, Gran#, Gran%, MCH and RDW-CV in pregnant women than the non-pregnant (table 4-6).

5.8.2. Comparison of hematological parameters of pregnant women based on trimesters

The mean ± SD gestational age of pregnant women was 24.28±11.59 weeks ranged from 7-41 weeks and the mean ±SD number of family size was 3.0±1.1 ranged 1-8. In this study, an increased trend in the value of hematologic parameter as the gestational age progressed from the 1st to 2nd and then to 3rd trimester for: WBC count, Gran#, Gran%, MCH, RDW-SD, RDW-

CV, MPV and PDW. However, a decreased trend for PLT count and Lymph% as the gestational age decreased from the 1st to 2nd and then to 3rd trimester was observed (Figure 6). Gestational-based reference interval of pregnant women for the hematological parameters at are shown in table 7

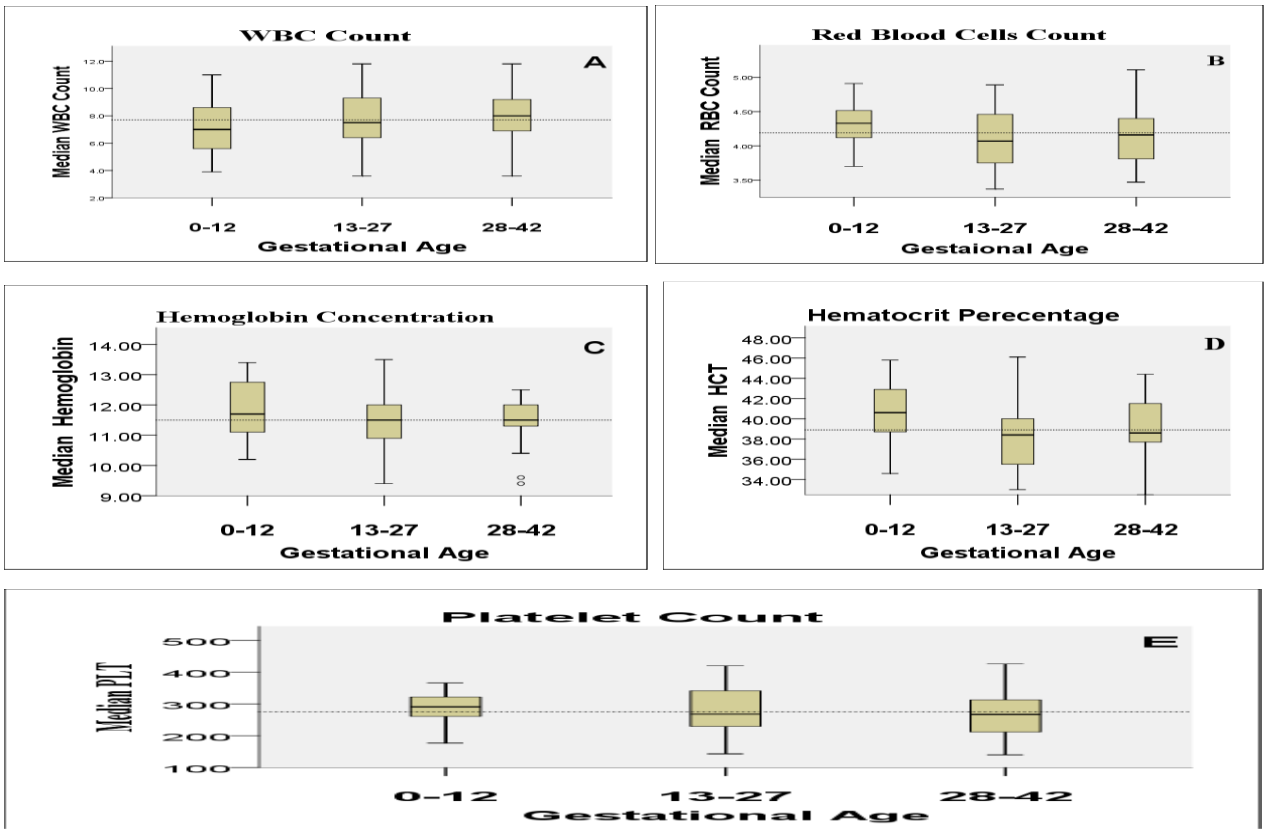

Figure 5 Whiskers and box plot comparison of hematologic parameter value among pregnant women based on gestational age of Dire Dawa population, Ethiopia of from January-May, 2019

A= WBC Count (p value: 1st & 2nd = 0.375, 1st & 3rd = 0.229 and 2nd & 3rd= 0.811)

B= RBC count (p value: 1st & 2nd= 0.131; 1st & 3rd= 0.378; 2nd & 3rd= 0.525

C= Haemoglobin (p value: 1st & 2nd= 0.007; 1st & 3rd= 0.042; 2nd & 3rd= 0.339)

D= Haematocrit (p value: 1st & 2nd = 0.776; 1st & 3rd = 0.769; 2nd & 3rd= 0.545);

E= Platelet count (p value: 1st & 2nd= 0.005; 1st & 3rd= 0.038; 2nd & 3rd= 0.387)

Table 7: Median and 95% RI of haematological values over the three trimesters of pregnant women of study participants of Dire Dawa, Ethiopia of from January-May, 2019

				Median(2.5th- 97.5th)					
Parameter		Trimester-1		Trimester-2		Trimester-3	***p-value***		
	N		N		N		1st & 2nd	1st & 3rd	2nd & 3rd
WBC	40	7.75(2.8-12.0)	49	7.4(3.3-11.7)	62	8.0(4.1-12.0)	0.375	0.229	0.811
Lymph# **(**x10^3/µl)	39	1.90(1.05-2.67)	49	1.80(1.01-2.63)	63	1.80(1.01-2.60)	**a** 0.940	**a** 0.987	**a** 0.947
Gran# (x10^3/µl)	39	5.40(1.19-8.83)	49	4.90(1.58-8.84)	63	5.70(2.28-9.17)	**a** 0.549	**a** 0.404	**a** 0.857
MID#(x10^3/µl)	41	0.60(0.18-0.91)	50	0.60(0.15-1.12)	65	0.6(0.24-1.06)	**b** 0.077	**b** 0.018*****	**b** 0.660
Lymph%	39	26.0(12.7-40.3)	49	23.8(12.5-38.4)	63	21.6(11.5-33.7)	**b** 0.401	**b** 0.003*****	**b** 0.020*****
Gran (%)	40	65.4(47.5-80.8)	50	67.5(50.6-81.2)	62	70.4(55.582.2)	**a** 0.405	**a** 0.139	**a** 0.612
MID (%)	41	8.2(4.08-12.1)	47	8.2(5.1-11.4)	64	7.9(3.8-12.2)	**a** 0.516	**a** 0.248	**a** 0.030*****
RBC**(**x10^6/µl)	41	4.38(3.65-5.21	48	4.08(3.19-5.02)	60	4.09(3.22-4.98)	0.131	0.378	0.525
Hgb (gm/dl)	39	12(9.7-14.0)	49	11.4(9.2-13.2)	57	11.8(10.0-13.3)	0.007*****	0.042*****	0.339
HCT (%)	39	41.5(34.6-47.7)	50	37.8(31.5-44.2)	59	38.8(32.1-46.1)	0.776	0.769	0.545
MCH(pg)	40	27.40(24.4-30.4)	45	27.6(23.6-32.3)	62	27.65(22.9-32.6)	**b** 0.537	**b** 0.551	**b** 0.823
MCHC(gm/dl)	41	29.2(27.3-30.9)	46	29.3(26.6-32.8)	61	29.0(26.3-32.3)	**b** 0.423	**b** 0.391	**b** 0.101
MCV (fl)	40	94.4(85.0-103.8)	45	94.0(84.9-102.9)	62	94.4(83.8-104.8)	**a** 0.867	**a** 0.148	**a** 0.160
RDW-SD	40	48.9(39.1-59.3)	48	50.6(41.9-58.3)	62	51.0(41.6-59.6)	**a** 0.269	**a** 0.565	**a** 0.525
RDW-CV	40	13.6(11.4-16.3)	46	13.8(12.0-16.0)	62	14.2(11.7-17.4)	**b** 0.221	**b** 0.004*****	**b** 0.072
Platelet **(**x10^3/µl)	41	304(185-404)	47	268(137-430)	61	267(133-404)	0.005*****	0.038*****	0.387
MPV (fl)	39	8.80(7.4-10.2)	49	9.00(7.6-10.2)	64	9.05(7.6-10.4)	**a** 0.911	**a** 0.387	**a** 0.280
PDW	42	15.70(14.9-16.6)	49	15.8(15.1-16.4)	62	15.9(15.4-16.6)	**b** 0.687	**b** 0.005*****	**b** 0.000*****
PCT	42	0.26(0.15-0.35)	47	0.23(0.13-0.37)	60	0.25(0.14-0.35)	**b** 0.657	**b** 0.231	**b** 0.492

a One-way analysis of variance (ANOVA); **b** Kruskall-Wallis; *****p *values* < 0.05: statistically significant different

5.9. Correlations of hematological indices with BMI of study participants

The correlation of BMI with WBC count, using Pearson correlation, indicated that weak positive correlation in male and female with no statistical significance (Male: *r*= 0.006; *p*=0.922, Females: *r*= -0.010; *p*=0.883). Similarly weak positive correlation in male while weak negative correlation for females was observed for absolute lymphocyte count (Male: *r*= 0.023; *p*= 0.684, Females: *r*=*-0.004*; *p*=0.958). Regarding the red blood cells parameter, the correlation of BMI with Hgb, RBC count, HCT, MCH and MCHC values were achieved, but weak positive and negative correlation among study subjects. For instance, Hgb showed weak negative correlation in both male and females with no significance value (Male: *r*= -0.045; *p*=0.337, Females: *r*= -0.111; *p*= 0.362). RBC count showed weak negative correlation in male and no correlation in adult female participants (Male: *r*= -0.148; *p*= 0.010, Females: *r*= 0.002; *p*=0.979). Weak negative correlation with BMI was noted in all study categories for Haematocrit percentage with significant level in male and female study subjects (Male: *r*= -0.118; *p*= 0.038, Females: *r*= -0.021). There was no statistically significant correlation between BMI and MCV for both groups (Male: *r*= -0.008; *p*= 0.894, Females: *r*= 0.006). Related to platelet count, weak negative correlation with BMI was observed in female while no correlation in male (Male: *r*= 0.006; *p*= 0.917, Females: *r*= -0.045; *p*= 0.589).

5.10. Comparison of hematological parameter RI with currently practiced and African studies

Comparison of this finding with currently practiced by most of laboratories in Dire Dawa town for the out of range in the RIs for Hgb, 16% in males and 7.2% in females; RBC count, 8.4% in males and 6.0% in females; WBC count, 10.7% in males and 12% in females; platelet count, 17.4% in males and 22.9% in females were indicated.

Comparison of this study reference interval with studies conducted from different geographic location Africa, higher Hgb in this study for the lower and upper limit (12.4-17.5gm/dl) than from the study conducted in Ghana (11.3-16.4gm/dl) in males, but lower in this study (10.7-15.2gm/dl) than for the upper limit in Ghana (9.8-16.0) in females. The RBC count of this study in males (4.46-6.15 $x10^3$/µl) is higher in the upper limit than from studies conducted in Ghana (3.79-5.96 $x10^3$/µl), Mali (4.1-6.2 $x10^3$/µl) and Asmara (4.2-6.07 x103/µl) but lower than from a study conducted in Zimbabwe (4.4–6.7$x10^3$/µl) as shown table 8.

