ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

ESTABLISHMENT OF HEMATOLOGICAL REFERENCE INTERVALS FOR APPARENTLY HEALTHY ADULT RESIDENTS IN ASELLA TOWN, SOUTH EAST, ETHIOPIA.

BY: SOLOMON TADESSE (BSc in MLS)

ADVISORS: ASTER TSEGAYE (MSc, PhD)

: JEMAL ALEMU (MSc, PhD candidate)

A 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 SCIENCES (HEMATOLOGY and IMMUNOHEMATOLOGY).

June, 2019

Addis Ababa, Ethiopia

Addis Ababa University

School of Graduate Studies

This is to certify that the thesis prepared by Solomon Tadesse, entitled: **Establishment of hematological reference intervals for apparently healthy adult residents in Asella town, southeast Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

Signed by the Examining Committee:

Examiner_____ __ __ Signature _____Date _____

Examiner_____ Signature _____ Date _____

Advisor_____ __ ____Signature _____ Date _____

Advisor_____ __ _____Signature _____Date _____

_____

Chairman of the Department or Graduate Program Coordinator

ACKNOWLEDGEMENT

I would like to express my deep gratitude to my Advisors Dr. Aster Tsegaye, Mr. Jemal Alemu (PhD candidate), Addis Ababa University, My Department, Arsi University Asella Referral and Teaching Hospital and especially the laboratory staff and also study participants for all to the support to undertake the study and develop this thesis.

Lastly but not least I would like to thank my lovely family (especially my **Son).** I really surprised when three years baby understand my feeling and say ok when I need silence.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS

AAU	Addis Ababa University
AUARTHL	Arsi University Asella referral and teaching hospital laboratory
CI	Confidence interval
CLSI	Clinical Laboratory Standards Institute
EDTA	Ethylene diamine tetra acetic acid
HCT	Hematocrit
HGB	Hemoglobin
IFCC	International federation for Clinical Chemistry
ISO	International organization for Standardization
LYM	Lymphocytes
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
MLS	Medical Laboratory Sciences
MPV	Mean platelet volume
MXD	Mixed (monocytes, eosinophils and basophils)
NEUT	Neutrophils
PDW	Platelet distribution width
PLCR	Platelet large cell ratio
PLT	Platelets
RBC	Red blood cells
RDW CV	RBC distribution width coefficient of variation
RDW SD	RBC distribution width standard deviation
RI	Reference Interval
STI	Sexually transmitted infection
WBC	White blood cells

ABSTRACT

Background: About 70% of medical decisions made by physicians are based on the information presented by laboratory results. However, test results by themselves are valueless unless reported with the appropriate reference interval or medical decision limit. Currently, Ethiopia use reference intervals adopted from textbooks that refer mainly to Caucasian subjects. The country having heterogeneous population, there is a need to establish locally derived hematological reference interval that could be used in Asella town, Arsi zone, Ethiopia.

Objective: To establish hematological reference intervals for apparently healthy adults in Asella town, southeast, Ethiopia.

Methods: A cross sectional study was conducted from January to March 2019 on apparently healthy individuals in Asella town aged from 18-60 years. Predesigned and structured questionnaire was used for collection of data on socio-demographic characteristics and dietary pattern of the reference population. Systematic random sampling technique was used**.** About 3ml of EDTA whole blood was collected and analyzed using Sysmex KX 21N automated hematology analyzer which analyses 60 tests per hour. The data was entered and analyzed by appropriate statistical software (Epi Info and SPSS) and interpreted using non-parametric methods, by which central 95% of the measured values was included in the intervals.

Result: a total of 494 participants were recruited and 424 participants were involved in this study with the median age of 28 years. Except WBC (3.4 to 10.1 $x10^9$/L) which showed no significant difference, other men and non-pregnant women reference intervals are: RBC (4.77 to 6.07 $x10^{12}$/L; 4.18 to 5.29 $x10^{12}$/L), HGB (14.7 to 18.1 gm/dL; 12.7 to 15.7gm/dL), HCT (42.1 to 51.3%; 37.1 to 44.4%) and PLT (159 to 336 $x10^9$/L; 177 to 376 $x10^9$/L), respectively. Pregnant women's WBC, RBC, HGB and PLT are (4.9 to 13.2 $x10^9$/L, 3.58 to 4.9 $x10^{12}$/L, 11.0 to 14.6gm/dL and (138 to 368 $x10^9$/L), respectively.

Conclusion: most of the hematological RI of this study was significantly different from currently in use in Asella referral and teaching hospital laboratory. The difference was also observed in studies of other African countries as well as studies from different parts of Ethiopia.

Key words: hematological RI, Asella, men, non-pregnant women, pregnant women.

1. INTRODUCTION

1.1. Background

About 70% of medical decisions made by physicians are based on the information presented by laboratory results (1, 2). Laboratory test result by itself is valueless unless it is reported with the appropriate information for its interpretation. Usually, this information is presented in the form of reference interval or medical decision limit. A reference interval is defined by Ceriotti "as an interval that, when applied to the population serviced by the laboratory correctly includes most of the subjects with characteristics similar to the reference group and excludes the others"(3).

International organizations like International Federation of Clinical Chemistry and laboratory medicine (IFCC), recommend that all clinical laboratories should establish their own biological reference intervals(4). Furthermore, the ISO 15189:2012 requires that biological reference intervals should be reviewed periodically and whenever there is any change in laboratory technology. But currently, only few laboratories follow these recommendations because of its difficulty, large time consumption and expensiveness(5).

The philosophy of IFCC, later used by the Clinical Laboratory Standard Institute (CLSI) and manufacturers, proposed the near exclusive use of Reference Limits on laboratory reports as a guide for interpretation. Its principal advantage is, it clearly differentiates Reference Limits (or Intervals), as purely descriptive of a well-defined population (closest to the patient), from Decision Limits. Because ''they are based on other scientific and medical knowledge and they may be related to a specific medical condition'', decision Limits are different as stated by the IFCC and the CLSI. On the other hand, Reference Intervals are calculated by different statistical methods (parametric or non-parametric) from a reference sample defined as an adequate number of persons selected for testing on the basis of well-defined criteria (exclusion and partition) and of an appropriate questionnaire(6).

1.1.1. Reference Interval determination approaches

For the first time, Harris and Boyd(7) refer Wootton *et al.* (8) (who in 1951 applied parametric statistics) approach to the calculation of the reference intervals. But this statistical model was only applicable in a minority of situations as later realized by the authors and after two years, they

proposed logarithmic transformation of data to achieve a Gaussian-like distribution (9). Unfortunately, the incorrect practice of defining the reference interval as the mean ±2SDs, without any prelude verification of the shape of the distribution of the data has continued for many years and is still sometimes used.

In 1987 IFCC documents(4) represent a milestone paper after several publications on the use of fractiles for the definition of reference intervals appeared in the 1970s and 1980s (10-13). This document clearly defined that: first, it promoted the random choice of using the central 95% of the distribution for the reference interval calculation. Even though few different opinions, such as Jorgensen *et al*(14) who proposed widening the interval to include 99.8% of the observed data to reduce false-positives in cases where a large battery of tests is requested, this approach is broadly acknowledged till now. Secondly, the IFCC document recommended that reference limits should always be presented together with their 90% CIs. As the number of evaluated subjects increase the width of the CIs decreases and represents a reliable indicator of the uncertainty of the reference limits. This document also recommends the use of a nonparametric statistical method to calculate the reference limits. Even though parametric methods are theoretically more reliable, particularly if the sample of subjects is small, the uncertainty on the real 'Gaussianity' of the original data (or after its transformation) increases the uncertainty of the final reference limits(4).

Based on the knowledge of the biology of the analyte and the available resources there are two ways by which decision is made regarding the choice of reference selection criterion, which is recommended in the IFCC document (15). First, a priori selection criterion (decide in advance which individuals to select and how to partition them) and secondly, a posteriori selection criterion (to collect a relevant number of subjects, analyses them and decide thereafter which are to be kept in the reference population and how to partition them). However, this document does not explicitly exclude the possibility of utilizing the existing data, primarily not collected for the scope of defining reference intervals, according to Martin *et al* (16).

An indirect reference value is another way of selection criterion. This approach is based on the use of existing databases containing thousands or even millions of patients' records which several authors currently work on(17). If appropriate software is used this approach is less costly and less time consuming procedure. The indirect method differs from posteriori approach by that the

database not contains detailed clinical information. In 1960s, the first papers using this criterion were published and based on the postulate that most of the laboratory test results are 'normal' (18, 19).

Because of several relevant limitations that may have a negative influence, care has to be taken in using the indirect method for reference interval determination. These limitations include in the first place it does not fulfill the fundamental principle of the theory of reference values, which means the characteristic of the reference population is not defined or the subjects under study are not well known (20). Even though the applied statistical calculation is based on a presumed distribution of the results of the studied population, in some cases the assumed distribution of data may not be correct as in the case of skewed distribution (21). Secondly, almost all pre-analytical variables are not controlled and lastly because of its difficulty to provide any demonstration of the metrological traceability of the obtained results, the calculated intervals cannot be adopted by other laboratories (22).

As an alternative, there are other statistical methods used to perform reference intervals of individuals beside their drawback. These are the robust method (proposed by Horn in 1998 for small sample size), bootstrap method and regression analysis method (23) (for small sample size).

Generally, even though information technology provides a powerful means of calculation, the indirect approach cannot be endorsed as the best way of defining reference intervals. For the laboratory results to be able to provide traceable and reliable clinical data the original data should be obtained with carefully controlled methodology. Mostly, this approach only represent a means to confirm and validate the findings obtained with the more scientifically sounds a priori selection.

Harris and Boyd (7, 24) proposed the most popular partitioning criterion which is later endorsed by the CLSI document C28-A2 (15). They first considered the idea that inter-individual variability should be reduced by partitioning to subgroups as compared with that of the entire data group. But they found that reducing inter-individual variability was hardly achieved, even with large differences between means of subgroups. So, they focus on proportions of the subgroups outside the 95% reference limits of the entire population. The concern remaining was whether a single pair of limits, derived from a combined sample of subpopulations, come close enough to satisfying this criterion of 2.5% below and 2.5% above for each subpopulation (24).

They also proposed an alternative acceptability limit that if the percentage is higher than 4% or lower than 1%, it is necessary to define different reference limits. It appears valid since this criterion considers both means and standard deviations of the subgroups, as a different standard deviation by itself may produce different reference limits. However, this approach will not work well other than Gaussian distributions and with subclasses of similar size and standard deviation (25). To overcome these limitations Lahti *et al*. (26) have proposed a method based on similar concepts, but allowing the estimation specifically of the percentage of subjects in a subclass outside the reference intervals of the entire population in any situation. Following the criteria based on biological variability, Gowans *et al*. (27) proposed the creation of a subclass when more than 4.1% or less than 0.9% of the subjects of the subgroup fall outside the limits of the entire group.

The presence of outliers can affect significantly the reference limits even with effective method of reference interval calculation. Very simple but effective method to detect outliers is visual inspection of the distribution of the data (28). Dixon(29) applies the most popular statistical methods to justify exclusion of outliers which is based on the D/R ratio, where D is the absolute value of the difference between the outlier and the next or preceding value and R represents the entire range of the observations (maximum–minimum), outlier included. According to Reed *et al.* (10) the CLSI C28-A2 document proposes one-third as the limit for the above ratio. However, this method is not very sensitive; particularly when there are more than one outlier, the presence of a less extreme outlier may mask the other(s).

More sophisticated two-step algorithm proposed by Horn *et al.* (30) that the data are first transformed using the Box and Cox method(31), to obtain a Gaussian distribution, then the outlier identified using the Tukey robust approach(32). The central 50% distribution method identifies the extremes by eliminating the confounding effects of more outliers. It then involves the computation of lower and upper quartiles (25th and 75th percentiles) of the transformed data (Q1 and Q3) from which the interquartile range (IQR) (Q3–Q1) is calculated. Lastly, the lower and upper 'fences' are calculated in such a way that the lower fence as Q1 – 1.5 * IQR and the upper fence as Q3 + 1.5* IQR. Any data point outside the fences is considered as an outlier and discarded.