Table 8: Comparison of adult hematological reference values obtain from currently practiced RI in Dire Dawa and African studies with this study of study participants of Dire Dawa, Ethiopia of from January-May, 2019

Parameter	Sex	This study		Currently the labs using		African studies							
		N	RI	RI	% Out of range	N	Ghana (75)	N	Zimbabwe(56)	N	Mali(57)	N	Asmara(33)
Hgb (g/dl)	Male	158	12.4-17.5	11-17	16.4%(26/158)	316	11.3-16.4	412	13.2–18.3	173	12.4-17.6	295	12.6–17.8
	Female	152	10.7-15.2	11-17	7.2%(9/152)	308	9.8-16.0	357	10.2–15.9	40	12.0-14.9	296	12.5–17.6
RBC (10/L)	Male	154	4.46-6.15	3.5-5.50	8.4%(13/154)	316	3.79-5.96	412	4.4–6.7	173	4.1-6.2	295	4.2-6.07
	Female	149	3.81-5.49	3.5-5.50	6.0%(9/149)	307	3.09-5.30	357	3.9–5.9	40	3.9-5.7	296	4.0-5.7
HCT (L/L)	Male	159	43.8-58.5	NA	NA	316	33.2-50.5	412	42.0–55.1	173	33.2-54.6	295	40.5-55
	Female	151	37.7-52.0	NA	NA	309	26.4-45	357	33.9–48.7	40	26.8-52.5	296	37.9-52
MCV (fl)	Male	150	86-104	80-100	10% (15/150)	316	70-98	412	72.8-102.6	173	72.3-97.7	295	85.7-100
	Female	141	83-104	80-100	9.1% (13/143)	309	73-96	357	68.8–100.7	40	79-118	296	85.5-100
MCH (pg)	Male	153	24.6-31.1	27-34	21.3% (33/155)	316	22.7-33.5	412	22.9–33.5	173	22.8-33.7	295	28-33
	Female	143	23.0-30.1	27-34	44.0% (63/143)	307	22.3-33.6	357	20.7–32.1	40	23.1-34.8	296	26.5-32.6
MCHC (g/dl)	Male	155	27.6-30.0	32-36	50% (76/150)	315	30.6-36.0	412	29.8–35.4	173	30.9-34.9	295	30.4-33.7
	Female	150	26.7-29.9	32-36	60.6 (91/150)	305	30.4-36.5	357	29.2–34.3	40	30.9-34.5	296	30-33.7
WBC ($x10^3$/µl)	Male	158	3.5-10.3	4-10	10.7 (17/158)	311	3.5-9.2	412	2.8–8.1	173	3.7-11.1	295	3.7-9.3
	Female	150	3.8-10.2	4-10	12% (18/150)	309	3.5-9.2	357	3.3–8.3	40	3.8-12.5	296	3.3-8.9
Gran# ($x10^3$/µl)	Male	159	1.4-6.8	2.0-7.0	15.7% (25/159)	313	1.5-5.9	412	0.77-3.9	173	1.0-4.4	295	NA
	Female	151	1.4-7.0	2.0-7.0	10% (15/151)	309	1.4-5.5	357	1.1-4.4	40	1.2-7.4	296	NA
Lymph# ($x10^3$/µl)	Male	157	1.2-3.8	0.8-4.0	2.5% (4/157)	316	1.2-5.2	412	1.1-3.2	173	1.2-3.8	295	NA
	Female	149	1.3-4.0	0.8-4.0	4.0% (6/149)	308	1.2-4.4	357	1.3-3.7	40	1.4-4.6	296	NA
Gran (%)	Male	156	33.1-72.8	50-70	44.8% (70/156)	316	30.2-69.9	412	22.1–62.8	173	26-66	295	31.7-73.6
	Female	147	39.9-71.2	50-70	34.0% (50/147)	309	33.3-67.5	357	27.1–62.0	40	26-67	296	33.5-70.5
Lymph (%)	Male	157	18.2-54.8	20-40	36.9% (58/157)	315	24.0-57.2	412	24.1–60.3	173	NA	295	22-59.9
	Female	152	18.1-55.1	20-40	32.8% (50/152)	309	26.9-58.3	357	28.4–59.0	40	NA	296	22.3-58.2
PLT ($x10^3$/µl)	Male	155	164-447	100-350	17.4% (27/155)	316	88-352	412	125-357	173	133-460	295	128-318.6
	Female	148	177-442	100-350	22.9% (38/148)	309	89-403	357	163-431	40	151-460	296	145-351

Ghana, age: 18-59 years, author: *Dosoo DK, et.al*. Year: 2012; **Zimbabwe**: age: 18-55, author: *Samaneka WP, et.al*. Year: 2016; **Mali**, age: 18-59 years, author: *Kone B, et*.al. Year: 2017 **Asmara**, age: 18-49 years, author: *Siraj N, et.al*. Year: 2018

RI, Reference Interval; WBC#, White Blood Cells; LYM#, Lymphocytes counts; Gran#, Granulocytes count; MID#, Mixed Cells count; RBC, Red Blood Cell count; Hgb, Hemoglobin; HCT, Hematocrit; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin; MCHC, Mean Corpuscular Hemoglobin Concentration; PLT#, Platelet Count;

6. Discussion

This study is the first of its kind to be conducted in the eastern part of the country, which aimed to establish hematologic parameter reference interval of Dire Dawa population after collecting and analysis of whole blood from apparently healthy adult individuals of both sexes and pregnant women. The participants' ages ranged from 18 to 65 years, which comprised both males and females for adult category and pregnant women ranged from 15-49 years. We found statistically significant difference ($p<0.05$) for most of hematologic parameters based on gender and between pregnant and non-pregnant women. Moreover, variation in hematologic RI based on trimester among pregnant women was observed in this study. Although no significant difference ($p>0.05$) among age group of both genders for all hematologic parameters, a trend of decreasing or increasing among different age groups also observed.

The hematopoietic system is modestly affected by ageing, and these effects become particularly notable after the age of 65 years (76). In this study, all hematologic parameter of adult male and female groups were not significantly ($p>0.05$) vary across the age groups. The minor differences with no statistical significance among the different age groups of both genders for hematologic parameter reference interval of our study is in agreement with study conducted in North American and Oman (53,54). Moreover, this minor difference in cellularity across age in study is also in line with the general knowledge that a decreasing trend with age for RBC parameters. Therefore, it is merely important of introducing age specific hematologic reference intervals for adult population between 18-65 years.

Significantly ($p<0.005$) higher mean values of RBC, Hgb and HCT in adult males than females was observed. This could be explained on the basis of the physiological effects of androgens on erythropoiesis. The total body iron storage is generally lower in women because of losses in iron during normal menstruation and the iron depletion intensify the degree of haemoglobin level to decrease in females than males (50). This variation is by the direct effect of sex hormones, both oestrogen and androgens, on erythropoiesis. However, since there is no difference in erythropoietin levels between the sexes, this effect most likely takes place in the kidney, rather than in the bone marrow. Oestrogens dilate and androgens constrict the renal microvasculature: dilation and vasoconstriction in vessels respectively increase and decrease the haematocrit in blood in arterioles, capillaries and venules, altering the oxygen delivery per

unit red cell mass, and providing a mechanism for varying the red cell mass without compensatory changes in erythropoiesis (15). This suggests the importance of gender specific hematologic parameter reference intervals. Our finding is consistent with the reported mean value by Oman and Thailand (54,55) and studies conducted in different geographical locations of Africa (56,57,75,78–80).

This study also illustrated that the reference intervals determined were partly comparable with some but not comparable for majority of the hematologic parameters as compared from the reviewed African literatures as shown in table 8. Comparison based on gender for RBC count, Hgb, and HCT values were higher in males than females observed in our study is consistent from previous African studies (56,57,75,78–80). Regarding the reference interval, the red cell parameters of this study is consistence with Malian and Eastern and Southern Africa males (57,78) and with Zimbabwean females (61). The RI of this finding for the above parameters is higher in both upper and lower limit than Middle Belt of Ghana males (64), but the Hgb is lower than that observed among Zimbabwean and Botswana males (56,80). The reference interval for Hgb of adult females of this study is lower than Malian and Botswana females (57,80), but higher than Middle Belt of Ghana and Eastern and Southern Africa females (75,78). This variation could be attributed by the socio demographic, dietary and genetic difference among different localities. Moreover; interregional, inter-country and inter-racial differences in hematological reference values are well documented (23).

Comparison of studies conducted in different part of Ethiopia indicated that higher RI for both genders, except slightly lower for RBC count in male, higher for Hgb HCT, MCV, MCH and MCHC than this study (34). Except for HCT in both genders, higher for all red cell parameters was noted in the study conducted in Southwest Ethiopia than this study (59). This could be the geographical location, which Debremarkos is at higher altitude (1800m above sea level), and the three towns (Jimma, Mizan, and Bonga) of Southwest Ethiopia is also located at higher altitude favoured for higher to majority red cells parameters than this study. This incomparable reference interval is also supported by a study conducted in Addis Ababa, Akaki, which found higher than this study for most red cell indices and similar studies conducted in Gondar and Bahir Dar, also indicated higher for most of red cell parameter, except for HCT, than this study (36,60,61). Studies indicated high altitude has an effect on blood count parameters especially it favours for higher red cell parameters values (81–83). In order to compensate for the low partial pressure of oxygen altitude, the human body undergoes a number of physiological

changes. A vital component in this process is the increase in the concentration of circulating haemoglobin. The role of Hypoxia Inducible Factor-1 (HIF-1) alpha, erythropoietin and red blood cells in this acclimatisation process, together with the fall in plasma volume that increases the concentration of haemoglobin in the early stages of hypoxic exposure (28).

The finding of this study is consistence with the study conducted at Addis Ababa, whic found no significant difference in age and the value for RBC, Hgb, HCT, and MCHC were significantly higher in males than females, whereas platelet count was higher in females. However, with regard to the RI, higher RBC count in upper limit for male and in both upper and lower limit in female; higher Hgb in upper and lower limit in females; higher HCT in lower limit in females; higher MCH in lower limit for both gender; and higher in both upper and limit of both gender than this study (35).

Regarding the white cells parameters, the higher mean value of the WBC count in females than males is consistent with a study conducted in Thailand (55). Except for Lymph%, which is higher in adult male than females, the RI determined in this study is comparable with a study conducted in multi-national African population for all hematologic parameter (84). However, as indicated in table 8, slight variations in WBC count among different African population supports the necessity of establishing a local reference interval, to avoid any adverse consequences by wrong classification or misdiagnosis of hematologic parameters. Evidences also indicated that a region on chromosome-1 where increased local African ancestry completely accounted for the presence of an African-derived variant causing low WBC. Moreover, the low neutrophil count is predominantly responsible for low WBC among African (85).

The white blood cells parameter is comparable with study a conducted in Debremarkos and Bahdar (34,61). Except for Gran#, which is lower than this study, comparable WBC count and its differential was observed with the one reported by a study conducted at Akaki (36). Moreover, with slight lower WBC count than this study, comparable WBC differential with the study conducted in Southwest Ethiopia (59). The slight variation for WBC count in different part of the country also supports the need to establish regional based reference interval even for the same country with different geographical location.

Significantly higher mean platelet count in females than males observed in our study. Although gender wise difference is consistent between the present study and a study conducted in United States that females were higher in mean platelet count than males, the platelet count for both genders is higher than this study (86). Evidences indicated that there is a micro-heterogeneity in platelet parameters even among apparently ethnically homogeneous subjects living in the same country and even in the same region (87).

Comparable platelet count reference interval was indicated from the previous studies conducted in Ethiopia (34,35,59,60). However, the lower platelet limit in this study is higher than that reported by Tsegaye et al from Akakai (36). The slight variations for WBC and platelet counts in different part of the country also support the need to establish regional based reference interval of the same country with different geographical location. Of note, varying degrees of misclassification is noted for most hematologic parameters when comparing the reference intervals determined by this study to that currently in use in Dil Chora Hospital, necessitating for the need for locally established RI.

In this study, a significance decreased during the 3rd trimester for MID % (p=0.030); PLT count during the 2nd (p=0.005) and 3rd (p=0.038) trimester and Lymph % during 3rd (p=0.003), a significance increased for MID# during 3rd (p=0.018); RDW-CV during 2nd (p=0.004); and PDW during 2nd (p=0.005) and 3rd (p=0.000) trimester. The decreased in MID% (eosinophil, basophil and monocytes) significantly in the 3rd trimester and platelets count in the 2nd and 3rd trimesters is consistent with a study conducted in Northwest Libya (88). Although no statistical significant differences was observed for WBC count, a progressive increase with gestational age and progressive decline in Hgb concentration from the 1st to the 3rd trimester, but a drop from 1st to the 2nd trimester was observed in this study. This finding is similar with a study conducted in Nigeria, Lagos, which reported statistically significant relationship between white blood cell counts and gestational age (89).

Higher WBC and Gran# with increasing trimester but lower platelets count and haemoglobin concentration value from 1st to 3rd trimester are also consistent with another study conducted in Nigeria (65). Higher WBC and Gran# with increasing trimester but lower platelets count and haemoglobin concentration value from 1st to 2nd and slowly rose during 3rd trimester are also consistent with study conducted in India (90). However, higher for the mean values of platelet

count in this study than Caucasian and non-Caucasian pregnant women in a conducted at United States (63). This may be attributed for the ethnic difference.

The findings of the current study do not support a study conducted in Bankura, which indicated no significant difference in the value of all hematological parameters when compared at different trimesters of pregnancy. The difference could be due to small sample size taken by the study than from our study for each trimester, which it was conducted on 30 pregnant women (10 in each trimester) (62).

Despite hemodilution, there is usually no change in MCV and MCHC (19). As there was no significant change in MCV, it is likely that the cellular changes are as a result of plasma accumulation and not due to nutrition deficiencies like insufficient iron of vitamin B_{12} deficiencies, which is consistent with a study conducted in central Uganda (91).

Except for RDW-CV (%), which increased progressively from 1st to 2nd and then 3rd trimester, our finding is consistent for all hematologic parameter based on trimester with a study conducted in Addis Ababa, Ethiopia (68). The progressive decreasing in platelet count and the decreased in 2nd trimester from the 1st and return to increase in 3rd trimester for HCT is similar; however, higher WBC count, and higher red cell indices (RBCs, Hgb, and HCT) values than from our finding is inconsistent with a study conducted North West Ethiopia (67).

There are a number of profound physiological changes during normal pregnancy, which is characterized by a change in hematologic parameter to accommodate the demands of the developing foetus. Some of these can induce significant alterations in laboratory values that in a non-pregnant woman would be considered distinctly abnormal (49). During pregnancy there was significant decrease in PCV values and a significant increase in WBCs in comparison to non-pregnant and by considering the haemodilution factor (21).

Red cell mass also increases, but relatively less, compared with the increase in plasma volume, the net result being a dip in haemoglobin concentration haematocrit and red blood cell count. Thus, there is dilutional anaemia. The drop in haemoglobin is typically by 1-2gm/dl and haematocrit disproportionate increase in plasma volume up to 50% increase consequently by the late second trimester and stabilizes thereafter in the third trimester, when there is a reduction in maternal plasma volume (owing to an increase in levels of atrial natriuretic peptide) (18).

There is a 10 to 20 fold increase in folate requirements, a two-fold increase in the requirement for vitamin B_{12} and a marked demand of extra iron during pregnancy especially in the second half of pregnancy (18–20). Thus, it is important to know and differentiate between normal physiological changes and disease pathology variation in hematological profile during the entire course of pregnancy(17,19).

In this study, except for MID%, MID #, MCV and MPV a statistically significant difference was observed between non-pregnant and pregnant women. Findings of the present study is consistent with studies conducted in East Africa (Sudan) and Nigeria pregnant women which they reported that higher WBC and Gran# but lower platelets, Hgb and Lymph# in the pregnant compared to the non-pregnant women (65,92). There are also exact similarities for non-pregnant and pregnant women in all hematologic parameter with a study conducted in Beijing (64). Leucocytosis, occurring in pregnant women than the non-pregnant is an indication for adequate bone marrow response to an increased drive for erythropoiesis. By 4 weeks post-delivery, typical WBC ranges are similar to those in healthy non-pregnant women (18).

During pregnancy, there is a progressive elevation of the innate immune system by suppression of the adaptive immune system and results in the activation of maternal granulocytes, which had greater potential to synthesize pro-inflammatory cytokine interleukin 8 (IL-8), which is an important inducers of WBC production. This modulation would assure immediate protection from pathogens and suppress specific immune responses (e.g., T cell functions) to tolerate the semi-allogenic conceptus. Evidences indicated that the T lymphocytes did not have any characteristics of the activated state and showed a decreased in pro-inflammatory cytokine interleukin 6 (IL-6) production (93). This supports for significantly lower lymphocyte count and lymphocyte percentage in pregnant women than the non-pregnant.

The lower platelet count in pregnant than non-pregnant can be explained by the increased consumption of platelets as well as decreased life span in the utero placental circulation resulted in reduced number of circulating platelets during pregnancy (17). Our finding is consistent with studies that have reported a gradual decrease in platelet count as normal pregnancy progressed with gestational age (64,65,88–90,92,94–97). A decreasing trend with gestational age from 1st to 2nd and then to 3rd trimester for platelet count observed in this study also consistent with studies conducted in Ethiopia, Gondar and Addis Ababa (67,68). There are a number of profound physiological changes during normal pregnancy, which is characterised by a change

in hematologic parameter to accommodate the demands of the developing foetus that result. Some of these can induce significant alterations in laboratory values that in a non-pregnant woman would be considered distinctly abnormal (49).

This study has demonstrated, for the first time in Dire Dawa town that differences in hematologic parameter between gender, between pregnant and non-pregnant women and among pregnant women based on trimester. It confirms previous findings and contributes additional evidence that suggests gender, pregnancy and gestational age of pregnancy has effect on hematologic parameters. In addition, it is relevant to both health care workers to utilise separate reference interval for each group of patient management of hematologic disorders and policy-makers. However, these results may not be applicable to all age groups and further studies will be required for geriatrics and paediatrics.

7. Strength and Limitation

7.1. Strength

The strengths of this study include the use of the same instruments with identical operational settings to obtain CBC results. This study allowed data collection from a more representative demographic population with a broader age distribution in the community. Moreover; this study includes adequate sample size for reference interval determination and adhered to follow strict study protocol.

7.2. Limitation

Antibody testing was employed to screen TTI's, which may limited to screen early acquired infectious agent and stool examination for intestinal parasite detection was not performed. In addition, this study was conducted on adults of both genders with age group of 18-65 years only and it has limited for geriatric and paediatric and not incorporated all age groups.

8. Conclusion and Recommendation

8.1. Conclusion

There is extensive variation in hematological reference values between genders for almost all hematologic parameter, hence this suggest for the importance of utilizing the gender specific reference interval. However, no significant difference among the age group of both genders, this suggests it is not important to establish age specific reference interval for adults.

This research has also investigated that a trend of increasing or decreasing of hematologic parameters among pregnant women; thus, it is important to understand changes in hematologic parameter based on their gestational age. Hence it is vital to use trimester specific RI to monitor changes during pregnancy and help to avoid pregnancy related hematologic complication.

The red cells parameter is lower than from all study conducted in Ethiopia. This variation can be attributed by geographical condition for lower red cells parameters than from different part of the country and this need caution in reporting these parameters for low land users. However, the reference interval of platelet count is well comparable, while the WBC count, slight differences were noted with this study for limited parameters in each study, this emphasising the importance of each region of different locality needs to establish its own reference interval.

The RI obtained from this study different from the clinical practice currently utilized by most laboratory of Dire Dawa region.

8.2. Recommendation

The present study tried to give a baseline reference interval for hematological parameters, which help for better management, diagnosis and monitoring of hematologic parameters for adults of both genders and pregnant women. More research is needed to better understand the variation across geriatric age groups and paediatrics by conducting similar study for hematologic reference interval for these age group**s**. The findings of this study also have a number of important implications for hematologic disorder management and suggested to be utilized by all health facilities of Dire Dawa town.

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10.Annex

Annex I Information Sheet (English version)

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Participant Information sheet

Principal Investigator: Teklay Mengistu Sissay (BSc.)
Advisors: Dr. Aster Tsegaye (MSc, PhD)
Melatwork Tibebu (MSc, PhD candidate)

Sponsor: Addis Ababa University (AAU), Ethiopia

1. **Study Title:**

   Determination of hematological parameters reference interval for adults of Dire Dawa Population, Dire-Dawa

2. **Introduction:**

   Hello!

   My name is Teklay Mengistu Sissay and I am a second year MSc student of Clinical Laboratory Science in Hematology and Immuno-hematology specialty at Addis Ababa University, Collage of health science, Department of medical laboratory science. I am conducting a study to Establish Hematological Reference Intervals for Dire Dawa population aged ≥18years of age.

3. .**Purpose of the research:**

   The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population. Therefore, the purpose of this proposed study is to Establish Hematological Reference Intervals for Dire Dawa population aged ≥18years of age.

You have been chosen for this study. Therefore, I invite you to take part in this study and contribute to the establishment of indigenous reference values which is needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of the adult population at large in Dire Dawa.

4. Procedures:

After agreeing that you can take part, the Health Extension workers will ask you some questions which will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide urine and fresh stool on a particular container we provide. We will collect 5ml venous blood from you by sterile-disposable vacutainer tube and needle (2ml in tube containing EDTA). We will conduct laboratory examination to determine different hematological parameters. You will be asked to provide urine (for pregnant women)

5. Confidentiality:

The information obtained during the study will remain confidential. Disclosure of any of the data to third parties other than those allowed in the Informed Consent form will not be permitted. The results of the research study may be published, but participants' names or identities will not be revealed. To maintain confidentiality, the investigator will keep records in locked cabinets in a locked room at the office and the results of the tests will be coded to prevent identification of the volunteers. Access to data entered into computerized files will be permitted only for authorized personnel directly involved with the study and will be password protected. Individual-specific information may be provided to responsible local medical personnel only with your permission. Blood and Urine (from pregnant) collected will not be used for other purposes. The leftover samples will be stored at the Dil chora Hospital laboratory for additional tests as needed. Finally, all the biological wastes, after analysis will be safely disposed in an environmentally friendly manner.

6. Risks and Discomfort:

However, there might be some minimal risk and discomfort when we take venous blood. Nevertheless, we will try to minimize the discomfort as much as possible, as the blood samples will be taken by experienced clinical nurse working as Health Extension Worker.

7. Safety:

The venous blood sample will be collected using sterile vacutainer tube/syringe and needle by experienced health professional after disinfecting the site of picture by 70% ethanol.

Moreover, leftover blood sample (that is not stored) will be discarded following the guideline of bio-safety.

8. Benefits:

By participating in the study, you will directly benefit by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. Establishing the reference interval will be used in the future to improve the general health status of Dire Dawa population.

9. Incentives:

Any positive finding in your stool/urine/blood will be taken care of by referring you to the nearby health institution; you will get all the laboratory investigation results for free. However, we will not pay you for taking part in this study as well as your treatment costs. But, we will thank you for your participation.

10. Right to refuse or withdraw:

We assure you that our best care will be taken if you agree to take part in the study. You should also know that you are free to withdraw from the study at any time and that you will not be discriminated in any form of service like health.

Whom to contact:

Please direct any questions or problems you may encounter during this study to

Teklay Mengistu
Department of Medical Laboratory Science
College of Health Science
Addis Ababa University
Mobile +251 915753485
E-mail: teklaymengistu@gmail.com

For additional information, please contact AAU, Medical Faculty Institutional Review Board (IRB) office.