The values represent a specific population and thus they rely upon the choice of the subjects on which they were obtained is the basic concept. First of all, the criteria for the selection of reference

individuals have to be clearly defined; those individuals represent the reference population from which a reference sample group is selected, on which the reference values are measured. The obtained values will assume a certain distribution (reference distribution) and, by analyzing it with appropriate statistical methods, reference limits can be calculated. The central 95% of the measured values are included in the limits. The reference limits define reference interval and reference interval includes reference limits(6).

Because of many different factors that affect reference interval it is not possible to have common hematological values for all countries. These factors include age, sex, geographic origin, altitude, and ethnic background in addition to hemoglobin abnormalities (thalassemia, sickle cell disease and hemoglobin C) or pathologic conditions (malaria, HIV and other viral infections)(33). Because of the positive effect of androgens and inhibitory effect of estrogens on erythropoietin and hence on erythropoiesis, males have significantly higher RBC parameters than females. In addition, menstruation also contribute for reduction of RBC parameters. As we go to high altitude the oxygen pressure decreases which result in tissue hypoxia. As a result the kidney produces erythropoietin and end up with physiologic increase in RBC to compensate for the adjustment of high altitude. Therefore, it is recommended to use hematological reference interval derived locally for patient management in routine clinical care(34) (35).

The concentrations of hematological analytes can also be affected by other factors like chronic disease (hypertension, diabetics, gastritis, TB, allergy and others), infections (HBV, HCV, Syphilis and others), parasites and others including obesity and history of surgical procedures. For example, many chronic disease can induce the production of pro-inflammatory cytokines (IL-1, IL-6 and TNF-α) and these cytokines enhance hepatocytes to produce acute phase protein like hepcidin which bind ferroportin and accelerates its degradation and inhibits iron absorption and export from macrophages. Moreover, cytokines like interferons (IFN-α, IFN-β and IFN-γ) are produced against viral infection which inhibits erythropoietin production and erythropoiesis (8). Pregnancy causes physiological iron and folate deficiency which leads to physiologic anemia of pregnant women. Obesity can make gastric plication which then causes cobalamin malabsorption in addition to leading to venous thrombosis and tissue hypoxia. Helminthes also affect blood cell count (especially eosinophils and lymphocytes (adaptive immunity-IgE)) and should be excluded from reference interval determination (8) (35-37).

Even though samples collected for reference interval studies must be collected under conditions representative of those used in routine laboratory tests, the practice of the pre-analytical phase is usually poorly standardized. Because of this, the pre-analytical conditions should be accurately defined and described when performing a reference interval study to allow others to reproduce the same situation and to understand the effects of certain factors (e.g. the collection device or the posture of the individual at the time of collection)(21).

The analytical aspect on reference intervals is also neglected in many publications. The IFCC document dealing specifically with this topic gave a series of recommendations for documenting the operating procedures, focusing on how internal quality control should be practiced during production and application of reference values. These IFCC recommendations were not very effective in providing procedures which define reference intervals that are 'transferable' to different laboratories, other than the laboratory which defined them. This is because, it allows the baseline conditions to be properly fixed and then to understand whether the modification of certain analytical aspects may change the reference intervals(38).

1.2. Statement of the Problem

Most African countries including Ethiopia use text book reference intervals generated from Caucasian adult populations living in developed countries for analysis of hematological parameters during routine clinical activities(34). However, many differences are reported in African population compared to similar population in Western counterparts in values of hematological parameters. Even though it was not practiced in many developing countries, there are plenty of evidences that clinicians and medical researchers should use method-specific reference ranges in their laboratories which account for gender differences and ethnic variances of the local society (39).

There is an increasing number of clinical trials taking place in Africa which are seeking to identify safe and effective prevention and treatment strategies to combat the heavy burden of infectious diseases in this region. These trials are facing challenges since there is limitation of well-established reference intervals. It is important to establish local hematological reference intervals for appropriate diagnosis, treatment, and prognosis of patients because in the absence of locally derived reference intervals, clinicians and researchers forced to use reference values of western population's(40).

Currently, reference intervals adopted from textbooks that refer mainly to Caucasian subjects are in use in Ethiopia. Some studies conducted in different parts of Ethiopia showed lower RBC (lower limit), WBC and platelet values of healthy Ethiopians than the adopted reference values (41, 42). In addition, in pregnant women Physiologic anemia, thrombocytopenia, and neutrophilia are common hematological changes reported during normal pregnancy. Many African countries, including Ethiopia, lack reference intervals specific to pregnant women and use reference intervals derived from the Western population for routine clinical practices (43, 44). Pregnant women have higher WBC and lower RBC and PLT count than non-pregnant women (15). This is why this study is going to establish hematological reference interval for normal pregnant women.

Generally, there are many factors that affect hematological reference intervals including age, sex, altitudes, ethnicity, nutritional status, genetics, race, pregnancy, intense exercise, blood donation and other socio-demographic variables (18, 34). Thus, the main aim of this study was to establish locally derived hematological reference values that could be used in Arsi zone of Ethiopia.

1.3. Significance of the Study

Asella town gets its own RIs by which our physicians confidently decide on delivered results from laboratory and interpret accordingly. People of Asella town (and even Ethiopia) have been treated by reference intervals established elsewhere which may differ by many factors (especially. altitude and race). Therefore, our community will get the representative RIs for the right medical decision-making process. Moreover, unnecessary "High" "Low "flags based on company derived values will be avoided, which will reduce avoidable smear reviews as well as workload to the laboratory professionals.

This study also benefits researchers, policy makers and other stakeholders by serving as a baseline paper in the study area for further studies give fruitful results in policy making process and others stake holders like public and private health facilities will be going to use this RIs for better interpretation of laboratory results.

2. LITERATURE REVIEW

2.1. Reference Interval concept

Grasbeck and Saris introduced the concept of reference values in 1969 to describe fluctuations of blood analyte concentrations in well-characterized groups of individuals(45). It was intended to replace the more ambiguous concept of normal values (46, 47) and to ‘‘establish a well-defined nomenclature and recommended procedures in the field” (45). In this first publication, there was a clear distinction between healthy reference values measured in healthy populations or individuals and patient reference values measured in patients having various diseases. It is now commonly accepted that reference values describe fluctuations observed in healthy populations or individuals, which makes the definition of health or characterization of health status a critical step(47).

The first introduced philosophy of Reference values have gained universal acceptance as one of the most powerful tools in laboratory medicine to aid in the clinical decision-making process (6, 47, 48). However, the recommendations for establishing RIs described in the original series of articles published by the IFCC and Laboratory Medicine were sometimes considered too complicated to be applicable in practice; and thus, they have been used erroneously, if used at all (4, 38, 49-52).

The difficulties seen in these original recommendations led to a necessary revision (53, 54) and the publication of common IFCC and CLSI guidelines (C28-A3) in 2008 (6). Previous recommendations were reinforced in the latter document, which were to establish RIs with at least 120 observations using the nonparametric ranking method. However, it is also acknowledged that RI determination is difficult, time-consuming, and costly, and therefore, ‘‘it is ideal to expect each laboratory to develop its own RIs.’’ The new document now allows individual laboratories to adopt, by transference and verification, RIs established elsewhere. In addition, alternate statistical approaches, such as the robust method, make it possible to establish RIs using smaller reference sample sizes; even though, ‘‘the working group is hesitant to recommend that it be done (with fewer than 80 observations), except in the most extreme instances” (6).

2.2. Selection of Reference population

Selection of the reference individuals represents the starting point. Usually, healthy subjects are selected, but the question remains by what it means and how health is defined. According to World

Health Organization, health is defined as 'a state of complete physical, mental and social wellbeing and not merely absence of disease or infirmity' (55) cannot be a realistic starting point. In contrast, the concept of health can be different in different cultures and countries. In 1975, the Scandinavian Committee on Reference Values tried to define a list of pathological conditions to be excluded to consider an individual 'healthy' (56). However, it was impractical (57), while they tried to apply this recommendation especially if aged subjects were involved. When Horn and Pesce (54) looked at the third National Health and Nutrition Examination Survey, they found that no more than 10% of the subjects aged 70–80 fell into the 'healthiest' category.

Therefore, a more pragmatic approach is needed and health should be judged subjectively as the absence of signs of disease specifically related to the measurand(s) (58). As clearly stated in the IFCC document (49), the first step should be the definition of the scope for use of reference intervals and, secondly the definition of the method used to select the reference individuals. When selecting individuals, it is necessary to take into account all variables that can affect the concentration of the analyte: including gender, age, environment, lifestyle, and ethnicity. In the example of hemoglobin, in addition to gender and age, stratification according to the altitude of living and smoking habits is also important. All these biological aspects can be used as partitioning criteria.

2.3. Reference Intervals: Global, Regional and Local experiences

The reference interval done in different parts of Africa shows better similarities with each other than with that derived from Europe and USA. However, there were still discrepancies between African studies (59, 60). For instance, reference interval established in Tanzania, Uganda and Ethiopia shows better similarity of WBC value (3.0-7.9 $x10^9$/L, 3.4-8.7 $x10^9$/L and 3.0-9.8 $x10^9$/L, respectively) than RI listed in wintrobe's (4.4-11.3 $x10^9$/L). In addition, the female lower RBC limits of Tanzania (3.84 $x10^{12}$/L), Uganda (3.70 $x10^{12}$/L) and Ethiopia (3.70 $x10^{12}$/L) shows better similarity than Caucasians (4.5 $x10^{12}$/L) (41, 61-62).

Similarly, the male lower and upper limits PLT (150-450 $x10^9$/L) of Caucasians are higher than that of Tanzania (147-356 $x10^9$/L), Uganda (80-288 $x10^9$/L) and Ethiopia (98-324 $x10^9$/L) (41, 61-62). Study conducted in Nigeria (3.6-9.6 $x10^9$/L) shows that the lower and upper limits WBC of pregnant women different from study conducted in Ethiopia (4.0-14.1 $x10^9$/L). And this studies

show that WBC (4.0-14.1 $x10^9$/L; 3.2-8.8 $x10^9$/L), RBC (3.39-5.35 $x10^{12}$/L; 3.53-6.25 $x10^{12}$/L) and PLT (95-367 $x10^9$/L; 128-432 $x10^9$/L) of pregnant women significantly different from non-pregnant women respectively (42, 44).

Due to the influence of androgen (stimulatory effect) and estrogen (inhibitory effect) on erythropoiesis and menstrual blood loss, significant gender differences in red blood parameters (RBC, hemoglobin, hematocrit and mean cell volume), were seen with males having higher values than females (59, 60). Hematological reference intervals conducted in Mozambique showed lower than those derived from the United State population, which is consistent with studies of similar age groups conducted in western Kenya and Uganda. This study also shows that the lower limits of many hematological parameters were lower than those derived from those two African countries (61-63).

Similarly studies conducted in different parts of Ethiopia shows statistically significant difference between genders due to the facts mentioned above. And also there were discrepancies between hematological reference intervals among different African countries and even among Ethiopians living in different location (42, 64-67). In pregnant women hematological values were varied from first trimester to third trimester. Different studies indicated that the results of pregnant women RBC indices are not the same as compared to non-pregnant women (43, 44, 68).

Generally the above reviewed literatures indicated that there were remarkable differences of hematological reference intervals between age groups, gender, pregnancy, altitude, ethnic groups, race, nutritional status and other socio-demographic variables.

3. OBJECTIVES

3.1. General Objective

To establish hematological reference intervals for apparently healthy adult residents in Asella town southeast, Ethiopia.