IRB address:

Addis Ababa University
College of Health Science
Telephone: +251 -11-896-13

Annex II Information sheet (Amharic Version)

የፕሮጀክቱ ርዕስ፡ "እድሜአቸው ከአስራ ስምንት ዓመትና ከዚያ በላይ ለሆኑ የድሬዳዋ ጤናማ ሰው ደም ሪፈረንስ ኢንተርቫል መስራት።"

የፕሮጀክቱ ዋና ተመራማሪ: ተክላይ መንግስቱ (ቢ.. ኤስ. ሲ)

ስፖንሰር (ወጪውን የሸፈነው)፡ አዲስ አበባ ዩኒቨርስቲ

መግቢያ:

ጤና ይስጥልኝ! ስሜ ተክላይ መንግስቱ ስሆን፣ በአዲስ አበባ ዩኒቨርስቲ የህክምና ላቦራቶሪ ሳይንስ ትምህርት የሁለተኛ ዲግሪ ትምህርት እየተከታተልኩኝ በመሆኑ እድሜአቸው ከአስራ ስምንት ዓመትና ከዚያ በላይ ለሆኑ የድሬዳዋ ጤናማ ሰው ሪፈረንስ ኢንተርቫል እያካሄድኩኝ ነው።

የምርምር ጥናቱ አላማ:

የህክምና ላቦራቶሪ በጤናው አገልግሎት ውስጥ ከፍተኛ ሚና ይጫወታል። ምርመራን ለማረጋገጥ፣ ህሙማን ለመድሃኒቶች ምላሽ መስጠታቸውን ክትትል ለማድረግ፣ የበሽታዎችን ስርጭት ለማጥናት፣ በሽታ ለመከላከል እና ስለበሽታዎች ምንጭ ምርምር ለማድረግ አስተዋፅዎ ያደርጋል። በተለይም በአገራችን የጤናማ ሰው የላቦራቶሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ።ስለሆነም የዚህ ጥናት ዓላማ የድሬዳዋ ጤናማ ሰው የሄማቶሎጂና ውጤት ማወዳደሪያ ሪፈረንስ ኢንተርቫል እድሜአቸው ከአስራ ስምንት ዓመትና ከዚያ በላይ ለሆኑ መሥራት ነው።

እርስዎም ለዚህ ጥናት ተመርጠኃል። ስለዚህ በዚህ ጥናት እንዲሳተፉና የጤናማ ሰው የሄማቶሎጂና ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንዲያደርጉ ተጋብዘኃል። ስለዚህ የዚህ ጥናት ውጤት ኢትዮጵያ ውስጥ የአዋቂ ሰዎች ጤናን ለማሻሻል ይረዳል።

የጥናቱ አካሄድ:

በጥናቱ ለመሳተፍ ከተስማሙ የጥናቱ አባል/አባላት 15 ደቂቃ የሚወስድ ጥያቄ ይጠይቁዎታል። ክብደት፣ ቁመት፣ የክንድ እና የደም ግፊት ልኬት ይወሰዳል። ሽንት (ሴት ከሆኑ) በምንሰጠው እቃ እንድትሰጡን እንጠይቃለን። በንፁህ ቫኩቴይነር ብልቃጥ እና

መርፌ እንቀዳለን (3ሚሊ ሊትር በባዶ ቲዩብ፣ 2ሚሊ ሊትር ደም እንዳይረጋ የሚያደርግ ንጥረ ነገር ፣ኢዲቲኤ፣ ባለበት ቲዩብ)፡፡ የሄማቶሎጂ፣ ሴሮሎጂ፣ ምርመራዎችን እናካሂዳለን፡፡

ሚስጥር ስለመጠበቅ:

በዚህ ጥናት የሚሰበሰብ መረጃ በሙሉ በሚስጥር ይጠበቃል፡፡ መረጃ በዚህ የስምምነት ቅፅ ከተፈቀደው ውጪ ለሶስተኛ ወገን ተላልፎ አይሰጥም፡፡ የዚህ ጥናት ውጤት ሊታተም ይችላል ነገር ግን የጥናቱ ተሳታፊዎች ስምና ማንኛውም መለያ አይገለፅም፡፡ ሚስጥራዊነቱን ለመጠበቅ የዚህ ጥናት አባል መረጃዎችን በተቆለፈ ክፍል በተቆለፈ ካቢኔት ውስጥ ያስቀምጣል፣ የፈቃደኛ ተሳታፊዎችን ማንነትን ላለማሳወቅ ውጤቶችም በኮድ ይቀመጣሉ፡፡ በኮምፒዩተር ውስጥ ለተቀመጡ ፋይሎች ለጥናቱ ተመራማሪ ብቻ የሚፈቀዱና በሚስጥር ቁልፍ የሚጠበቁ ይሆናል፡፡ የተሳታፊ ውጤት ለህክምና ባለሞያ ሊተላለፍ የሚችለው በተሳታፊው ፈቃድ ብቻ ነው፡፡ የተሰበሰበው ደምና ሽንት(ሴት ከሆኑ) ለሌላ አገልግሎት አይውልም፡፡ የሚተርፉት ናሙናዎች በድልጮራ ሆስፒታል ላቦራቶሪ ደህና ቦታ ተቀምጠው ለተጨማሪ ምርመራዎች እንደ አስፈላጊታቸው ጥቅም ላይ ይውላሉ፡፡ በመጨረሻም ተሰርቶባቸው የተራረፉ የሚደፉ ናሙናዎች አካባቢን በማይበክል መልኩ በጥንቃቄ ይወገዳሉ፡፡

ጥናቱ የሚያስከትላቸው የጤና ችግሮችና አለመመቸት:

ደም በሚቀዳበት ጊዜ መጠነኛ መጎዳትና የተወሰነ አለመመቸት ሊኖር ይችላል፡፡ ይሁን እንጂ በተቻለ መጠን ልምድ ያለው የጤና ኤክስቴንሽን ባለሞያ በመጠቀም አለመመቸቱን ለመቀነስ እንሞክራለን፡፡ሆኖም ሽንት (ሴት ከሆኑ) በመስጠት የሚደርስ መጠነኛ አለመመቸት ሊኖር ይችላል፡፡

ደህንነት:

የደም ናሙና በሚወሰድበት ጊዜ በንፁህ የደም መቅጃ በመጠቀም የሚቀዳውን ቦታ በ70% አልኮል በማፅዳት ልምድ ባለው የጤና ኤክስቴንሽን ባለሙያ ይከናወናል፡፡ በተጨማሪም ጥቅም ላይ ከዋሉ በኋላ ለማስቀመጥ የማይሆኑ የሚደፉ ደምና ሽንት (ሴት ከሆኑ) ትራፊዎች የላቦራቶሪ ደህንነት መመሪያ በመከተል ይወገዳሉ፡፡

ጥቅማ ጥቅሞች:

በዚህ ጥናት በመሳተፍ የደምና ምርመራ በማድረግ የጤንነት ሁኔታ ማወቅ ይቻላል፡፡ የጤናማ ሰው የሄማቶሎጂ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል እድሜአቸው ከአስራ ስምንት ዓመትና ከዚያ በላይ ለሆኑ የድሬዳዋ ጤናማ ሰው ደም መሰራቱ የጤና ሁኔታ ለማሻሻል ይረዳል፡፡

በጥናቱ ለመሳተፍ ማትጊያ:

በደም ምርመራ ጤናማ ያልሆነ ውጤት ከተገኘ በአቅራቢው ወደ ሚገኝ ጤና ተቋም ይላካሉ፣ የላቦራቶሪ ውጤቶቹን በነፃ ያገኛሉ፡፡ ይሁን እንጂ በዚህ ጥናት ለመሳተፍም ሆነ ለመድሃኒት ክፍያ አይሰጥም፡፡ ስለተሳትፎዎ ግን እናመሰግናለን፡፡

ያለመሳተፍ መብት:

በዚህ ጥናት ከተሳተፉ የቻልነውን ሁሉ እንክብካቤ እናደርጋለን፡፡ በማኛውም ሰዓት ከጥናቱ መውጣት እንደሚቻልና ይህም በሚያገኙት አገልግሎት ላይ (ለምሳሌ የጤና አገልግሎት) ምንም አይነት ልዩነት አይደረግም፡፡

ጥያቄ ካለ ለማነጋገር:

ምንም ዓይነት ጥያቄ ካለ የደም ናሙና የሰጡትን ሰው መጠየቅ ይቻላል በሚከተለው አድራሻ መጠየቅ ይቻላል፡፡

ተክላይ መንግስቱ

ሞባይል፡ +251 915753485

ኢሜይል: teklaymengistu@gmail.com

ለተጨማሪ

በአዲስ አበባ ዩኒቨርስቲ

የጤና ሳይንስ ኮሌጅ

የምርምር ስነምግባር

ቢሮ ስልክ፡ +251915 -75-34-85

Annex III Consent form (English Version)

Code No:_____

I have read the information above, or it has been read to me and clearly understood the purpose and anticipated benefit of the research. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. I voluntarily consent that I would participate in this study.

To collect my blood ☐

To give urine sample (non-pregnant women) ☐

Be a participant in this study ☐

And understand that I have the right to withdraw from the study at any time. ☐

_____ ____ /____ /_____ _____

Name of Study Participant DD/ MM/ YY Signature/thumb impression

Phone number: _____

If illiterate;

_____ ____ /____ /_____ _____

Name of independent literate witness DD/ MM/ YY Signature/thumb impression

Phone number: _____

Name of PI/Consent collector: _____

Date: ____ /____ /_____

Signature: _____

Annex IV Consent form (Amharic Version)

ከላይ የተገለፀውን መረጃ አንብቤአለሁ /ወይም ተነቦልኛል ፣የጥናቱን አላማ በግልጽ ተረድቻለሁ ጥያቄ ለመጠየቅ ዕድል ተሰጥቶኝ ጠይቄ በሚያረካ መልኩ ተመልሶልኛል፡፡ በዚህ ጥናት ለመሳተፍ በፈቃደኝነት ተስማምቻለሁ፡፡

ደም ለመቀዳት ☐

የሽንት ናሙና ለመስጠት (እርጉዝ ላልሆኑ ሴቶች) ☐

እና በዚህ ጥናት ተሳታፊ ለመሆን፣ ☐

በማንኛውም ሰዓት ከጥናቱ ለመውጣት መብት እንዳለኝም ተረድቻለሁ ☐

_____ _____ /_____/_____ _____

የተሳታፊ ስም፣ (ቀን/ወር/ዓመተ ምህረት) *ፊርማ (ወይም አሻራ)*

ስልክ ቁጥር : _____

ያልተማሩ ከሆኑ;

የተማሩ ገለልተኛ እማኝ ሰው ስም፣ ቀንና ፊርማ

_____ _____/_____/_____ _____

እማኝ ሰው ስም፣ (ቀን/ወር/ዓመተ ምህረት) *ፊርማ (ወይም አሻራ)*

ስልክ ቁጥር : _____

የተመራማሪው/ የመረጃ ሰብሳቢ ስም: _____

ቀንና: _____ /_____ /_____

ፊርማ _____

Annex V Questionnaire (English version)

Questionnaires to be filled by health professionals

Part I. General Information

Questionnaire Code Number:_____ Name of Village: _____
Sample Code Number: _____ Kebele: _____

S/Nº	Questions	Response	Skip
Part II. Personal Information			
0201	Age (in years)	_____	
0202	Sex	1. Male 2. Female	
0203	Place of Birth	_____	
0204	For how long did you live in the birthplace?	_____years	
0205	How long do you live in this specific area? (If different from the birth place)	_____years	
Part III. Socio-demographic Information			
0301	Educational status	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above	
0302	Occupation	1. Student 2. House wife 3. Government employee 4. Private employee 5. Farmer 6. Unemployed 7. Others (specify) _____	
0303	Your monthly income?	|___|___|___|____| ETB	
0304	Marital status	1. Single 2. Married 3. Widowed 4. Divorced	
0305	Religion	1. Orthodox Christian 2. Muslim 3. Protestant 4. Catholic 5. Others (Specify) _____	
0306	Ethnicity	1. Amhara 2. Oromo 3. Somali 4. Others (Specify) _____ 5. If mixed, specify _____	
0307	Residence	1. Urban 2. Rural	
0308	Family Size (Number of People)	_____	
0309	Source of water	1. Pipe	

		2. Spring water 3. Well water 4. River 5. Other sources (specify)
0310	Type of house	1. Mud 2. Cement 3. Wood 4. Bricks 5. others/specify _____
0311	Presence of or contact with Pet animals (e.g. Cat, Dog)	1. Yes 2. No
0312	Presence of domestic animals	1. Yes 2. No
	Additional questions to Students	
0313	Father's Age	_____ Years
0314	Mother's Age	_____ Years
0315	Father's Educational Level (from Q^{n} # 301)	
0316	Mother's Educational Level (from Q^{n} # 301)	
0317	Father's Occupation (from Q^{n} # 302)	
0318	Mother's Occupation (from Q^{n} # 302)	
0319	Monthly income (in birr collected from salary, rent, and other income)	|___|___|___|____| ETB
Part IV.Medical and Clinical Information		
0401	Do you consider currently healthy?	1. Yes 2. No
0402	Have you been sick with in the past 4 months?	1. Yes 2. No
0403	Did you take any type of drug for any illness for the last three month?	1. Yes 2. No
0404	If yes to Q^{n} 403, what type of drug? (more than one answer possible)	1. Anti-protozoa 2. Anti-helminthic 3. Anti-allergy 4. Birth control pills 5. Anti-bacterial 6. Anti-TB 7. Other (specify) _____
0405	Are you taking prescribed medication?	1. Yes 2. No
0406	If yes, what?	_____
0407	Have you been immunized in the last 6 months	1. Yes 2. No
0408	If yes what vaccine	_____
0409	Did you take over-the-counter for iron tablets or vitamins	1. Yes 2. No
0410	Do you lose your weight recently?	1. Yes 2. No
0411	If yes how much?	_____Kg
	History of common diseases	
0412	History of diabetes	1. Yes 2. No
0413	History of Hypertension	1. Yes 2. No
0414	History of Blood transfusion for the last 1 yr	1. Yes 2. No
0415	history of blood donation in the last 6 moths	1. Yes 2. No
0416	History of Hospital Admission for the last 1yr	1. Yes 2. No
0417	History of Surgical procedure for the last 3yr	1. Yes 2. No
0418	History of chronic gastritis	1. Yes 2. No

0419	History of Malaria for the last 6 month	1. Yes 2. No
0420	History of TB for the last two years	1. Yes 2. No
0421	History of Cancer	1. Yes 2. No
0422	History of Cardiac illness	1. Yes 2. No
0423	History of Bleeding disorders	1. Yes 2. No
0424	History of allergy	1. Yes 2. No
0425	History of Wheezing	1. Yes 2. No
0426	Is there any inherited health disorder in your family?	1. Yes 2. No
0427	If yes, what?	_____

Part V. Life Style and Nutritional Habit

0501	Have you been exposed drug abuse	1. Yes 2. No
0502	If yes, what drug?	_____
0503	Have you been exposed in hazardous chemicals in your job?	1. Yes 2. No
0504	If yes, What chemical?	_____
0505	Do you have Fasting habit?	1. Yes 2. No
0506	If Yes, How is your fasting habit?	1. Eating vegetable food only 2. Complete abstinence from food then eating all kinds of food 3. Complete abstinence from food then eating vegetable food only
0507	Did you eat undercooked/raw meat?	1. Yes 2. No
0508	Do you have the habit of physical Exercise?	1. Yes 2. No
0509	If yes, how many times do you do the exercise per week?	_____
0510	Any sexual contact	1. Yes 2. No
0511	If yes to Q 0510, condom use`	1. Yes 2. No

How often do you eat the following food? (put a "√ " mark)

A= Once/day; **B**= More than Once/ day; **C**= 2-3 times/week; **D**= Occasionally (e.g holidays, special ceremonies); **E**= Never

		A	B	C	D	E	Remark
0512	Roots and Tuber (Potato, sweet potato, Enset, Cassava)						
0513	Legumes (Beans, peas, chicken pea, etc)						
0514	Cereals (Corn, Teff, Wheat, sorghum, etc)						
0515	Vegetables (Tomato, cabbage, etc)						
0516	Fruits (Orange, banana, etc)						
0517	Meat (including poultry, fish, etc)						
0518	Milk and Milk products (Butter, yoghurt, cheese, etc)						
0519	Egg						
0520	Tea and/or coffee						

How frequent do you consume/use the following (put a √ mark)

A= Once/day (Regular); B= More than once/day; C=2-3 times/week; D= Once a week; E= Occasionally (holiday, special ceremony); F=Never

		A	**B**	**C**	**D**	**E**	**F**
0521	Alcohol						

0522	*Khat*						
0523	Cigarettes						

Part VI. Females (Non-Pregnant)		
0601	Are you still menstruating?	1. Yes 2. No
0602	If No, when was your last period?:__day	
0603	How often stay on average for menstruating	_____# of days
0604	Are you taking oral contraceptive or implant contraceptives?	1. Yes 2. No
0605	If yes, details?	
0606	Have you been taken over-the-counter oral contraceptives	1. Yes 2. No
0607	If yes, what _____(specify)	
0608	Are you breast feeding?	1. Yes 2. No
Part VII. Females (Pregnant)		
0701	Gestation	_____(weeks)
0702	Do you have vaginal bleeding currently	1. Yes 2. No
0703	Iron supplementation:	1. Yes 2. No
0704	Folate supplementation	1. Yes 2. No
0705	Iron and folate combined supplementation	1. Yes 2. No
0706	Parity	
0707	did you encountered with pregnancy & obstetrics complication	
Part VIII. Anthropometric Measurement		
0801	Height	_____(in cm)
0802	Weight	_____ (in kg)
0803	Blood pressure	_____(mm Hg)

❖ We thank you for your cooperation!

Interview Date:_____

Interviewer's Name_____ Signature_____

Annex VI Questionnaire (Amharic Version)

የመጠይቁ መለያ_____
የደም ናሙናው መለያ ቁጥር:_____

በጤና ባለሞያዎች የሚሞላ ቃለ መጠይቅ

መመሪያ:

በቅድሚያ ይህንን ቃለ መጠይቅ ለመሙላት ለሰጡን ጊዜና ትብብር አድናቆቴን እገልፃለሁ። የዚህ ቃለ መጠይቅ አላማ "የጤናማ ሰው ደም ውስጥ የሚገኙ የሜማቶሎጂ ምርመራዎች ሪፈረንስ ኢንተርቫል እድሜአቸው ከአስራ ስምንት እስከ ስልሳ አምስት ዓመትና እርጉዝ ለሆኑ ለሆኑ የድሬዳዋ ነዋሪ ለመስራት" መረጃ ለመሰብሰብ ነው። የዚህ ጥናት ተመራማሪ በአዲስ አበባ ዩኒቨርስቲ የህክምና ላቦራቶሪ ትምህርት ክፍል ሁለተኛ ዲግሪ ተማሪ የሆኑት አቶ ተክላይ መንግስቱ ሲሆኑ የእርስዎ ቅን ትክክለኛ መልስ በሰዓቱ መስጠት የዚህን ጥናት ስኬት ይወስናል። ስለሆነም ይህንን ቃለ መጠይቅ ሃቀኝነትና ሃላፊነት በተሞላው መንገድ እንዲሞሉ በትህትና እጠይቃለሁ።

አመሰግናለሁ!!!

ክፍል 1. አጠቃላይ መረጃ

የመጠይቁ ኮድ: _____ **የመንደሩ ስም:**_____
የናሙናው መለያ ኮድ:_____ **ቀበሌ:** _____

ቁጥር	ጥያቄ	ምላሽ
	ክፍል 2. የግል መረጃ	
0201	እድሜ	_____ዓመት
0202	ጾታ	1. ወንድ 2. ሴት
0203	የትውልድ ቦታ	_____
0204	በትውልድ ቦታዎ ለምን ያህል ጊዜ ኖረዋል?	_____ዓመት
0205	አሁን ያሉበት ቦታ ለምን ያህል ጊዜ ኖረዋል? (ከትውልድ ቦታዎ የተለየ ከሆነ)	_____ዓመት
	ክፍል 3. ማህበራዊና ኢኮኖሚያዊ መረጃ	
0301	የትምህርት ደረጃ	1. ያልተማሩ 2. ማበብና መፃፍ 3. አንደኛ ደረጃ (1-8) 4. ሁለተኛ ደረጃ (9-12) 5. ኮሌጅ ዲፕሎማ/ዲግሪ እና ከዚያ በላይ
0302	ሥራ	1. ተማሪ 2. የቤት እመቤት 3. የመንግስት ሠራተኛ 4. የግል ተቀጣሪ 5. ገበሬ 6. ስራ ፈላጊ 7. ሌላ ካለ ይግለፁ _____
0303	ወርሃዊ ገቢ	|___|___|___|____| ብር
0304	የጋብቻ ሁኔታ	1. ያላገቡ 2. ያገቡ 3. የተፋቱ 4. ባል/ሚስት የሞተባቸው
0305	ሃይማኖት	1. ኦርቶዶክስ ክርስቲያን 2. ሙስሊም 3. ፕሮቴስታንት

		4. ካቶሊክ 5. ሌላ ካለ ይግለፁ _____
0306	ብሄረሰብ	1. አማራ 2. ኦሮሞ 3. ሶማሌ 4. ሌላ_____ 5. ድብልቅ ከሆኑ ይግለፁ_____
0307	መኖሪያ ቦታ	1. ገጠር 2. ከተማ
0308	የቤተሰብ ብዛት	_____
0309	የውሃ ምንጭ	1. ቧንቧ 2. የምንጭ 3. የጉድጓድ 4. የወንዝ 5. ሌላ ካለ ይግለፁ_____
0310	የቤት አይነት	1. ጭቃ 2. ሲሚንቶ 3. እንጨት 4. ጡብ/ሸክላ 5. ሌላ ካለ ይግለፁ _____
0311	የቤት ውስጥ ለማዳ እንስሳ መኖር ወይም ንክኪ (ለምሳሌ ድመት፣ ውሻ)	1. አለ 2. የለም
0312	የቤት እንስሳት መኖር	1. አለ 2. የለም
	ተጨማሪ ጥያቄዎች ለተማሪዎች	
0313	የአባት እድሜ	_____ዓመት
0314	የእናት እድሜ	_____ዓመት
0315	የአባት የትምህርት ደረጃ(ከተ/ቁ 301 ይምረጡ)	
0316	የእናት የትምህርት ደረጃ(ከተ/ቁ 301 ይምረጡ)	
0317	የአባት ሥራ(ከተ/ቁ 302 ይምረጡ)	
0318	የእናት ሥራ (ከተ/ቁ 302 ይምረጡ)	
0319	ወሃዊ ገቢ (በብር ከደሞዝ፣ ኪራይ፣ እና ሌሎች ገቢዎች)	___|___|___|____|
ክፍል 4. የጤንነት ሁኔታና የህክምና ታሪክ መረጃ		
0401	ጤነኛነት ይሰማዎታል?	1. አዎ 2. አይ
0402	ባለፈው አራት ወር ታመው ነበር?	1. አዎ 2. አይ
0403	ባፉት ሶስት ወራ ለማንኛውም ዓይነት ህመም ማንኛውንም ዓይነት መድሃኒት ወስደኋል?	1. አዎ 2. አይ
0404	ለተራ ቁጥር 403 መልስዎ ወስጃለሁ ከሆነ የትኛውን ዓይነት መድሃኒት ነው ወሰዱት? (ከአንድ በላይ መልስ ይቻላል)	1. ፀረ-ፕሮቶዞአ 2. ፀረ-ሄልሚንትስ 3. ፀረ-አለርጂ 4. የወሊድ መከላከያ ኪኒን 5. ፀረ-ባክቴሪያ 6. ፀረ-ቲቢ 7. ሌላ ካለ ይግለፁ _____
0405	በሀኪም የታዘዘ መድሃኒት እየወሰዱ ነው	1. አዎ 2. አይ
0406	አዎ ከሆነ፣	ምን አይነት መድሃኒት_____
0407	ባለፉት 6 ወራት ጊዜ ክትባት ወስደዋል?	1. አዎ 2. አይ
0408	አዎ ከሆነ፣	የምን ክትባት ነው የወሰዱት_____
0409	ከመጠን ያለፈ መድሃኒ፣የደም ማነስ ክኒን ወይም ቪታሚን ወስደው ያውቃሉ?	1. አዎ 2. አይ
0410	ባልፈው 3 ወር ክብደት ቀንሰው ነበር?	1. አዎ 2. አይ
0411	አዎ ከሆነ፣	ምን ያህል _____?(ኪሎ ግራም)
	የሚከተሉት የህመም ዓይነቶች አሞዎት ያውቃል?	