3.2. Specific Objectives

- To determine sex specific hematological reference intervals for apparently healthy adults aged from 18 to 60 years in Asella town, southeast, Ethiopia
- To establish hematological reference intervals for apparently healthy pregnant women in Asella town, southeast, Ethiopia

4. HYPOTHESIS

There is no significant difference between hematological reference intervals of apparently healthy Asella town residents and reference intervals currently used in Asella town laboratories which is adopted from text book mainly of Caucasian population.

5. MATERIALS AND METHODS

5.1. Study area

The study was conducted at Asella town, which is located in the Arsi zone of Oromia regional state about 175km southeast of the capital city Addis Ababa. This town has a latitude and longitude of 7°57′N 39°7′E, with an elevation of 2,430 meters above sea level. According to the 2007 Ethiopian census report and based on annual population growth rate, Asella has an estimated total population of 101,739 and almost half of them 51,159(50.3%) are males. The majority of the inhabitants (67.43%) are followers of Ethiopian Orthodox Christianity, while 22.65% of the populations were Muslim, and 8.75% of the populations were Protestant. In 2010 E.C, Asella town's population estimate was 111,433 and about 23,215 households under 8 kebeles. (69)

5.2. Study design and Period

A cross sectional study was conducted from January to March 2019 in Asella town.

5.3. Population

5.3.1. Source population

Adult population of Asella town

5.3.2. Study population

Selected apparently healthy individuals who live in the randomly chosen kebeles and households in Asella town aged between 18 to 60 years and fulfill the eligibility criteria.

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion criteria

Those apparently healthy individuals aged between 18 to 60 years and who volunteer to participate in the study.

5.4.2. Exclusion criteria

The exclusion criteria were, infections (including HBsAg, HCV and Syphilis), chronic diseases including hypertension, diabetics, gastritis, TB), intestinal parasites, lactating mothers, women on menstruation, blood donors less than three months and others like obesity and history of surgical procedures.

5.5. Study Variables

5.5.1. Dependent variables

- Hematological reference interval

5.5.2. Independent variables

- Age, Sex, Altitude, BMI
- Nutritional factors
- Other Socio-demographic variables

5.6. Measurement and Data collection

5.6.1. Sample size determination

The CLSI document recommendation was used to calculate sample size. This document put minimum sample size of 120 for reference interval calculation by using 90% confidence interval (6). But this study enrolled 182 individuals in each partition because of 34% prevalence of various diseases including common viral infection and syphilis and other factors that lead to participant exclusion (41). There were 3 partitions: adult Male, adult non-pregnant women, and pregnant women. Thus, a total of 494 individuals were involved in this study.

N*(100%) = 120 → N*(100%-34%) = 120 → N*(66%) = 120 → N = 120/0.66= **182**

5.6.2. Sampling method

Systematic random sampling technique was used to efficiently represent reference population**.** The 3 kebeles were selected from 8 kebeles and total population of selected kebeles were divided by sample size. K was obtained by dividing the total households for sample size. The number between 1 to K^{th} was randomly selected and continue sampling by $(B+K)^{th}$ houses on the road. Where B was randomly selected number of household and starting point.

K = 8994/546 **= 17**

Therefore, the sample size was distributed to kebeles (from Chilalo 2811 households and 170 participants, Hunde guddina 3572 households and 217 participants and Welkesa 2611 households and 159 participants) total population by probability proportionate to size.

5.6.3. Data collection procedure

Predesigned and structured questionnaire was used for data collection on socio-demographic characteristics (age, sex and pregnancy) and dietary information of the reference population.

5.6.3.1. *Sample collection and analysis*

Blood sample of about 13 ml was collected in EDTA vacutainer and plane tubes using multisampling needle. To minimize diurnal variation of some analytes blood samples were collected before 11:00 am. Whole blood was used for hematological analysis and hemo-parasites identification while serum samples for clinical chemistry profiles as well as for screening HIV, HBV, HCV, and syphilis. Stool sample was collected for parasitological analysis and urine for Urinalysis and determining pregnancy status. Leak proof clean containers were used to collect urine and stool samples. All samples were labeled with unique identification number (AS-001 to 494)

5.6.4. Principles of each Laboratory analysis, procedures and interpretation

5.6.4.1. *DC Detection Principle*

Blood sample is aspirated, measured to a predetermined volume, diluted at the specified ratio, and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both side of the aperture, there are the electrodes between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to change between the electrodes. As direct current resistance changes, the blood cell size is detected as electric pulses. Blood cell count is calculated by counting the pulses, and a histogram of blood cell sizes is plotted by determining the pulse sizes. Also, analyzing a histogram makes it possible to obtain various analysis data.

5.6.4.2. *Non-Cyanide Hemoglobin Analysis*

Non-cyanide hemoglobin analysis method rapidly converts blood hemoglobin to Oxyhemoglobin and contains no poisonous substance, making it suitable for automated method. Being capable of analyzing met-hemoglobin, this method can accurately analyze control blood, etc. which contain methemoglobin.

5.6.4.4. Interpretation of the results

The result was interpreted according to CLSI recommendation by which central 95% was the reference interval.

5.7. Data Quality Assurance

5.7.1. Pre-analytic phases

Table 1 below shows the most important pre-analytical conditions that were considered while blood analyte is evaluated.

Table 1: - Main pre-analytical factors to be considered in the production of reference values

Subject preparation	Methodological factors	
	Specimen collection	Specimen handling
Fasting vs. non-fasting	Time of day	Transportation
Drug regimen	With or without tourniquet	Time before centrifugation
Physical activity	Body posture during phlebotomy	Centrifugation time and Speed
	Type of anticoagulant	Storage conditions before measurement
	Sampling equipment	
	Freedom from hemolysis	
	Sample volume	

5.7.2. Analytic phases (Quality control)

5.7.2.1. Precision testing or test verification

Reproducibility testing at the reliability level of 95% of week one of all ten runs was within company precision limits. For instance WBC, RBC, HGB and PLT repeatability test were 1.6% (<=3.5%), 0.5% (<=2.0%), 0.3% (<=1.5%) and 5.8% (<=6.0%), respectively for current study and company's limit. Reproducibility testing at the reliability level of 95% of week three of all ten runs was within company precision limits. For instance WBC, RBC, HGB and PLT repeatability test are 0.64% (<=3.5%), 1.1% (<=2.0%), 0.3% (<=1.5%) and 2.8% (<=6.0%), respectively (70) (Table 2).

Table 2: Precision testing of three weeks

Parameters	Week one				Week two				Week three			
	Mean	SD	Precision	Company precision	Mean	SD	Precision	Company precision	Mean	SD	Precision	Company precision
WBC	5.44	0.09	1.64	<=3.5%	6.72	0.13	1.94	<=3.5%	13.08	0.08	0.64	<=3.5%
RBC	5.48	0.03	0.54	<=2.0%	4.56	0.04	0.81	<=2.0%	5.29	0.06	1.12	<=2.0%
HGB	16.66	0.05	0.33	<=1.5%	14.04	0.05	0.39	<=1.5%	16.44	0.05	0.33	<=1.5%
HCT	46.34	0.25	0.54	<=2.0%	40.7	0.37	0.90	<=2.0%	46.14	0.48	1.03	<=2.0%
MCV	84.52	0.08	0.10	<=2.0%	89.22	0.23	0.26	<=2.0%	87.22	0.19	0.22	<=2.0%
MCH	30.4	0.10	0.33	<=2.0%	30.8	0.27	0.89	<=2.0%	31.08	0.40	1.27	<=2.0%
MCHC	35.98	0.13	0.36	<=2.0%	34.52	0.32	0.93	<=2.0%	35.62	0.45	1.26	<=2.0%
PLT	192.4	11.08	5.76	<=6.0%	238.6	8.47	3.55	<=6.0%	245.2	6.80	2.77	<=6.0%
LYM#	1.98	0.04	2.26	<=15.0%	2.74	0.05	2.00	<=15.0%	1.7	0.07	4.16	<=15.0%
MXD#	0.38	0.04	11.77	<=30.0%	0.7	0.07	10.10	<=30.0%	1.16	0.15	13.1	<=30.0%
NEUT#	3.08	0.04	1.45	<=15.0%	3.28	0.13	3.98	<=15.0%	10.24	0.15	1.48	<=15.0%
LYM%	36.44	0.57	1.56	<=15.0%	40.98	1.27	3.09	<=15.0%	13.14	0.52	3.94	<=15.0%
MXD%	6.88	0.44	6.45	<=30.0%	10.18	0.80	7.84	<=30.0%	8.98	1.14	12.7	<=30.0%
NEUT%	56.68	0.98	1.72	<=15.0%	48.84	1.24	2.54	<=15.0%	77.88	1.19	1.53	<=15.0%
RDW-SD	40.66	0.73	1.80	<=4.0%	45.66	0.71	1.56	<=4.0%	42.32	0.34	0.79	<=4.0%
RDW-CV	12.82	0.08	0.65	<=4.0%	13.2	0.07	0.54	<=4.0%	12.66	0.11	0.9	<=4.0%
PDW	12.68	0.58	4.54	<=12.0%	12.24	0.38	3.14	<=12.0%	11.56	0.68	5.85	<=12.0%
MPV	10.14	0.15	1.50	<=5.0%	10.5	0.22	2.13	<=5.0%	9.5	0.12	1.29	<=5.0%
P-LCR	26.74	1.13	4.22	<=20.0%	28.52	1.44	5.03	<=20.0%	21.36	1.08	5.04	<=20.0%

5.7.2.1.Inter-laboratory quality assessment

5.7.2.1.1. One world accuracy performance result

Arsi University Asella Referral and Teaching Hospital (AUARTH) Laboratory is participating on EQA on a quarterly basis from One world accuracy through the Ethiopian Public Health Institute (EPHI). The three consecutive (Apr 2018, July 2018 and Oct 2018) participation of inter laboratory quality assessment result showed 100% performance rate for WBC, RBC, HGB and PLT and 87% for HCT value. Therefore, these indicates that performance of AUARTH laboratory Sysmex KX 21N CBC analyzer was in acceptable limits for WBC, RBC, HGB, PLT and HCT. The performance rate of each tests should be equal or greater than 80% to be acceptable (71).

5.7.3. Post Analytic phases

- Reference result interpretation
- Archiving results
- Specimen retention

5.8. Data analysis and interpretation

The data was entered and analyzed by appropriate statistical software (Epi Info™ 7 and SPSS 23) and interpreted by using median, mean and central 95%. Mann-Whitney U test and Analysis of Variable (ANOVA) also used as a generation of P-value ($P<0.05$ indicate significance) to compare median and mean of groups, respectively. Age and sex specific reference interval was determined using a non-parametric procedure as recommended by CLSI. The upper and lower reference limits covering 95% of the reference value of each parameter was determined. Distribution of clinical laboratory parameters was checked and the non-parametric procedure was used.

5.9. Ethical considerations

The study protocol was reviewed by the Departmental Research and Ethics committee of Department of Medical Laboratory Sciences of College of Health Science, Addis Ababa University. Support letter was written to Arsi University and then each administrative unit hierarchically wrote support letter to each level up to kebeles. The aim of the study was explained to all study participants. Questionnaire to collect socio-demographic data, Information sheet, consent form was translated into local languages. The participants were informed of the minor discomfort associated with phlebotomy, their benefits and rights to withdraw from the study

anytime. Data confidentiality was maintained through pass word protection for electronic data and limiting access of hard copies by keeping them in locked cabinet. Codes were used for all biological samples. Questionnaires containing names were kept locked for linking results for those study participants who need their result. HIV testing was performed following national guidelines but none were positive. Participants with positive laboratory findings for tests other than HIV were linked to the respective health facilities. Participants got all their laboratory results for free.