0412	የስኳር ህመም?	1. አዎን 2. የለም					
0413	የደም ግፊት ከፍ ማለት?	1. አዎን 2. የለም					
0414	ባለፈው 1 ዓመት ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም					
0415	ባለፈው 6 ወር ጊዜ ደም ለግሰው ነበር?	1. አዎን 2. የለም					
0416	ባለፈው 1ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1. አዎን 2. የለም					
0417	ባለፉት 3ዓመታት የቀዶ ህክምና ተደርጎልዎ ያውቃል?	1. አዎን 2. የለም					
0418	የቆየ የጨጓራ ህመም አለብዎት?	1. አዎን 2. የለም					
0419	ባፉት 6ወራት የወባ ህመም አጋጥሞዎት ያውቃል?	1. አዎን 2. የለም					
0420	ባለፉት 2ዓመታት የቲቢ ህመም ኖሮዎት ያውቃል?	1. አዎን 2. የለም					
0421	ካንሰር ህመም	1. አዎን 2. የለም					
0422	የልብ ህመም	1. አዎን 2. የለም					
0423	የመድማት ችግር/ህመም	1. አዎን 2. የለም					
0424	አለርጂ (የሰውነት መቆጣት)	1. አዎን 2. የለም					
0425	የመተንፈስ ችግር (ሲተነፍሱ ሲር ሲር የሚል ድምፅ)	1. አዎን 2. የለም					
0426	በቤተሰብ የሚተላለፍ የጤና እክል አለቦት?	1. አዎን 2. የለም					
0427	አዎ ከሆነ፣ምንድን ነው	_____					
ክፍል 5. የአመጋገብ እና የህይወት ልምድ							
0501	አደገኛ እጽ ተጠቅመው ያውቃሉ	1. አዎን 2. የለም					
0502	አዎ ከሆነ፡	መቼ_____ (ወር) ምን አይነት_____					
0503	በስራ ቦታዎ ለአደገኛ ኬሚካል ተጋልጠው ያውቃሉ?	1. አዎን 2. የለም					
0504	አዎ ከሆነ ፣ ለምን አይነት ኬሚካል?	_____					
0505	የመፆም ልምድ አለዎት?	1. አዎን 2. የለም					
0506	መልስዎ አዎን ከሆነ፣ የመፆም ልምድዎ እንዴት ነው?	1. አትክልቶችን ብቻ መመገብ 2. በአጠቃላይ ከምግብ መታቀብ ከዚያም ያገኙትን መመገብ 3. በአጠቃላይ ከምግብ መታቀብ ከዚያም አትክልቶችን መመገብ					
0507	በደንብ ያልበሰለ ወይም ጥሬ ሥጋ ይመገባሉ?	1. አዎን 2. የለም					
0508	የሰውነት እንቅስቃሴ የማድረግ ልምድ አለዎት?	1. አዎን 2. የለም					
0509	መልስዎ አዎ ከሆነ በሳምንት ለምን ያህል ጊዜ ይንቀሳቀሳሉ?	1. አዎን 2. የለም					
0510	የግብረ ሥጋ ግኑኝነት አድርገው ያውቃሉ	1. አዎን 2. የለም					
0511	ለተ/ቁ 0510 መልስዎ አዎን ከሆነ፣ ኮንዶም ይጠቀማሉ?	1. አዎ 2. የለም					
የሚከተሉትን የምግብ ዓይነቶች ምን ያህል ጊዜ ይመገቧቸዋል ? (“√ “ ይህን ምልክት ያስቀምጡ)1=በቀን አንድ ጊዜ፣2=በቀን ከአንድ ጊዜ በላይ፣3=በሳምንት ከ 2 እስከ 3 ጊዜ 4=አልፎ አልፎ (ለምሳሌ፣ ለበዓል፣ ልዩ ዝግጅቶች ሲኖሩ)፣ 5=ተጠቅሜ አላውቅም							
		1	2	3	4	5	ማብራሪያ
0512	ሥራ ሥር (ድንች፣ስኳር ድንች፣እንሰት ካሳቫ ወዘተ)						
0513	አባዝርት (Legumes፡ ባቄል፣አተ፣ ሽንብራ ወዘተ)						
0514	ጥራጥሬ (በቆሎ፣ ጤፍ፣ ስንዴ፣ ማሽላ)						
0515	አትክልት (ቲማቲም፣ ጎመን፣ ወዘተ)						
0516	ፍራፍሬ (ብርትኳን፣ ሙዝ፣ ወዘተ)						
0517	ሥጋ (የዶሮ፣ የ አሳን ጨምሮ)						
0518	ወተትና የወተት ተዋፅዎ (እርጎ፣ቅቤ፣አይብ፣ወዘተ)						
0519	እንቁላል						
0520	ሻይ እና/ወይም ቡና						
የሚከተሉትን ምን ያህል ይበላሉ/ይጠቀማሉ (√ ይህን ምልክት ያስቀምጡ)							

1=በቀን አንድ ጊዜ (ሁልጊዜ)፣2=በቀን ከ1 ጊዜ በላይ፣3=በሳምንት ከ 2 እስከ 3 ጊዜ፣4=በሳምንት 1 ቀን፣5=አልፎ አልፎ (ለምሳሌ፣ ለበዓል፣ ልዩ ዝግጅቶች ሲኖሩ)፣ 6=ተጠቅሜ አላውቅም							
		1	2	3	4	5	6
0521	አልኮል						
0522	ጫት						
0523	ሲጋራ						
ለነፍሰጡር ላልሆኑ ሴቶች ብቻ ነው							
0601	አሁን የወር አበባ ላይ ነሞት?	1. አዎ 2. የለም					
0602	አይ ከሆነ፣ የመጨረሻ የወር አበባ ያዩበት ጊዜ?	_____በቀን					
0603	ለምን ያህል ጊዜ የወር አበባዎ በአማካኝ ይቆያል?	_____በቀን					
0604	የአርግዝና መከላከያ ኪኒን ወይም ብክርን የሚቀበር እየወሰዱ ነው	1. አዎ 2. የለም					
0605	አዎ ከሆነ፣ የትኛውን?	_____					
0606	ከመጠን በላይ የሆነ የእርግዝና መከላከያ ኪኒን ወስደው ያውቃሉ?	1. አዎን 2. የለም					
0607	አዎ ከሆነ ምን አይነት የመከላከያ	_____(ግለጹ)					
0608	ጡት እያጠቡ ነው?	1. አዎን 2. የለም					
ለነፍሰጡር ላልሆኑ ለሆኑ ሴቶች ነው							
0701	ከፀነሱ ስንት ጊዜዎ ነው?	____በሳምንት					
0702	የብልት መድማት እልቦት	1. አዎን 2. የለም					
0703	ተጨማሪ ብረት ንጥረነገር	1. አዎን 2. የለም					
0704	ተጨማሪ ፎሌት ንጥረነገር	1. አዎን 2. የለም					
0705	ተጨማሪ የብረት ንጥረነገር ና ፎሌት	1. አዎን 2. የለም					
0706	ለስንተኛ ጊዜ ነው የፀነሱት?						
0707	ለተ/ቁ 0706 ከአንድ በላይ ከሆነ፣ ከእርግዝና እና ወሊድ ጋር የተያያዘ ህመም አጋጥሞት ነበር?	1. አዎን 2. የለም					
ክብደት፣ ቁመት፣ የክንድና የደም ግፊት ልኬት							
0801	ቁመት	_____ሴንቲ ሜትር					
0802	ክብደት	_____ኪሎ ግራም					
0803	የክንድ መሃለኛው ክፍል ዙሪያው (MUAC)	_____ሴንቲ ሜትር					
0804	የደም ግፊት (በሚሊሜትር ሜርኩሪ)	_____(mm Hg)					

❖ ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን:__ __ / __ __ / __ __ __ __
ቀን/ ወር/ ዓመተ ምህረት

ቃለ መጠይቁን ያካሄደው:

ስም፡ _____
ፊርማ፡ _____

Annex VII Questionnaire (Somali Version)

Su aalahan waxaa buuxinaya xirfadlayaasha caafimaadka

Qaybta I. Macluumaadka Guud

Tirade Su aalaha:_____ Magaca Tuulada: _____
Sample Code Number: _____ Qabalaha: _____

S/N^{o}	Su aalo	Jawaab	Skip
	Qaybta II. Macluumaad Shaqsi ahaaneed		
0201	Imisa jir baa tahay ?	_____ Sanno	
0202	Jinsi	1. lab 2. Dhedig	
0203	Meeshaad ku dhalatay	_____	
0204	Meeshaad ku dhalatay muddo intee le eg ayaad ku noolayd?	_____sanno	
0205	Muddo intee le eg ayaa halkan ku nooshahay? (hadii ayka duwan tahay meeshaad ku dhalatay)	_____sanno	
	Qaybta III. Macluumaadka Dadka Iyo Dhaqaalaha		
0301	Waa maxay heerkaaga waxbarasho ee u sareeya?	6. Jaahil 7. Waxna qora waxna akhriya 8. Dugsiga hoose (1-8) 9. Dugsiga dhexe (9-12) 10. Kuliyad diploma/degree iyo waxka badan	
0302	Waa maxay shaqadaada	8. Arday 9. Guri joogto 10. Shaqaale dowladeed 11. Shaqaale gaar ah 12. Beeroley 13. Shaqo la aan 14. Tukale _____	
0303	Waa imisa dhammaan daqliga bishii ku soogala (Birka. Eth)?	|___|___|___|____| ETB	
0304	Waa maxay xaalada xiriirka / guurka ee waqti xaadirkan?	5. Kali ah 6. Guursaday 7. Carmali ah 8. Kala tegay	
0305	Waa maxay diintaadu ?	1. Ortodhoks 2. Muslim 3. Brotestanti 4. Katolik 5. Tukale _____	
0306	Waa maxay qoomiyadaadu ?	1. Amxaaro 2. Oromo 3. Somali 4. Tukale	

		5. Isku dhejir
0307	Waa halkee degaankaagu ?	2. Magaalo 2. Miyi
0308	Baaxada Qoyska (tirade dadka)	_____
0309	Ilaha Biyaha	1. biyaha dur dur 2. Biyaha ceelasha 3. Webi 4. Tukale
0310	Nooca aqalka	1. Dhoobo 2. Sibidh 3. Looxaan 4. Marmar 5. Tukale
0311	Xayaanka dabjoog	1. Haa 2. Maya
0312	Heerka waxbarasho ee Aabaha	1. Haa 2. Maya
	Su aalo dheeri ah Ardayda	
0313	Da ada Aabaha	_____ Sanno
0314	Da ada Hooyada	_____ Sanno
0315	Heerka waxbarasho ee Aabaha (Su aasha # 301)	
0316	Heerka waxbarasho ee hooyada (from Q^{n} # 301)	
0317	Shaqada aabaha (from Q^{n} # 302)	
0318	Shaqada hooyada (from Q^{n} # 302)	
0319	Waa imisa dhammaan daqliga bishii ku soogala (Birka. Eth)?	|___|___|___|____| ETB
	Qaybta IV. Macluumaad ku sabsan xaalada caafimaad iyo daaweynta	
0401	Hada ma caafimaad qabtaa?	1. Haa 2. Maya
0402	Afartii bilood ee lasoo dhaafay miyaad xanuunsatay?	1. Haa 2. Maya
0403	Saddexdii bilood ee la soo dhaafay xanuunkasta daawokasta miyaad isticmaashay?	1. Haa 2. Maya
0404	Haddii su aasha 403 jawaabtu haa tahay daawanoocee ah (waxka badan Haddii su aasha 403 jawaabtu haa tahay daawanoocee ah (waxka badan halsu aal waa suurtagal?	8. Daawada protozoa 9. daawaada-helminthic 10. daawada Anti-allergy 11. dhalmada joojisa 12. daawada bacterial 13. daawada TB 14. tukale _____
0405	ma qaadataa daawada dhaqtarkuu qoro?	1. Haa 2. Maya
0406	Hadii ay haa tahay maxay?	_____
0407	Talaada waqti dhow ma qaatatay	1. Haa 2. Maya
0408	Hadii haa tahay talaal maxay ah	_____
0409	Ma qaadatay daawo aan dhaqtar kuu qori iron ama vitaamo ah	1. Haa 2. Maya
0410	Muddooyinkii udambeeyay miisaankaagu hoos ma u dhacay?	1. Haa 2. Maya
0411	Hadii haa tahay in intee le eg?	_____Kg
	Xanuunadan soo socda ma kugu dhaceen	