5.10. Dissemination of Result

The result will be disseminated to Arsi University, Asella town administration, Arsi Zone health office, Oromia regional health bureau, Addis Ababa University College of Health Sciences, Department of Medical Laboratory Sciences and Federal Ministry of Health. Data will also be communicated to the medical/scientific community through conferences, seminars. Manuscripts will be published on peer reviewed journals.

5.11. Operational Definitions

Adult is a matured person aged from 18 years up to 60 years.

Apparently Healthy is individuals who are without sign of disease.

Decision limits (cut-offs, cut-points, or consensus values) are thresholds used to classify patients into diseased vs. non-diseased states or to identify when medical action is advised, regardless of the reference limit.

Hematological parameters: include WBC, differential, RBC, HGB, HCT, MCV, MCH, MCHC, RDW, MPV, PCT, PDW, PLT and P-LCR.

Observed value is a value of an analyte obtained by observation or measurement of a test subject, which should be compared with reference values, a reference distribution, reference limit or reference interval

Parameter is a quantity that defines certain characteristics of a population (eg, the mean of a population) and does not vary among individuals.

Posteriori sampling, the process of exclusion and partitioning also takes place after sampling and analyte testing rather than before.

Priori sampling is a method that requires well-defined exclusion and partitioning criteria before the selection of the reference individuals.

Reference distribution is the distribution of reference values.

Reference individual is a person selected for testing on the basis of well-defined criteria.

Reference interval is defined by threshold values between which the test results of a specified percentage (usually 95%) of apparently healthy individuals would fall.

Reference population is a group consisting of all possible reference individuals.

Reference sample group is an adequate number of persons selected to represent the reference population.

Reference value is the value, or test result, obtained by the observation or measurement of a particular type of quantity on a reference individual.

References limits are the values derived from the reference distribution and are used for descriptive purposes.

Resident person who lives at particular place for at least 5 (five) years

6. Result

A total of 494 participants were involved in this study to establish hematological reference intervals for adult men, non-pregnant women and pregnant women of Asella town with 98% (486) response rate. Of these, 170 were adult men and 159 non-pregnant women while 157 were pregnant women. Figures 1, and Tables 3-5 showed socio demographic, dietary pattern, BMI distribution and history of common diseases of the participants.

6.1.Socio demographic characteristics

The median and mean age of study participants were 28 and 30.5 years respectively with minimum of 18 and maximum of 60 years. The participants' educational status was: about 297 (61%) of them were tertiary level, 87 (18%) secondary level, while 23 (5%) were illiterate. About 266 (55%) were government employee, 120 (25%) were house wife, 64 (13%) were students and 28 (6%) were private employee. Majority of study participants were married, 360 (74%) and Orthodox Christians, 259 (53%) (Table 3).

Table 3: Socio-demographic characteristics of study participants of Asella town, from January-March 2019 (N=486)

Variables	Categories	Frequency	Percentage (%)
Age	18-25	169	34.8
	26-35	210	43.2
	>=36	107	22.0
Sex	Female	316	65.0
	Male	170	35.0
Educational status	illiterate	23	4.7
	read and write	7	1.4
	primary level	71	14.6
	secondary	87	17.9
	tertiary level	297	61.1
Occupation	student	64	13.2
	house wife	120	24.7
	government employee	266	54.7
	private employee	28	5.8
	farmer	3	0.6
	other (labor)	4	0.8
Marital status	single	117	24.1
	married	360	74.1
	divorced	5	1.0
	widowed	2	0.4
Religion	Orthodox	253	52.1
	Muslim	166	34.2
	Protestant	59	12.1
	Catholic	1	0.2
	other (*Waqefeta*)	6	1.2

6.2.Dietary pattern of study participants

As mentioned in table 4, the major of dietary pattern of the study participants were Cereals (84%), Tea and Coffee (77%) and Legumes (72%) which consumed more than once per day. Roots and Tubes (66%), Vegetables (54%) and Fruits (51%) are eaten 2-3 times per week. About 74% of participants consume milk and milk products and only 47% of respondents take egg at least 2-3 times per week. Remarkably, only 17% of study participants consume meat at least 2-3 times per week.

Table 4: Dietary pattern of study participants of Asella town, from January-March 2019 (N=486)

Variables	More than once/day	Once/day	2-3 times/ week	Occasional	Never
Cereals	410 (84%)	71 (15%)	5 (1%)	0	0
Legumes	350 (72%)	70 (14%)	61 (13%)	5 (1%)	0
Roots and Tubes	32 (7%)	104(21%)	322(66%)	26(6%)	2(0)
Vegetables	103(21%)	101(21%)	261(54%)	20(4%)	1(0)
Fruits	15(3%)	19(4%)	246(51%)	205(42%)	1(0)
Meat	2(0)	6(1%)	76(16%)	394(81%)	8(2%)
Milk	99(20)	95(19%)	168(35%)	115(24%)	9(2%)
Egg	5(1%)	9(2%)	215(44%)	248(51%)	9(2%)
Tea and Coffee	374(77%)	75(16%)	24(5%)	3(1%)	8(2%)

The body mass index (BMI) of adult men and non-pregnant women showed that 64.7% (214) were normal (18.5-24.9), 9.1% (30) were underweight (<18.5), 24.6% (79) were overweight (25-29.9) while 1.6% (5) were obese (>29.9). Since the data of BMI was distributed normally (Mean= 22.56, Median = 22.20 and Mode = 22.22) the central 95% of the results are X ± 2SD (22.56 ± 6.4) (Table 5).

Table 5: Body Mass Index (BMI) of study participants' of Asella town, from January-March 2019 (N=486)

Body Mass Index (BMI)						
BMI (kg/m^2))	Male	Percentage	Female	Percentage	Total	Percentage
<18.5	16	9.4	14	8.9	30	9.1
18.5-24.9	134	78.8	80	50.6	214	64.7
25-29.9	19	11.2	60	38.0	79	24.6
>29.9	1	0.6	4	2.5	5	1.6
Total	170	100.0	158	100.0	328	100.0

6.3. History of common disease

Data on the history of common disease among respondents revealed that about 11 (2%) had hypertension, 4 (1%) chronic gastritis, 3 (1%) diabetics, 3 (1%) history of surgical procedure, 3

(1%) history of allergy and 2 (0%) had history of TB. Among infections, Hepatitis B was detected in 26 (5.3%), Syphilis 16 (3.2%) and Hepatitis C in 1 (0.2%).

6.3.1. Blood pressure

The mean ± SD and median diastolic and systolic blood pressure of study participants were [76 ±14mmHg] and [112± 22mmHg] and 80mmHg and 115mmHg, respectively. The mean ± SD and median blood pressure of pregnant women were [73±14mmHg] and [106±18 mmHg] and 75mmHg and 105mmHg, respectively (Figure 1).

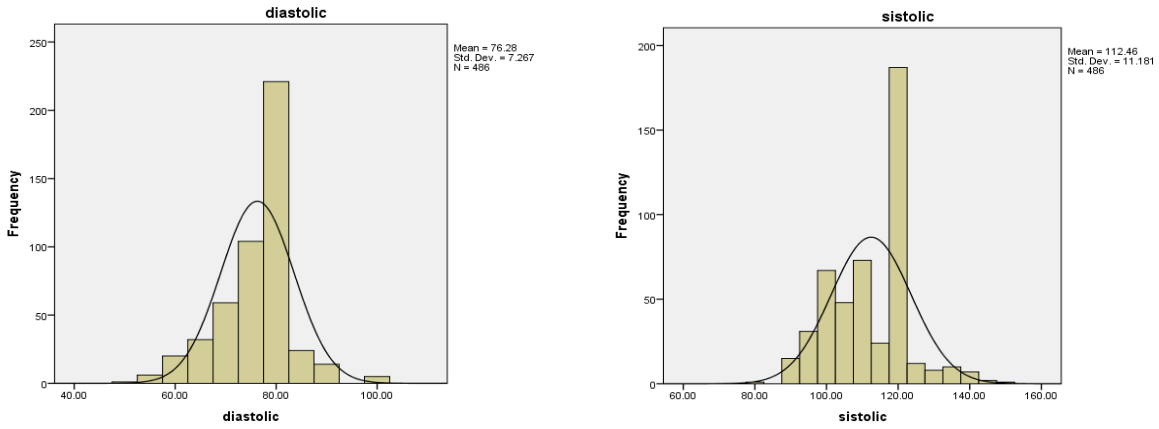


Figure 1: Histogram shows diastolic and systolic blood pressure of study participants of Asella town, January – March 2019 (N=486)

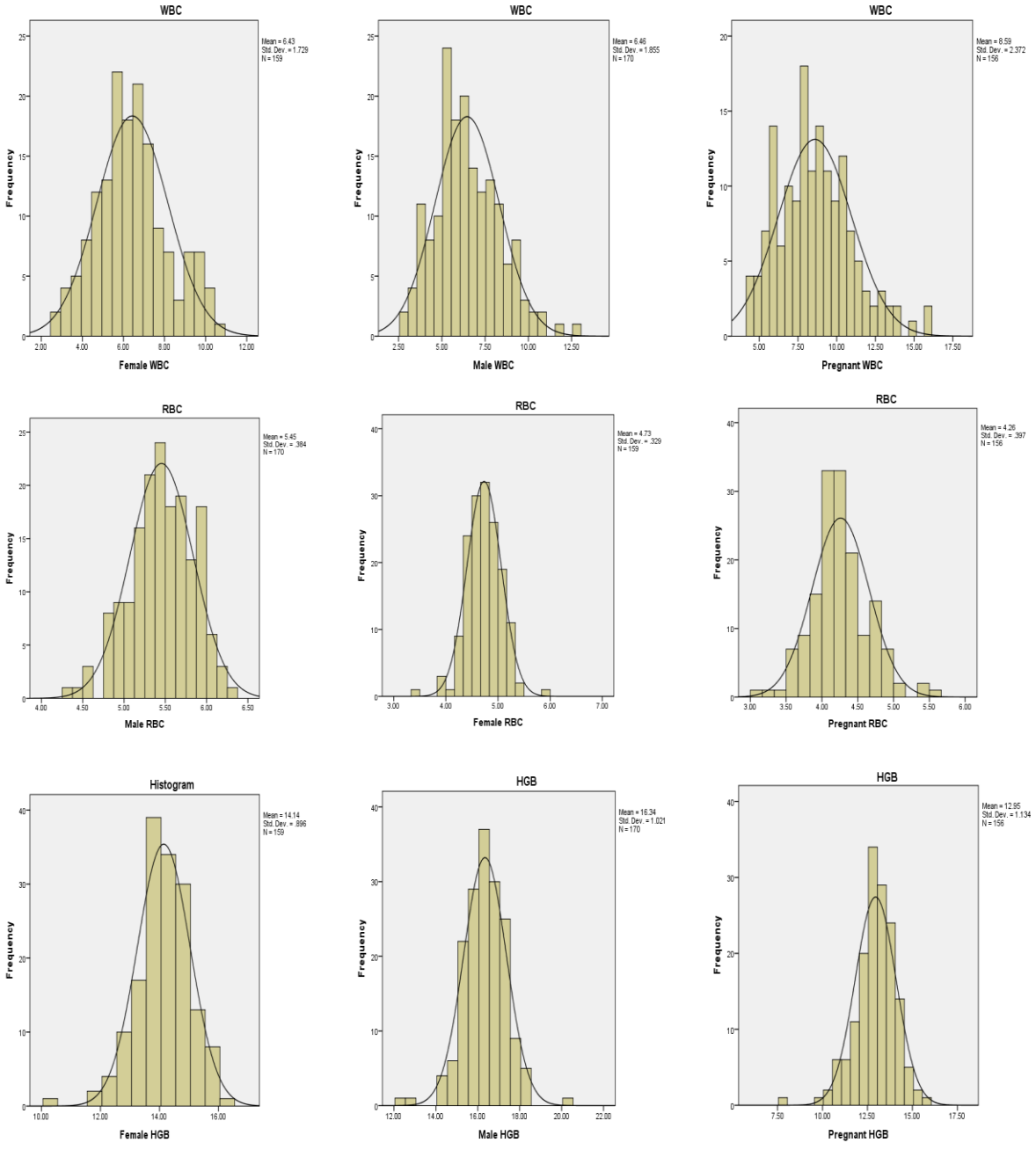
6.4.Hematological results of all partitions

After rejection of 12.8% (62) individuals due to different reasons, 424 participants (147 adult men, 136 non pregnant women and 141 pregnant women) were left to be included in this reference interval study. The reasons were infections (HBsAg, HCV and Syphilis), chronic diseases (hypertension, diabetics, gastritis, TB and others), intestinal parasites (esp. *teania species and H.nana*) and others like obesity and history of surgical procedures. Moreover, test dependent outlier exclusion was done and sample size in such cases is shown in the respective tables. Even though no positive result is obtained, blood film for hemoparasites was performed. In all cases, the final sample size is more than what has been recommended by CLSI (which recommends a minimum of 120 participants per partition)

6.4.1. Frequency distributions for selected hematological parameters

The following figure shows frequency distributions of men, non-pregnant women and pregnant women WBC, RBC, HGB and PLT. Even though nonparametric procedure was planned to analyze

these data, most of the hematological results are normally distributed as indicated by histogram, so that both parametric (ANOVA table; mean comparison) and nonparametric (Mann-Whitney U test; median comparison) procedures were used for analysis of these data to get more reliable output (Figure 2).



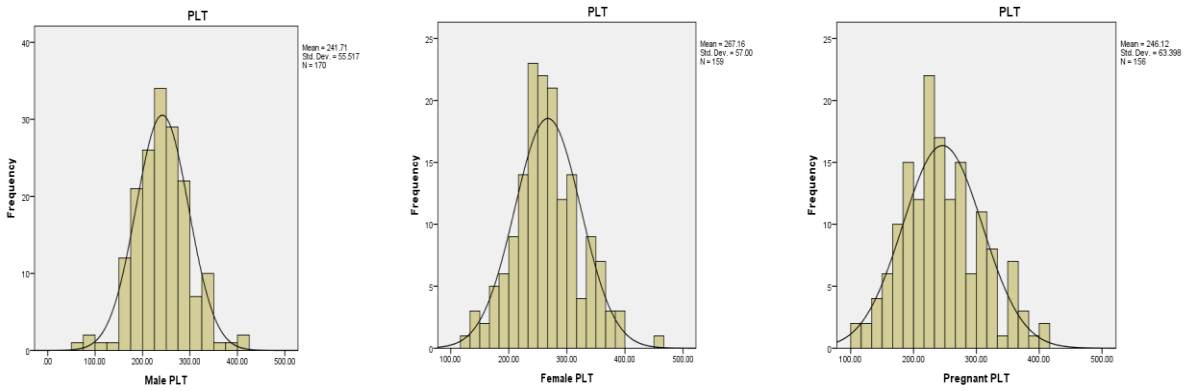


Figure 2: histogram distribution of WBC, RBC, HGB and PLT of Men, non-pregnant women and pregnant women of study participants of Asella town, January – March 2019

6.4.2. Hematological parameter results of Men and non-pregnant women

Sysmex KX 21N which is a three Diff hematology analyzer generates 19 parameters and are displayed in Table 6. Table 6 summarizes the comparison between men and non-pregnant women of median hematological parameters using the Mann-Whitney U test. So, more than half of the parameters showed statistically significant difference between the two sexes. Of these, the median WBC of men and non-pregnant women were not significantly different (P=0.926) whereas median of men and non-pregnant women of RBC, HGB, HCT, MCV, MCH, MCHC, LYM, MXD, MXD%, RDW SD and PLT were different with statistical significance (P<0.05).

As a result, considering the common hematological parameters, common WBC reference interval is obtained (3.4 to 10.1 $x10^9$/L) for both men and non-pregnant women. The men and non-pregnant women RBC, HGB, HCT and PLT reference intervals were: (4.77 to 6.07 $x10^{12}$/L; 4.18 to 5.29 $x10^{12}$/L), (14.7 to 18.1 gm/dL; 12.7 to 15.7gm/dL), (42.1 to 51.3%; 37.1 to 44.4%) and (159 to 336 $x10^9$/L; 177 to 376 $x10^9$/L), respectively.

Table 6: Results of hematological reference interval of men and non-pregnant women of Asella town residents, from January – March 2019

Parameters	Partitions	Unit	N	Min	Max	Median	P-value	Mean ±2SD	95% (RI)	
									2.50%	97.50%
WBC	Men	10^9/L	147	2.8	12.8	6.1	0.926	6.5±3.6	3.7	10.1
	NPW	10^9/L	136	2.7	10.2	6.3		6.5±3.4	3.4	9.8
RBC	Men	10^{12}/L	147	4.35	6.33	5.49	0.0001*	5.5±0.8	4.76	6.07

	NPW	10^{12}/L	136	3.48	5.86	4.71		4.7±0.6	4.11	5.29
HGB	Men	g/L	145	12.8	20.2	163	0.0001*	164±18	146	183
	NPW	g/L	136	10.3	16.4	141		142±16	125	157
HCT	Men	%	147	37.8	55.4	46.5	0.0003*	46.8±5.0	41.9	51.5
	NPW	%	136	33.8	37.2	40.8		40.9±4.3	36.4	45.2
MCV	Men	fl	147	76.5	95.8	85.6	0.027*	85.7±7.2	79.1	94.0
	NPW	fl	133	77.3	94.7	86.4		86.8±5.9	80.5	91.9
MCH	Men	pg	147	25.0	33.7	30	0.729	30.1±2.7	27.8	32.8
	NPW	pg	133	24.5	33.5	29.8		30.1±2.6	27.2	32.6
MCHC	Men	g/L	147	327	370	352	0.0001*	352±16	335	366
	NPW	g/L	136	305	374	346		347±17	327	364
PLT	Men	10^9/L	142	158	380	246	0.00003*	247.4±102.2	158	336
	NPW	10^9/L	134	138	400	265		273.7±106.4	165	377
LYM#	Men	10^9/L	146	1.1	3.8	2.2	0.043*	2.4±1.1	1.3	3.4
	NPW	10^9/L	136	1.2	4.7	2.4		2.5±1.2	1.6	4.0
MXD#	Men	10^9/L	144	0.2	1.5	0.7	0.003*	0.7±0.5	0.3	1.4
	NPW	10^9/L	135	0.2	1.4	0.6		0.6±0.4	0.3	1.2
NEUT#	Men	10^9/L	145	0.9	7.8	3.1	0.891	3.3±2.8	1.1	6.5
	NPW	10^9/L	136	0.5	6.5	3.3		3.4±2.7	0.9	6
LYM%	Men	%	147	12	65.1	37.8	0.145	38.8±22.4	19.7	61.2
	NPW	%	135	18.2	61.5	38.3		40.1±19.9	25.1	60.8
MXD%	Men	%	146	4.9	29	10.7	0.0001*	11.7±6.9	6.5	24.0
	NPW	%	135	4.6	22.8	9.4		9.7±4.7	5.5	19.7
NEUT%	Men	%	146	22.8	81.6	50.3	0.620	50.0±24.4	27.3	70.3
	NPW	%	135	17.1	70.5	50		49.8±22.4	28.9	67.6
RDW-SD	Men	fl	147	36.2	49.2	42.2	0.025*	42.6±5.4	37.8	47.9
	NPW	fl	135	38	52.4	42.8		43.2±5.1	38.6	48.1
RDW-CV	Men	%	146	11.5	14.8	13.05	0.245	13.1±1.2	12	14.3
	NPW	%	135	11.8	16.7	12.9		13.0±1.3	11.9	15.2
PDW	Men	fl	146	8.8	21.4	12.45	0.989	12.9±3.5	10	18.2
	NPW	fl	135	9.6	19.3	12.3		12.9±3.2	10.1	16.8
MPV	Men	fl	146	7.9	14.0	10.2	0.297	10.5±2.0	8.7	12.9
	NPW	fl	135	8.8	13.1	10.3		10.5±1.9	9.1	12.7
PLCR	Men	%	146	10.4	47.8	26.6	0.336	28.4±15.5	15.3	44.8
	NPW	%	135	15.4	47.8	27.2		29.1±15.1	16.9	46.5

* Shows P-value with statistical significance using the Mann Whitney U test; NPW = non-pregnant women

6.4.3. Hematological parameter results of pregnant women as compared to non-pregnant women

Majority of median hematological parameters of pregnant women and non-pregnant women were significantly different. The difference of only two (MCV (P=.091) and PDW (P=0.838)) of the nineteen parameters were not statistically significant. The reference intervals of WBC, RBC, HGB and PLT of pregnant women and non-pregnant women are (4.9 to 13.2 $x10^9$/L; 3.4 to 9.8 $x10^9$/L), (3.58 to 4.9 $x10^{12}$/L; 4.18 to 5.29 $x10^{12}$/L), (11.0 to 14.6gm/dL; 12.7 to 15.7gm/dL) and (138 to 368 $x10^9$/L; 177 to 376 $x10^9$/L) respectively (Table 7).

Table 7: Results of hematological reference interval of Non-pregnant women and pregnant women versus P-value of Asella town residents, January – March 2019

Parameters	Partitions	Unit	N	Min	Max	Median	P value	Mean ±2SD	95% (RI)	
									2.50%	97.50%
WBC	NPW	10^9/L	136	2.7	10.2	6.3	0.0003*	6.5±3.4	3.4	9.8
	Pregnant	10^9/L	141	4.4	16.1	8.4		8.9±4.5	4.9	13.7
RBC	NPW	10^{12}/L	136	3.48	5.86	4.71	0.00005*	4.7±0.6	4.11	5.29
	Pregnant	10^{12}/L	140	3.12	5.59	4.2		4.31±0.7	3.56	5.02
HGB	NPW	g/L	136	103	164	141	0.0002*	142±16	125	157
	Pregnant	g/L	140	78	159	130		129±19	108	146
HCT	NPW	%	136	33.8	37.2	40.8	0.00006*	40.9±4.3	36.4	45.2
	Pregnant	%	140	26.2	46.1	36.6		36.6±5.2	31.1	41.2
MCV	NPW	fl	133	77.3	94.7	86.4	0.091	86.8±5.9	80.5	91.9
	Pregnant	fl	140	69.5	98.9	87.2		87.0±8.2	76.3	94.9
MCH	NPW	pg	133	24.5	33.5	29.8	0.0002*	30.1±2.6	27.2	32.6
	Pregnant	pg	139	24.3	34.9	30.8		30.7±3.6	25.8	33.7
MCHC	NPW	g/L	136	305	374	346	0.0002*	347±17	327	364
	Pregnant	g/L	138	298	373	352		352±18	331	369
PLT	NPW	10^9/L	134	138	400	265	0.016*	273.7±106.4	165	377
	Pregnant	10^9/L	141	106	413	241.5		246.1±124.1	138	368
LYM#	NPW	10^9/L	136	1.2	4.7	2.4	0.0001*	2.5±1.2	1.6	4.0
	Pregnant	10^9/L	139	0.8	2.6	2.0		2.0±0.8	1.3	2.8
MXD#	NPW	10^9/L	135	0.2	1.4	0.6	0.0001*	0.6±0.4	0.3	1.2
	Pregnant	10^9/L	140	0.2	1.4	0.7		0.7±0.4	0.4	1.2
NEUT#	NPW	10^9/L	136	0.5	6.5	3.3	0.0001*	3.4±2.7	0.9	6