0412	Sonkorta	1. Haa 2. Maya					
0413	Dhiikarka	1. Haa 2. Maya					
0414	Dhiig lagugu shubo sannad kii udanbaysay	1. Haa 2. Maya					
0415	Wax la xiriira dhiig shubid	1. Haa 2. Maya					
0416	Cisbitaal lagu dhigo sannadkii u danbaysay	1. Haa 2. Maya					
0417	Qaliin muddo saddex sanno ah	1. Haa 2. Maya					
0418	Gastriig muddo dheer ah	1. Haa 2. Maya					
0419	Xanuunka duumada muddo lixdii bilood ee u danbaysay	1. Haa 2. Maya					
0420	TB muddo labo sanno ah	1. Haa 2. Maya					
0421	Xanuun kansarka	1. Haa 2. Maya					
0422	Wadna xannuun	1. Haa 2. Maya					
0423	Xanuunada dhiiga	1. Haa 2. Maya					
0424	Xanuun aalerge ah	1. Haa 2. Maya					
0425	Xanuunka naqaska	1. Haa 2. Maya					
0426	Majiraa xanuun aad waalidiintaada ka dhaashay?	1. Haa 2. Maya					
0427	Hadii haa tahay waa maxay ?	_____					
	Qaybta V. Macluumaad kusaabsan Nafaqaddaada iyo hab nololeedkaaga						
0501	Waligaa daawaayin si khaldan ma usticmaashay	1. Haa 2. Maya					
0502	Hadii haa tahay dawadee?	_____					
0503	Goobta shaqada kiimiko qadar is maku wayeelaysay?	1. Haa 2. Maya					
0504	Hadii haa tahay kiimikadee?	_____					
0505	Ma soomtaa?	1. Haa 2. Maya					
0506	Hadii haa tahay waa side soomkaagu?	4. Waxaa cunaa khudaar kaliya 5. Dhamaan cunto oo dhan baan joojiyaa kadibna cuntoodhan baan cunaa 6. Dhamaan cunto oo dhan baan iska joojiyaa kadibna khudaar oo kaliyaan cunaa					
0507	Ma cuntaa cunno aan aad loo Karin / ama hilib qaydhiin?	1. Haa 2. Maya					
0508	Isboorti ama dhaqdhaqaaq masamaysaa?	1. Haa 2. Maya					
0509	Hadii haa tahay imisa jeer baa todobaadkii isboorti samaysaa?	_____					
0510	Wax xidhiidh galmo ah masamaysaa	1. Haa 2. Maya					
0511	If yes to Q 0511 Hadii haa tahay S0510 cinjirka coondoomka ma isticmaashaa?`	1. Haa 2. Maya					
	Imisa jeer Imisa jeer baad badana cuntooyinkan soo socda cuntaa ? (dhig "√ " calaamada)						
	A= mar kaliya/maalintii; **B**= Marar badan/maalintii; **C**= 2-3 jeer/todobaadkii; **D**= maalmo qudha/ kedis ah (e.g ciidaha, xafladaha qaaska ah); **E**= marnaba						
		A	B	C	D	E	Remark
0512	xididlaha (baradho , midaadis, Enset, Cassava)						
0513	Legumes (Digir, peas, chicken pea, iwm)						

0514	Cereals (Corn, Teff, Wheat, sorghum, iwm)						
0515	xawaash (tamaad , kaabbash, iwm)						
0516	khudaar (liin macaan , muus, iwm)						
0517	Hilibka (ayka mid yihiin digaagad, kaluun iwm)						
0518	Caanaha iyo waa caanaha ka soobaaa (Butter burcad, yoghurt, subag, etc)						
0519	Ukunta						
0520	Shaaha iyo ama Bunka						

	Sideed Badanaa u istimaashaa kuwan soo socda (put a √ mark)						
	A= mar kaliya/maalintii; **B**= Marar badan/maalintii; **C**= 2-3 jeer/todobaadkii; **D**= maalmo qudha/ kedis ah (e.g ciidaha, xafladaha qaaska ah); **E**= marnaba						
		A	**B**	**C**	**D**	**E**	**F**
0521	khamri						
0522	*Jaad*						
0523	Sigaar						

Qaybta VI. Dhedig (uur lahayn)		
0601	Ilaa imika caadadu may kaa timaadaa?	1. Haa 2. Maya
0602	Hadii maya tahay, goormay ahayd waqtigii kuugu dambaysay?:__maalin	
0603	Badanaa celcelis ahaan imisa cishuu kaa iman?	_____# Maalmood
0604	Miyaad qaadataa daawada lagaga hortago/ irbadaha uurka?	1. Haa 2. Maya
0605	Hadii haa tahay, sharax?	
0606	Waligaa ma qaadatay daawada lagaga hortago uurka ee suuga	1. Haa 2. Maya
0607	Hadii haa tahay waa maxa (qeex)	
0608	Ma naas nuujisaa?	1. Haa 2. Maya
Qaybta VII. Dumarka (Uurka)		
0701	Da'da gestational	_____(toddobaadyo)
0702	Miyaad leedahay dhiigbax?	1. Haa 2. Maya
0703	Dheefinta birta:	1. Haa 2. Maya
0704	Kaalmooyinka sharraxaadda	1. Haa 2. Maya
0705	Kobcinta birta iyo folate isku dhafka ah	1. Haa 2. Maya
0706	Nidaamka	
0707	Miyaad kula kulantay dhibaatada uurka iyo dhibaatooyinka dhalmada?	
Qaybta VIII. Cabbiraadda Nalalka Aaladda		
0801	dhererka	_____(ee cm)
0802	miisaanka	_____ (ee kg)
0803	Dhiig kar	_____(ee mmHg)

❖ Waan kugu mahadnaqaynaa iskaashigaaga!

Taariikhda wareysiga: _____

Magaca Wareysiga:_____ saxiixa _____

Annex VIII Questionnaire (Oromifa Version)

Gaffi kanakan guutuukanbarnotta faaya baryani Gaffi waluu galdi qofa du

Lakkumsa : _____ Nannoolla: _____

Labri meghaa ithi fuddah : _____ Ganda : _____

S/NO		Yaada	dhufaa
0201	Umurii	_____	
0202	Salaa	1. dhira 2. Dhaalaa	
0203	Bakka dhaloota	_____	
0204	Bakka dhaloot Hangami Jiratanii	____wagga	
0205	Bakka Amma Jafan Hangaoni Jurdi	_____wagga	
Part I. Haaraa gaffi waliti Dhufanya Nanno			
0301	Sadarka baranota	1. Hin barnni 2. Barresuufi dubisuu 3. Sadarkaa tokoffa (1-8) 4. Sadarkaa lamaffa (9-12) 5. Diploma, digrii fii saaoli	
0302	Hojii	1. Barataa 2. Hojata manna 3. Hojarta motuuma 4. Hojata dhafaa 5. Qotee bulaa 6. Hoji hin qabu 7. Garii bbira yojiraat ibsi_____	
0303	Gaalin Ja.aati argetuu	|___|___|___|____| Qr.	
0304	Haala Gaa.illa	1. Hinfuna 1. Fudherra 2. Abban Mana kan irra du.ee 3. Hinkine jiraa adabone	
0305	Amanti	1. Ortodoksii 2. muslilama 3. protetance or beenxe 4. catolikni 5. bira waqafa	
0306	Gosaa ykffu lamii	1. Amaraa 2. oromoo 3. sulmaalew 4. aigiree _____ 5. Walili makaa	
0307	Bakka Jireenya	1. magalla 2. bidaaya	
0308	Baayira mati fakkl	_____	
0309	Argama Bishaani	1. boniba 2. Burqaa	

		3. Lagga 4. Kaa birra yoo jiratee ibsa 5. _____
0310	Gosa manaa	1. Dhoqe 2. Siminooto 3. Mukaa 4. biilekot 5._____ Kaa birra yoo ratee ibsa
0311	Manaa keesaa beladda wallin jirtan ni jira	1. Eyye 2. lakkii
0312	Beladda manna ni qabdoo	1. Eyye 2. lakkii
	Gaffin daabalata Baratootaf	
0313	Umurin Abakee	_____ waggan
0314	Umurin Hadhakee	_____waggan
0315	Sadarkan barnota abakee	
0316	Sadarkan barnota hadhakee	
0317	Hojin Abbaakee	
0318	Hojin Hadhakee	
0319	Gaalin baratin argaafani	|___|___|___|____|Qr
0401	Fayyummoran Isinitti dhagahoma	1. Eyyee 2. Miti
0402	Jioota afran darba dhuffub sattani turfan?	1. Eyyee 2. Miti
0403	Jioota sadan darbe dhukkube kamifiyyuu dawaa fudhataniru	1. Eyyee 2. Miti
0404	Tatiiba lakk 403 deebii fudha dheera yoo jette dawaa isa kami fudhatte (dubii tokko olni dandiama)	1. Fara protozoa 2. Farra helmentis 3. Farra alargii 4. Kiniina dhala ittisu 5. Farra jiibii 6. Kan biraa yoojiraate ibsaa 7. _____
0405	Dawaa doctoran ajajome fudhachaa jiruu?	1. Eyyee 2. Miti
0406	Eyyeen yoo ta e	Dawaa gosa kamil_____
0407	Ji oota 6 n darbe talaallii	1. Eyyee 2. Miti
0408	Eyyeen yoo ta e	Talaalii isa kami kan fudhattan? _____
0409	Dawaa humna keesanii olii kiniina hiriina dhiigaa, kn viitamina fudhattanni beektu?	1. Eyyee 2. Miti
0410	Ji oota sadan darbe ulfaatina hiristan turfan?	1. Eyyee 2. Miti
0411	Eyyee yoo jettan	_____hammam
	Gosa Dhukkuba hardoftani dhukkubs attanii beektu	
0412	Dhukkuba Sukakaaraa?	1. Eyyee 2. Hinjiru
0413	Dhiibbaan Dhhiqaa Dabaluu?	1. Eyyee 2. Hinjiru
0414	Waggaa Tokko Dhiigni Isniif?	1. Eyyee 2. Hinjiru
0415	Jioota 6`n darbe dhiiga Qrjoomtanii beektu?	1. Eyyee 2. Hinjiru
0416	Waggaa tokko Hospitaala Ciistanii beektu?	1. Eyyee 2. Hinjiru
0417	Waggaa Sodan(3) darbe tojaajilli beqaqsanii hodhuu isiniif godhamee jira?	1. Eyyee 2. Hinjiru
0418	Dhukkuba goraachaa isininna ture?	1. Eyyee 2. Hinjiru

0419	Jiòota johan (6) darbe Dhukkubini Busaa isin mudatee beeka?	1. Eyyee 2. Hinjiru					
0420	Waggoota laman (2) darbe Dhukkubni Tiibii (sombaa) isni qabee beeka?	1. Eyyee 2. Hinjiru					
0421	Dhukkuba Kaansarii?	1. Eyyee 2. Hinjiru					
0422	Dhukkuba Laghee?	1. Eyyee 2. Hinjiru					
0423	Rakkoo dhukkuba Dhiiguu?	1. Eyyee 2. Hinjiru					
0424	Alargii (Dallanw Qooma Namoo) ?	1. Eyyee 2. Hinjiru					
0425	Rakkina Hargansuu yoo harganan sir sir kan jedehu	1. Eyyee 2. Hinjiru					
0426	Mootiidhaan kan daddarbu rakkoo fayyaa qabdu?	1. Eyyee 2. Hinjiru					
0427	Eyyan yoo jettan maalinni	_____					
Part II. kuta 5:-Akkaataa nyaataa fi muuxannoo jireenyaa							
0501	Qawoo sammuu nama hadoochu fayyadamtonii beektu?	1. Eyyee 2. Hinjiru					
0502	Eyyeen yoo taè?	_____					
0503	Bakka hajiitti chemikaala cimaadhan hubantanii beektuu?	1. Eyyee 2. Hinjiru					
0504	Eyyee yoo jettan chemis	_____kaalo akkamiti?					
0505	Muuxannoo soomaa qabduu?	1. Eyyee 2. Hinjiru					
0506	Deebin eyyeen you taè muux annoon sooma keessani akkami?	1. Kudraafi muduraa qofa riyachuu 2. Waliiqala nyaatarra qusachii, isaan booda kan orgataa nyaachuu 3. Waliigalan nyaatarraa of qusachuu isaan bbda kuduraa nyaachuu					
0507	Sirritti kan hin bilchaanna yookin foon dheedhii ninyaattu?	1. Eyyee 2. Miti					
0508	Muuxannoo sosochtii qaamoo qabduu?	1. Eyyee 2. Miti					
0509	Eyyee yoo jettan forbanitti yeroo meeqa sochii qaamaa gootu?	_____					
0510	Wal-qunnamti saaloo raawwatonii beektuu?	1. Eyyee 2. Miti					
0511	Tartiba lakk 0510 deebiin keessan Eyee yoo tae	1. Eyyee 2. Miti					
	Gosoota nyaatoo kanpp gadii yeroo meeqa nyaattu? ("√ " mallattoo kana barreessaa)						
	A= Guyyaatti yeroo tokko; **B**= guyysstti yeroo tokkoo oli; **C**= torbanitti yeroo 2 hanga 3; **D**= darbee darbee (fakkenyof: guyyaa ayyaannaa, qophii addaa, yoojiraate) ; **E**= fayyadamee hin beeku						
		A	B	C	D	E	Ibsa
0512	Hundee (Dincha Dincha sukkaroo wargee)						
0513	Jirma (Boqolloo, Atara, shunbura)						
0514	Kanfalfaltomu (Boqolloo, Xaafi, Qamadii, misingaa)						
0515	Fuduraa (timaatima, raafuufi k.k.f						
0516	Kudura (Burtukaana, Muuzii fi k.k.f						
0517	Foon (Kan lukkuu, Kan qurxummii)						
0518	Annan (Itittuu, dhadhaa, Ayibii)						
0519	Killee						