	Pregnant	10^9/L	141	2.1	12.2	5.5		5.8±4.1	2.6	10.4
LYM%	NPW	%	135	18.2	61.5	38.3	0.0002*	40.1±19.9	25.1	60.8
	Pregnant	%	141	11.6	44.3	23.75		24.4±13.2	13.6	40.4
MXD%	NPW	%	135	4.6	22.8	9.4	0.002*	9.7±4.7	5.5	19.7
	Pregnant	%	141	2.4	15.9	8.2		8.4±4.7	4.1	14.8
NEUT%	NPW	%	135	17.1	70.5	50	0.0001*	49.8±22.4	28.9	67.6
	Pregnant	%	141	45	83	68.15		67.0±15.0	48.1	79.1
RDW-SD	NPW	fl	135	38	52.4	42.8	0.0003*	43.2±5.1	38.6	48.1
	Pregnant	fl	138	37.2	64.7	44.1		44.1±5.6	39.2	55.8
RDW-CV	NPW	%	135	11.8	16.7	12.9	0.0001*	13.0±1.3	11.9	15.2
	Pregnant	%	139	11.8	20.9	13.4		13.6±1.7	12.3	18.2
PDW	NPW	fl	135	9.6	19.3	12.3	0.838	12.9±3.2	10.1	16.8
	Pregnant	fl	141	9.0	21.7	12.5		13.0±4.1	9.9	18.0
MPV	NPW	fl	135	8.8	13.1	10.3	0 .050	10.5±1.9	9.1	12.7
	Pregnant	fl	141	7.9	14.2	10		10.2±2.1	8.5	12.3
P-LCR	NPW	%	135	15.4	47.8	27.2	0.025*	29.1±15.1	16.9	46.5
	Pregnant	%	141	9.9	46.9	26.1		27.5±16.5	14.4	43.5

* Shows P-value with statistical significance using the Mann Whitney U test; NPW = non-pregnant women

6.4.4. Pregnant women need for partitioning by gestational period

The need for partitioning of pregnant women by gestational period was tested by mean comparison of ANOVA table (Table 8). Therefore, the difference of only three (RBC (P=0.002), HGB (P=0.018) and HCT (P=0.007)) of the nineteen parameters have statistical significance and need partition by gestational period. However, there was no significant difference between second and third trimesters thus reference interval was established for first trimester with the rest gestational periods being merged (Table 8).

Table 8: Hematological Reference interval of pregnant women which need partitioning by gestational period of Asella town, from January – March 2019 (N=141)

Parameters	Gestational period	N	Median	Mean	P-value	Min	Max	95%	
								2.50%	97.50%
RBC $x10^{12}$/L	1st	22	4.59	4.54	0.0001*	3.98	5.38	3.98	5.38
	2nd & 3rd	119	4.18	4.21		3.12	5.59	3.53	5.02
HGB (g/L)	1st	22	137	136	0.003*	123	154	123	154

	2nd & 3rd	119	129	126		78	159	10.3	14.8
HCT %	1st	22	39.2	38.8	0.001*	34.3	43.7	34.3	43.7
	2nd & 3rd	119	36.4	36.6		26.2	46.1	31.1	41.2

*Shows P –value with statistical significance using ANOVA table

6.4.5. Summary of Hematological reference interval for the three groups

After testing the need for different reference interval among partitions, the following hematological reference interval was established for Asella town. The P values (P=<0.05) were not statistically significant for the following hematological parameters and hence there was no need for partitioning them into different groups. These are Male and Female WBC, MCV, MCH, NEUT#, LYM%, NEUT%, RDW-SD, RDW-CV, PDW, MPV and P-LCR (Table 9).

Table 9: Hematological reference interval with 90% confidence interval for Asella town, from January – March 2019

Parameters	Partitions	Unit	N	Median	Mean ±2SD	RI (95%)		90% confidence interval	
						2.50%	97.50%	Lower limits	Upper limits
WBC	M & NPW	10^9/L	283	6.3	6.5±3.6	3.4	10.1	3.2-3.6	9.9-10.3
	Pregnant	10^9/L	141	8.4	8.9±4.5	4.9	13.2	4.6-5.5	12.9-13.5
RBC	Men	10^{12}/L	147	5.49	5.5±0.8	4.76	6.07	4.71-4.81	6.02-6.12
	NPW	10^{12}/L	136	4.71	4.7±0.6	4.11	5.29	4.07-4.15	5.25-5.33
	Pregnant	10^{12}/L	140	4.2	4.31±0.7	3.56	5.02	3.51-3.61	4.97-5.07
HGB	Men	g/L	145	163	164±18	146	183	144-146	182-184
	NPW	g/L	136	141	142±16	125	157	124-126	156-158
	Pregnant	g/L	140	130	129±19	108	146	107-109	145-147
HCT	Men	%	147	46.5	46.8±5.0	41.9	51.5	41.6-42.2	51.2-51.8
	NPW	%	136	40.8	40.9±4.3	36.4	45.2	36.1-36.7	44.9-45.5
	Pregnant	%	140	36.6	36.6±5.2	31.1	41.2	30.8-31.4	40.9-41.5
MCV	M & NPW	fl	280	86	85.7±7.2	79.1	94.0	78.8-79.4	93.7-94.3
	Pregnant	fl	140	87.3	87.0±8.2	76.3	94.9	75.7-76.9	94.3-95.5
MCH	M & NPW	pg	282	29.8	30.1±2.6	27.2	32.6	27.1-27.3	32.5-.32.7
	Pregnant	pg	141	30.8	30.7±3.6	25.8	33.7	25.6-26.0	33.5-33.9
MCHC	Men	g/L	146	352	352±16	335	366	334-336	365-367
	NPW	g/L	133	346	347±17	327	364	326-328	363-365
	Pregnant	g/L	138	352	352±18	331	369	330-332	368-370
PLT	Men	10^9/L	142	246	247.4±102.2	158	336	151-165	329-343
	NPW	10^9/L	134	265	273.7±106.4	165	377	158-172	370-384
	Pregnant	10^9/L	141	241.5	246.1±124.1	138	368	129-147	359-177
LYM#	Men	10^9/L	146	2.2	2.4±1.1	1.3	3.4	1.2-1.4	3.3-3.5
	NPW	10^9/L	136	2.4	2.5±1.2	1.6	4.0	1.5-1.7	3.9-4.1
	Pregnant	10^9/L	139	2	2.0±0.8	1.3	2.8	1.2-1.4	2.7-2.9
MXD#	Men	10^9/L	144	0.7	0.7±0.5	0.3	1.4	0.27-0.33	1.37-1.43
	NPW	10^9/L	135	0.6	0.6±0.4	0.3	1.2	0.27-0.33	1.17-1.23
	Pregnant	10^9/L	140	0.7	0.7±0.4	0.4	1.2	0.37-0.43	1.17-1.23

NEUT#	M & NPW	10^9/L	281	3.2	3.3±2.8	0.9	6.5	0.8-1.0	6.4-6.6
	Pregnant	10^9/L	141	5.5	5.8±4.1	2.6	10.4	2.3-2.9	10.1-10.7
LYM%	M & NPW	%	282	38	38.8±22.4	19.7	61.2	18.7-20.7	60.2-62.2
	Pregnant	%	141	23.75	24.4±13.2	13.6	40.4	12.7-14.5	39.5-41.3
MXD%	Men	%	146	10.6	11.7±6.9	6.5	24.0	6.0-7.0	23.5-24.5
	NPW	%	135	9.15	9.7±4.7	5.5	19.7	5.2-5.8	19.4-20.0
	Pregnant	%	141	8.2	8.4±4.7	4.1	14.8	3.8-4.4	14.5-15.1
NEUT%	M & NPW	%	281	50	50.0±24.4	27.3	70.3	26.1-28.5	69.1-71.5
	Pregnant	%	141	68.15	67.0±15.0	48.1	79.1	47.1-49.1	78.1-80.1
RDW-SD	M & NPW	fl	282	42.15	42.6±5.4	37.8	48.1	37.5-38.1	48.8-48.4
	Pregnant	fl	138	44.1	44.1±5.6	39.2	55.8	38.8-39.6	55.4-56.2
RDW-CV	M & NPW	%	281	13	13.1±1.2	11.9	15.2	11.8-12.0	15.1-15.3
	Pregnant	%	139	13.4	13.6±1.7	12.3	18.2	12.0-12.6	17.9-18.5
PDW	M & NPW	fl	282	12.3	12.9±3.2	10.1	16.8	9.9-10.3	16.6-17.0
	Pregnant	fl	141	12.5	13.0±4.1	9.9	18.0	9.6-10.2	17.7-18.3
MPV	M & NPW	fl	282	10.3	10.5±1.9	9.1	12.7	9.0-9.2	12.6-12.8
	Pregnant	fl	141	10	10.2±2.1	8.5	12.3	8.4-8.6	12.2-12.4
PLCR	M & NPW	%	282	27	28.4±15.5	16.5	46.5	15.7-17.3	45.7-47.3
	Pregnant	%	141	26.1	27.5±16.5	14.4	43.5	13.3-15.5	42.4-44.6

M & NPW = men and non-pregnant women; NPW = non-pregnant women

6.4.6. Blood group and Rh factor

As displayed in table 10, the predominant blood group phenotype was O blood group (210 (44.4%)) and AB (43 (8.9%)) blood group is the least and also more than 94% (457) of study participants were Rh positive (D+).

Table 10: Blood group and Rh factors of study participants of Asella town, from January – March 2019 (N=486)

Blood Group and Rhesus factor	Frequency	Percentage %
A	118	24.3%
B	109	22.4%
AB	43	8.9%
O	216	44.4%
Total	486	100.0%
Rh positive (+)	457	94%
Rh negative (-)	29	6%
Total	486	100%

6.4.7. Mean comparison of current study and company

Table 11 illustrates the mean comparison of current study and mean provided by the company of Sysmex KX 21N. There was significant difference between the mean of men and non-pregnant women RBC, HGB, HCT and MCV. And also there was significant difference between the mean of men WBC which was 6.5 and 5.7, respectively. However, there was no significant difference between the mean of non-pregnant women WBC which is 6.5 and 6.7 respectively.

Table 11: Comparison of Current study and Company mean values

Parameters	Female		Male	
	Current mean	Company mean	Current mean	Company mean
WBC $x10^9$/L	6.5	6.7	6.5	5.7
RBC $x10^{12}$/L	4.7	3.9	5.5	4.45
HGB (g/dL)	14.2	11.8	16.4	13.5
HCT %	40.9	36.3	46.8	40.1
MCV (fl)	86.8	90.4	85.7	91.9
MCH (pg)	30.1	29.9	30.1	30.2
MCHC (g/dL)	34.7	33.4	35.2	34.1
PLT $x10^9$/L	274	281	247	255.5
LYM $x10^9$/L	2.5	1.9	2.4	1.8
MXD $x10^9$/L	0.6	0.9	0.7	0.8
NEUT $x10^9$/L	3.4	4.3	3.3	3.3
LYM1 %	40.1	30.4	38.8	32.7
MXD1 %	10.1	13.6	11.9	13.3
NEUT1 %	49.8	60.4	50	53.7
RDWSD (fl)	43.2	42.1	42.6	41.3
RDWCV %	13	13.1	13.1	12.9
PDW (fl)	12.9	13.8	12.9	13.9
MPV (fl)	10.5	10.5	10.5	10.3
PLCR %	29.1	29.1	28.4	27.9

6.4.8. Comparison of current study and Company reference intervals

Table 12 shows the comparison of reference interval of current study versus Company derived RI which is currently in use in Asella referral and teaching hospital laboratory including proportion of misclassification. So, there are significant difference between current study reference interval and currently in use company derived values of RBC, HGB, HCT, MCV, MCHC, PLT, absolute NEUT, LYM% and NEUT%.

6.4.9. Comparisons of mean, median and central 95% of current study and other studies

The comparison of current study reference interval with other studies in Ethiopia and other African countries revealed that no common reference interval could serve for all (tables 13 and 14).

Table 12: Comparison of current and company reference intervals with proportion of misclassification from January – March 2019

Parameters	Female (136)						Male (147)					
	Current RI		Company RI		Proportion misclassified		Current RI		Company RI		Proportion misclassified	
	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
WBC $x10^9$/L	3.4	10.1	3.1	10.3	2 (1.5%)	0 (0%)	3.4	10.1	2.6	8.8	6 (4.0%)	+14 (9.5%)
RBC $x10^{12}$/L	4.11	5.29	3.20	4.60	4 (3.0%)	+70 (51.5%)	4.76	6.07	3.60	5.30	4 (3.0%)	+93 (63%)
HGB (g/L)	125	157	99	136	3 (2.2%)	+92 (68%)	146	183	113	157	4 (3.0%)	+103 (70%)
HCT %	36.4	45.2	30.2	42.3	4	+29 (21%)	41.9	51.5	32.6	47.5	4 (3.0%)	+54 (37%)
MCV (fl)	79.1	94	78.6	102.2	0	2	79.1	94	80.3	103.4	-5 (3%)	4
MCH (pg)	25.8	33.7	25.2	34.7	0	0	25.8	33.7	26.0	34.4	0	0
MCHC (g/L)	327	364	313	354	1	+19 (14%)	335	366	318	363	3	+5
PLT $x10^9$/L	165	377	128	434	3 (2.2%)	3 (2.2%)	158	336	134	377	4 (3.0%)	4 (3.0%)
LYM $x10^9$/L	1.6	4	0.9	2.8	4	+31 (23%)	1.3	3.4	0.8	2.7	2	+32 (22%)
MXD $x10^9$/L	0.3	1.2	0.1	1.6	2	4	0.3	1.4	0.1	1.5	1	1
NEUT $x10^9$/L	0.9	6.5	1.6	6.9	-10 (7%)	0	0.9	6.5	1.2	5.3	-5 (3%)	+10 (7%)
LYM1 %	19.7	61.2	15	45.8	1	+37 (27%)	19.7	61.2	17.5	47.9	2	+27 (18%)
MXD1 %	5.5	19.7	1.3	25.9	4	4	6.5	24	1.9	24.6	3	0
NEUT1 %	27.3	70.3	43.7	77.1	38 (28%)	0	27.3	70.3	38.3	69.0	-25 (17%)	+4
RDWSD (fl)	37.8	48.1	35.3	48.9	0	0	37.8	48.1	33.4	49.2	4	4
RDWCV %	11.9	15.2	10.6	15.7	2	2	11.9	15.2	10.8	14.9	2	0
PDW (fl)	10	18.2	9.4	18.1	4	0	10	18.2	9.8	18.0	2	+1
MPV (fl)	8.5	12.9	8.5	12.4	0	+3	8.5	12.9	8.1	12.4	2	+4
PLCR %	14.4	46.5	14.3	44.0	0	+3	14.4	46.5	10.7	45.0	3	+3

'-'= results classified as abnormal in lower limits; '+'= results classified as abnormal in upper limits; 'number without sign'= results classified as normal in lower and upper limits

Table 13: Comparisons of mean, median and central 95% of current study and other studies in Ethiopia from January – March 2019

Parameters	Partitions	Current study			Ethiopia (Addis Ababa)[41]			Ethiopia (Gondar)[42]			Ethiopia (Southwest)[67]		
		Median	Mean	95% RI	Median	Mean	95% RI	Median	Mean	95% RI	Median	Mean	95% RI
WBC $x10^9$/L	M & NPW	6.3	6.5	3.4 -10.1	6.1	NA	3.0-10.2	5.1	NA	3.2-8.8	6.4	NA	3.3-11.6
	Pregnant	8.4	8.9	4.9-13.2	NA	NA	NA	9.05	9.2	4.0-14.1	NA	NA	NA
RBC $x10^{12}$/L	Men	5.49	5.5	4.76-6.07	5.1	NA	4.3-5.9	5.01	NA	3.53-6.93	5.32	NA	4.26-6.68
	NPW	4.71	4.7	4.11-5.29	4.5	NA	3.7-5.2	4.8	NA	3.45-6.25	5.02	NA	4.02-6.15
	Pregnant	4.2	4.31	3.56-5.02	NA	NA	NA	4.36	4.37	3.39-5.35	NA	NA	NA
HGB (g/L)	Men	163	164	146-183	161	NA	139-183	142	NA	115-180	155	NA	121-188
	NPW	141	142	125-157	143	NA	122-166	129	NA	110-167	146	NA	123-177
	Pregnant	130	129	108-146	NA	NA	NA	132	132	106-158	NA	NA	NA
HCT %	Men	46.5	46.8	41.9-51.5	48.3	NA	41.6-55.1	46.9	NA	36.2-58.6	45.2	NA	36.7-54.5
	NPW	40.8	40.9	36.4-45.2	42.0	NA	35.3-48.8	45.2	NA	32.1-56.6	43.1	NA	36.9-51.6
	Pregnant	36.6	36.6	31.1-41.2	NA	NA	NA	40.9	40.8	31.4-50.2	NA	NA	NA
MCV (fl)	M & NPW	86	85.7	79.1-94.0	NA	NA	NA	92	94	85-100	83	NA	75-93
	Pregnant	87.3	87.0	76.3-94.9	NA	NA	NA	93.7	94	84.7-103.3	NA	NA	NA
MCH (pg)	M & NPW	33.5	29.8	27.2-32.6	NA	NA	NA	29	NA	28.5-34.4	29	NA	25.6-32.8
	Pregnant	34.9	30.8	25.8-33.7	NA	NA	NA	NA	NA	NA	NA	NA	NA
MCHC (g/L)	Men	352	352	335-366	NA	NA	NA	31.3	NA	29.5-34.4	343	NA	321-365
	NPW	346	347	327-364	NA	NA	NA	30.8	NA	28.5-34.4	339	NA	320-360
	Pregnant	352	352	331-369	NA	NA	NA	32	32.6	27.4-37.9	NA	NA	NA
PLT $x10^9$/L	Men	246	247.4	158-336	206	NA	98-337	264	NA	128-432	275	NA	164-403
	NPW	265	273.7	165-377	206	NA	98-337	264	NA	128-432	288	NA	202-444
	Pregnant	241.5	246.1	138-368	NA	NA	NA	230	231	95-367	NA	NA	NA
LYM#	Men	2.2	2.4	1.3-3.4	NA	NA	NA	1.9	NA	1.0-3.5	2.1	NA	1.1-3.8
	NPW	2.4	2.5	1.6-4.0	NA	NA	NA	1.9	NA	1.0-3.5	2.2	NA	1.2-4.0
	Pregnant	2	2	1.3-2.8	NA	NA	NA	2.2	2.2	1.1- 3.3	NA	NA	NA
MXD#	Men	0.7	0.7	0.3-1.4	NA	NA	NA	0.5	NA	0.2-1.0	NA	NA	NA
	NPW	0.6	0.6	0.3-1.2	NA	NA	NA	0.5	NA	0.2-1.0	NA	NA	NA
	Pregnant	0.7	0.7	0.4-1.2	NA	NA	NA	NA	NA	NA	NA	NA	NA

NEUT #	M & NPW	3.2	3.3	0.9-6.5	NA	NA	NA	2.7	NA	1.6-5.1	3.3	NA	1.0-7.2
	Pregnant	5.5	5.8	2.6-10.4	NA	NA	NA	NA	NA	NA	NA	NA	NA
LYM %	M & NPW	38	38.8	19.7-61.2	36	35.8	17.0-59	38	NA	22-55	NA	NA	NA
	Pregnant	23.75	24.4	13.6-40.4	NA	NA	NA	NA	NA	NA	NA	NA	NA
MXD %	Men	10.6	11.7	6.5-24.0	NA	NA	NA	9.0	NA	6.0-13.0	NA	NA	NA
	NPW	9.15	9.7	5.5-19.7	NA	NA	NA	9.0	NA	6.0-13.0	NA	NA	NA
	Pregnant	8.2	8.4	4.1-14.8	NA	NA	NA	NA	NA	NA	NA	NA	NA
NEUT %	M & NPW	50	50	27.3-70.3	55	54.3	31-78	53	NA	36-69	NA	NA	NA
	Pregnant	68.15	67	48.1-79.1	NA	NA	NA	NA	NA	NA	NA	NA	NA
RDW-SD (fl)	M & NPW	42.15	42.6	37.8-48.1	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	44.1	44.1	39.2-55.8	NA	NA	NA	NA	NA	NA	NA	NA	NA
RDW-CV %	M & NPW	13	13.1	11.9-15.2	NA	NA	NA	14	NA	12-17	13.7	NA	12.5-17.6
	Pregnant	13.4	13.6	12.3-18.2	NA	NA	NA	NA	NA	NA	NA	NA	NA
PDW (fl)	M & NPW	12.3	12.9	10.1-16.8	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	12.5	13.0	9.9-18.0	NA	NA	NA	NA	NA	NA	NA	NA	NA
MPV (fl)	M & NPW	10.3	10.5	9.1-12.7	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	10	10.2	8.5-12.3	NA	NA	NA	NA	NA	NA	NA	NA	NA
P-LCR %	M & NPW	27	28.4	16.5-46.5	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	26.1	27.5	14.4-43.5	NA	NA	NA	NA	NA	NA	NA	NA	NA

M & NPW = men and non-pregnant women; NPW = non-pregnant women; NA = Not Available

Table 14: Comparisons of mean, median and central 95% of current study and other studies in Africa from January – March 2019

Parameters	Partitions	Current study			Kenya (Western)[62]			Nigeria (North central)[44]			Eritrea (Asmara)[72]		
		Median	Mean	95% RI	Median	Mean	95% RI	Median	Mean	95% RI	Median	Mean	95% RI
WBC $x10^9$/L	M & NPW	6.3	6.5	3.4-10.1	5.6	NA	3.3-9.7	4.5	4.6	2.5-8.7	5.6	5.6	2.6-10.5
	Pregnant	8.4	8.9	4.9-13.2	NA	NA	NA	6.4	6.6	3.6-9.6	NA	NA	NA
RBC $x10^{12}$/L	Men	5.49	5.5	4.76-6.07	5.3	NA	4.3-6.5	5.2	5.2	3.76-6.64	5.3	5.3	4.2-6.02
	NPW	4.71	4.7	4.11-5.29	4.5	NA	3.4-5.7	4.56	4.6	3.34-5.86	4.7	4.7	4.0-5.7
	Pregnant	4.2	4.31	3.56-4.90	NA	NA	NA	4.22	4.27	3.37-5.17	NA	NA	NA
HGB (g/L)	Men	163	164	147-183	142	NA	114-169	143	142	116-168	154	157	126-178
	NPW	141	142	125-157	121	NA	80-142	128	127.5	104-172	149	150	125-176
	Pregnant	130	129	108-152	NA	NA	NA	119	118	100-136	NA	NA	NA
HCT %	Men	46.5	46.8	42.1-51.3	41.7	NA	32.6-51.5	44.8	44.2	36.2-52.2	49.9	49.3	40.5-55.0
	NPW	40.8	40.9	37.1-45.4	35.8	NA	23.2-44.3	40.0	39.7	30.3-49.1	44.5	44.2	37.9-52.0
	Pregnant	36.6	36.6	31.4-41.2	NA	NA	NA	36.0	36.0	30.7-41.3	NA	NA	NA
MCV (fl)	M & NPW	86	85.7	79.1-94.0	NA	NA	NA	86.1	85.5	73.2-97.1	93.7	93.7	85.8-100
	Pregnant	87.3	87.0	76.3-94.9	NA	NA	NA	84.9	85.0	72.5-97.5	NA	NA	NA
MCH (pg)	M & NPW	33.5	29.8	27.2-32.6	NA	NA	NA	27.9	27.7	22.5-32.9	30.6	30.6	27.4-32.8
	Pregnant	34.9	30.8	25.8-33.7	NA	NA	NA	NA	NA	NA	NA	NA	NA
MCHC (g/L)	Men	352	352	335-366	NA	NA	NA	32.4	32.2	29.5-34.9	33.0	32.8	30.4-33.7
	NPW	346	347	330-362	NA	NA	NA	32.1	32.1	29.8-34.4	32.8	32.4	30.0-33.7
	Pregnant	352	352	333-369	NA	NA	NA	32.8	32.8	30.2-35.4	NA	NA	NA
PLT $x10^9$/L	Men	246	247.4	158-336	201	NA	102-307	213	216	106-326	211	216	128-318
	NPW	265	273.7	165-377	220	NA	88-439	236	241	121-361	227	230	145-352
	Pregnant	241.5	246.1	138-368	NA	NA	NA	207	215	99-331	NA	NA	NA
LYM#	Men	2.2	2.4	1.3-3.4	2.2	NA	1.0-3.5	NA	NA	NA	NA	NA	NA
	NPW	2.4	2.5	1.6-4.0	2.2	NA	1.3-3.8	NA	NA	NA	NA	NA	NA
	Pregnant	2.0	2.0	1.3-2.8	NA	NA	NA	NA	NA	NA	NA	NA	NA
MXD#	Men	0.7	0.7	0.3-1.4	NA	NA	NA	NA	NA	NA	NA	NA	NA