0520	Shaayii fi Buna						
	Kan Armaan gadii hammam nyaatu (fayyadamu) (√) mallottoo kan kaaàa.						
	A= guyyaatti yeroo tokkoo; B= guyyaatti yeroo tokkoo oli; C= torbanitti yeroo 2 hanga3 ; D=torbanitti yeroo tokko; E= Darbee darbee ; F=						
		A	**B**	**C**	**D**	**E**	**F**
0521	Alkkolii						
0522	*Jimaa*						
0523	Tamboo						
Part III. Dubartii Ulfa hin faare qofaaf							
0601	Amma Jiã marsaa lagu irro jirtuu?	1. Eyee 2. Miti					
0602	Lakki yoo taè-guyyaa dhumaa ? ____ guyyaan marsaa laguu itti argitan ?						
0603	Marsaa laguu gidduu galoon hammam isin irra tura?	_____# guyyaan					
0604	Kinina ulfa ittisu yookin harka keessa kan awwaalomu fudha cha jirtu	1. Eyyee. 2. Miti					
0605	Eyyeen yoo jettan isa kami?						
0606	Kinina ulfa ittisu humnoo oli fudhattanii beektuu	1. Eyyee 2.Miti					
0607	Eyyeenyoo yoo taè mala ittisaa _____ ibsa. Isa kami?						
0608	Harma hoosisaa jirtuu	1. Eyyee 2. Miti					
Part IV. Dubarti ulfaa taàn Qofaaf							
		_____torbanin					
0702	Erga ulfooftanii guyyaa meeqa?	1. Eyeee 2.Miti					
0703	Dhangaa nyaata dabalatoo ironii?	1. Eyeee 2.Miti					
0704	Dhagaa nyatoo dabalatoo foolatii	1. Eyeee 2.Miti					
0705	Dabalota nyaata foolatii fi ironnin badhaadhe?	1. Eyeee 2.Miti					
0706	Yeroo mwqaf kan ulfoofta?						
0707	Lakk 0706 tokko oli yoo taè ulfaafi dahuumsa irratti mudannoon dhukkubbii isin qunnamee beeka?						
Part V. Ulfaatina, Dheerina, Cigille fi Sa farttuu Dhiibabaa Dhiigaa							
0801	Dheerina	_____(Senti meter)					
0802	Ulfaatina	_____ (Kilo meter)					
0803	Dhiibbaa Dhiiga	_____(mmHg)					

❖ **Waan Nu gargaartanif Galatoomaa**

Guyyaa Gaafi fi deebi itti godhme :_____

Gaafii fi deebi kan gaggeesse

Maqaa :_____

Mallattoo :_____

Annex IX Protocol for determining reference intervals

1. Establish an appropriate list of biological variations and analytical interferences from medical and scientific literature
2. Establish selection (or exclusion) and partition criteria and an appropriate questionnaire designed to reveal these criteria in the potential reference individuals.
3. Execute an appropriate written consent form for participation in the reference interval study and have the reference individual complete the questionnaire.
4. Categorize the potential reference individuals based on the questionnaire findings and results of other appropriate health assessments.
5. Exclude individuals from the reference sample group based on the exclusion criteria or other assessments indicating a lack of good health.
6. Decide on an appropriate number of reference individuals in consideration of desired confidence limits.
7. Prepare, properly and consistently, the selected persons for specimen collection for the measurement of a given analyte consistent with the routine practice for patients.
8. Collect and handle the biological specimens properly and in a manner consistent with the routine practice for patient specimens.
9. Collect the reference values by analyzing the specimens according to the respective analytical methodology under well-defined conditions and consistent with the routine practice for patient specimens.
10. Inspect the reference value data and prepare a histogram to evaluate the distribution of data.
11. Identify possible data errors and/or outliers.
12. Analyze the reference values
13. Document all of the previously mentioned steps and procedures.

Annex X SOP for Blood Collection, Handling and Transportation

Purpose: A properly collected blood specimen is essential to reporting quality and accurate results in the laboratory

Principle: The EDTA process forms in insoluble calcium salt that prevents coagulation. EDTA is the most commonly used anticoagulant in hematology for tests such as the CBC

Materials Supplies

- EDTA: Lavender top
- Needle
- Syringe
- A tourniquet
- Alcohol prep pads
- Non-alcohol-based cleanser
- Gauze pads, adhesive bandages, or tape (including hypoallergenic adhesives)
- Gloves
- Sharps container
- personal protective equipment (lab coat)

Sample volume: 5ml

Sample retention: Samples will be discarded after 1day of collection.

Special Safety Precautions:

- Use standard precautions as outlined in the Blood borne Pathogen Plan.
- Place sharps container close to the collection site.
- Wear disposable gloves at all times during the procedure
- Wash your hands before you put on your gloves and after you remove your gloves.
- Change gloves between each study participants.

Procedure for whole blood collection:

Step 1: Prepare accession order: identify all paperwork and equipment

Step 2: Approach and identify the donor:

Step 3. Verify donor's diet restrictions and inquire if patient has a latex sensitivity.

Step 4: Assemble necessary supplies and select appropriate tubes according to test requests.

Step 5: Position the p.

Step 6: Apply the tourniquet and wrap the tourniquet around the arm 3 to 4 inches (7.5 to 10.0 cm) above the venipuncture site.

Step 7: Put on gloves

Step 8: Cleanse the venepuncture site.

Step 9: Venepuncture procedure, follow procedure is recommended:

a) Assemble the needle and syringe.
b) Hold the patient's arm firmly distal to the intended puncture site. The phlebotomist's thumb should be 1 or 2 inches (2.5 cm or 5.0 cm) below the venepuncture site.
c) Prepare the patient by informing him or her that the venepuncture is about to occur.
d) With the bevel up, puncture the vein with the needle at an angle of insertion of 30^{o}or less

Step 10: Use the correct order of draw.

Step 11: Release and remove the tourniquet.

Step 12: Place the gauze pad over the puncture site.

Step 13: Remove the needle, activating any safety feature

Step 14: Apply pressure to the site, making sure bleeding has stopped, and then make bandage the arm.

Step 15: Label the tubes and record the time of collection.

Step 16: Send properly labelled blood collection tubes to Dilchora hospital laboratory.

Sample Handling:

Whole blood specimens are kept covered (air tight) at all times to prevent possible exogenous contamination, evaporation, concentration changes, or possible spillage and aerosols.

- Labelling:
  - the participants first and last name;
  - a unique identification number which is similar to the assigned questionnaire number
  - the time and date of collection;
  - the initials of the person collecting the sample
- Temperature: at ambient temperature
- Sample Storage**:** at ambient temperature
- Sample retention: 24 hrs.
- Sample transportation: Specimens must be transported in the appropriate biohazard bags or containers to the laboratory in as short a time as possible.
- Sample transportation time: within two hours of after ambulatory collected whole blood.
- Sample tube orientation: tubes of blood should be kept in a vertical, closure-up position.

The package must be clearly labeled "DIAGNOSTIC SPECIMENS".

Annex XI SOP for Hematology analyser Mindray BC-3000Plus Operation

Introduction

The Mindray BC-3000 plus designed to automatically perform the following functions:

- Aspirate and dilute whole blood
- Count, size, and classify cell present in a whole blood specimen
- Analyze raw data collected
- Output result to display, printer, and on-line computer

Intended use

Evaluate anemia, leukemia, reaction to inflammation and infections, peripheral blood cellular characteristics, state of hydration and dehydration, polycythemia, hemolytic disease of the new born, inherited disorders of red cells, white cells, and platelets; manage chemotherapy decisions; determine qualitative and quantitative variations in white cell numbers and morphology, morphology of red cells and platelet evaluation.

Principle

Uses a volumetric metering unit to control the count cycle and to ensure that a precise volume of sample is analyzed. A test is performed using the two independent measurement method: -

1. The impedance method for determining WBC, RBC and Platelet data.
2. The colorimetric method for determining the Hemoglobin

Impedance method:

The measurement of changes in electrical resistance produced by a blood cell suspended by conductive diluents as it passes through an aperture of known dimension. An electrode is submerged in the liquid on both side of the aperture to create an electrical pathway. As each particle passes through the aperture, a transitory change in the resistance between the electrodes is produced. This change produces a measurable electrical pulse. The number of pulse generated indicates the number of blood cells that passes through the aperture. The amplitude of each pulse is proportional to the volume of each blood cell. Each pulse is amplified and compared to the internal reference voltage channel which only accepts the pulse of certain amplitude.

Colorimetric Method:

HGB is determined by the colorimetric method. The WBC/HGB dilution is delivered to the WBC bath where it is bubble mixed with a certain amount of lyses, which converts Hgb to Hgb complex that is measurable at 525 nm. An LED is mounted on one side of the bath and emits a beam of light, which passes through the sample and a 525nm filter, and then is measured by

a photo-sensor that is mounted on the opposite side. The signal is then amplified and the voltage is measured and compared to the blank reference reading (readings taken when there is only diluent in the bath). The HGB is expressed in g/L.

Clinical Significance:

- To asses hematological disorder.

Sample:

- Type: whole blood
- Amount: 2.5ml-3.5ml

Interpretation of test Result

- To investigate anemia, polycythemia, hemoglobinopathis, infections, bleeding disorders and blood cell disorders (leukemia's)

Performance Characteristics

Parameter	Linearity range	Precision (CV%)
WBC	0.0 - 99.9(*10^3/ul)	2.5%(7.0-15.0xl09/L)
RBC	0.00 - 8.00(*10^6/ul)	2%(3.5-6.0xl012/L)
HGB	0 - 300(g/L)	1.5% (110-180g/L)
MCV	40.0-150.0	0.5% (80.0-110.OfL)
PLT	10-999 (*10^3/ul)	5% (200-500xl09/L)

Quality control

The analyzer has 4 QC programs: L-J Analysis, $\bar{X}$ Analysis, $\bar{X}$-R Analysis and X-B Analysis. Normal background: WBC≤ 0.3*103/ul; RBC≤ 0.03*106/ul; HGB ≤ 1mg/dl; HCT≤ 0.5 %; PLT≤ 10*103/ul.

Abnormal QC Results

- Check the upper left corner of the screen for error messages.
- Check the L-J settings for inappropriate entries;
- Do the background check
- Re-run the control;
- Run another vial of control;
- Check if the analyzer needs to be calibrated.

Maintenance: Routine preventive maintenance and cleaning are required to keep the analyzer in good operating condition. The analyzer has automatic cleaning functions that are performed during normal operation. These built-in functions keep the fluidic system clean.

Annex XII Inter precision of BC-3000Plus Hematology analyzer

Analyte	Manufacturer's Precision	Observed Results (Between Day CV %)	Acceptability
WBC	2.5%	0.98%	Acceptable
RBC 10^{12}/L	2%	0.74	Acceptable
Hemoglobin (g/dL)	1.5%	1.01	Acceptable
MCV (fL)	0.5%	0.31	Acceptable
Platelets (K/uL)	5%	2.7	Acceptable
Between day precision (CV)			
No comparable value obtained from manufacturer			
Absolute Lymphocytes		2.16	
Absolute Granulocytes		3.08	
Asolute MID		2.3	
Neutrophil %		2.9	
Lymphocyte %		1.68	
MID %		4.45	
Hematocrit %		0.65	
MCH (pg)		0.97	
MCHC (g/dL)		1.11	
RDW %		1.72	
RDW-CV		1.58	
MPV (fL)		1.5	
PCT		0.56	

Declaration

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

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Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **Dr. Aster Tsegaye (MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **Melatwork Tibebu (MSc, PhD candidate)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.