	NPW	0.6	0.6	0.3-1.2	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	0.7	0.7	0.4-1.0	NA	NA	NA	NA	NA	NA	NA	NA	NA
NEUT #	M & NPW	3.2	3.3	1.1-6.3	2.3	NA	1.3-3.8	NA	NA	NA	NA	NA	NA
	Pregnant	5.5	5.8	2.6-10.4	NA	NA	NA	NA	NA	NA	NA	NA	NA
LYM %	M & NPW	38	38.8	19.7-61.2	NA	NA	NA	40.0	39.0	24.0-54.0	37.3	39.2	22.0-59.2
	Pregnant	23.75	24.4	13.6-38.5	NA	NA	NA	26.0	27.3	15.5-39.1	NA	NA	NA
MXD %	Men	10.6	11.7	6.5-19.0	NA	NA	NA	7.0	7.4	1.4-12.6	7.6	7.5	3.1-11.6
	NPW	9.15	9.7	5.5-13.9	NA	NA	NA	8.0	8.7	2.9-14.5	7.6	7.5	3.0-11.8
	Pregnant	8.2	8.4	4.1-13.3	NA	NA	NA	9.0	9.2	4.4-13.0	NA	NA	NA
NEUT %	M & NPW	50	50	27.3-70.3	NA	NA	NA	53.0	53.5	38.8-68.2	54.7	53.3	32.4-72.6
	Pregnant	68.15	67	50.5-79.1	NA	NA	NA	64.0	63.6	51.1-76.1	NA	NA	NA
RDW-SD (fl)	M & NPW	42.15	42.6	37.8-47.9	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	44.1	44.1	39.2-49.5	NA	NA	NA	NA	NA	NA	NA	NA	NA
RDW-CV %	M & NPW	13	13.1	12.0-14.3	NA	NA	NA	NA	NA	NA	13.5	13.6	12.3-15.6
	Pregnant	13.4	13.6	12.3-15.4	NA	NA	NA	NA	NA	NA	NA	NA	NA
PDW (fl)	M & NPW	12.3	12.9	10.1-16.8	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	12.5	13.0	9.9-18.0	NA	NA	NA	NA	NA	NA	NA	NA	NA
MPV (fl)	M & NPW	10.3	10.5	9.1-12.7	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	10	10.2	8.5-12.3	NA	NA	NA	NA	NA	NA	NA	NA	NA
P-LCR %	M & NPW	27	28.4	15.3-44.8	NA	NA	NA	NA	NA	NA	NA	NA	NA
	Pregnant	26.1	27.5	14.4-43.5	NA	NA	NA	NA	NA	NA	NA	NA	NA

M & NPW = men and non-pregnant women; NPW = non-pregnant women; NA = Not Available

7. Discussion

Until now Arsi University Asella referral and teaching hospital laboratory was using RI found in the CBC analyzer that was provided by the company, which was adopted from western countries (USA). Even though this is a national problem, there is an attempt to established RI at a national level. Unfortunately, many types of CBC analyzers like Sysmex KX 21N, Mindray and Cell DYN Emerald found in our hospital. RI also varies with these automated machines. But, Sysmex is the most predominantly used machine in the hospital and hence by this study as well. Thus, this study is the first community study to establish hematological RI for apparently healthy adult populations in Asella town. The study determined the 2.5^{th} and 97.5^{th} percentile RI for men, non-pregnant women, and pregnant women with 90% CI as recommended by CLSI (6).

7.1.Comparison within partitions

The WBC, MCV, NEUT, NEUT% and RDW of men and non-pregnant women of this study participants are similar to each other. But, there is statistically significant difference between RBC, HGB, HCT, MCHC and PLT of men and non-pregnant women. This could be because of erythropoiesis being stimulated by testosterone while down regulated by estrogen hormone (8). There is also significant difference between WBC, RBC, HGB, HCT, MCHC and PLT of non-pregnant women and pregnant women. Not only physiologic anemia, thrombocytopenia and neutrophilia but also decrease in absolute and percent lymphocyte count was observed in this study which is concordant with study in Gondar (15, 16). However, there is no statistically significant difference in the values of MCH and MPV between all study partitions.

7.2. Comparison of current study mean and RI with mean and RI currently in use

The mean of both men and non-pregnant women RBC, HGB, HCT, MCV, NEUT% and LYM% of this study were different from the company mean values which are currently in use. However, the mean of both men and non-pregnant women MCH, PLT, and P-LCR of current study and company (currently in use in the laboratory) are relatively similar. Similarly, the mean WBC of non-pregnant women also relatively concordant with company mean. But mean of men WBC is not similar with currently in use (70).

The lower and upper limits of men WBC of this study were higher than those being in use in the laboratory. The lower and upper limits of men and non-pregnant women RBC, HGB and HCT of this study were higher than company limits. This study's lower limits of both men and non-pregnant women PLT is higher than company limits but their upper limits are lower. And company limits are lower than current study LYM count and LYM% of both men and non-pregnant women but current study lower and upper limits of non-pregnant women and lower limits of men NEUT count and NEUT% are lower than company limits (70). Such variations between the company and currently established RIs underscore the need for establishing locally derived intervals (6, 8).

The upper limits of men and non-pregnant women WBC and PLT are lower than hematological RI listed in Wintrobe's clinical hematology. However, the lower and upper limits of men and non-pregnant women RBC, HGB and HCT are higher than what is mentioned in the text book which is commonly referred. The lower and upper limits of men absolute and percent neutrophils are lower. but the lower and upper limits of men absolute and percent lymphocytes were higher than Wintrobe's (8). This is because of variation in race, genetics, altitude, ethnicity, nutrition and other factors of study area and participants of the current study (8, 17).

7.3. Proportion of misclassification

The proportion of misclassified hematological parameters was analyzed in this study. Remarkable misclassifications were noted for RBC and HGB where 51.5%and 68% of non-pregnant women were classified as abnormal by the instrument based on the company upper limit. Moreover, about 9.5% men WBC, 63% RBC and 70% HGB values were misclassified as abnormally high results. Such high RBC and HGB values are expected for a locality like Arsi which has an altitude of 2430 m (8, 17).

There were also remarkably large percentage of non-pregnant women LYM% (27%) and absolute LYM (23%) misclassified as lymphocytosis by the company reference interval. On the other hand, about 28% of %NEUT values of non-pregnant women were classified as neutropenic when using the instrument RI even if they were not. There was also significant number of men absolute LYM (22%), absolute NEUT (7%) and %LYM (18%) that were misclassified as abnormal results. And also about 17% of men %NEUT values were misclassified as abnormally low results. Lower RI

limit for % NEUT which is lower than the company value is a common finding in Ethiopia as well as in other African countries (13, 69).

7.4.Comparison between studies in Ethiopia

The lower and upper limits of men and non-pregnant women WBC of current study results were relatively similar with study in Addis Ababa (41) and includes the RI determined in Gondar (42) and within the RI of Southwest Ethiopia (67), though slight difference was noted between the upper limits of men and non-pregnant women WBC compared to those studies in Gondar and Southwest Ethiopia. The RI of pregnant women WBC of this study was within RI of Gondar with few differences in lower and upper limits (43).

The current study RBC, HGB and HCT lower and RBC and HCT upper limits of all the three partitions were not similar with other studies in Ethiopia (Addis Ababa (41), Gondar (42) and Southwest Ethiopia (67). Even though there is higher lower limit of RBC, HGB and HCT of current study compared to studies in Gondar (42) and southwest Ethiopia (64), the lower and upper limits of this study are within their reference interval. This is mainly because of high altitude of Asella town as compared to Addis Ababa, Gondar and southeast Ethiopia.

7.5.Comparison between studies in Africa

The mean and median values of both men and non-pregnant women WBC of current study were greater than studies in Kenya (62), Eritrea (72) and Nigeria (44). The lower WBC limit of this study for men and non-pregnant women was higher than studies in Eritrea (72), Kenya (59) and Nigeria (44). Whereas, the upper WBC limit of the current study was higher than studies in Kenya (59) and Nigeria (44). Similarly, mean, median, lower and upper limits of WBC of the current study for pregnant women were higher than a study in Nigeria (44).

The mean, median and lower limits of this study for men RBC, HGB and HCT were higher than studies in Kenya (62), Eritrea (72) and Nigeria (44). Whereas, the mean, median and lower limit of non-pregnant women's RBC, HGB and HCT of the current study were higher than a study in Kenya (62). On the other hand, the non-pregnant women's upper limit for RBC, HGB, and HCT of current study were also higher than the upper limit in Kenya (62) but similar to Eritrea (69) and Nigeria (44). Besides, there was significant difference between lower limits of pregnant women RBC, HGB and HCT of current study and that from Nigeria (44).

With regards to platelets, the current study men and non-pregnant women lower limits were higher than Kenya (62), Eritrea (72) and Nigeria (44). Whereas, the upper PLT limit was higher than studies in Nigeria (44) and Eritrea (69). Compared to the available data for pregnant women, both the lower and upper PLT limits were higher than those reported from Nigeria (16).

Taken together, comparisons within or outside Ethiopia revealed that there were variations in the RI limits with no consistent pattern to use common intervals between countries necessitating the need for locally relevant reference intervals.

8. Strength and Limitation

8.1.Strength

This study is community based study. Various methods (Questionnaire, testing of TTI, HCG test, parasitological, urinalysis, physical examinations) were used to exclude non eligible participants. The precision test was used to detect random error by comparing with company reproducibility range. The hospital laboratory persistently passes external quality assessment (EQA) by One world accuracy.

8.2.Limitation

Sysmex KX 21N control sample and working reagents were stock out; however Adama regional laboratory supported the study by providing reagents. The research budget was not released timely. Even though second and third trimesters shows no significant difference, this study was not take sample size recommended by CLSI document to partition gestational age in to trimesters.

9. Conclusion and Recommendation

9.1.Conclusion

In conclusion most of the hematological reference interval of this study was significantly different from currently in use in Asella referral and teaching hospital laboratory (western populations). The difference was also observed not only in studies of other African countries but also studies done in different parts of Ethiopia. These lead us to the necessity of establishing reference interval in every laboratory.

9.2.Recommendation

Every laboratory should establish its own reference interval considering all partitions, which relatively could represent the majority of the population it serves. It is better to use this reference interval by health facilities in Asella town. Those having different equipment can perform transference and utilize it.

10. REFERENCES

1. Hallworth MJ. The '70% claim': what is the evidence base? : SAGE Publications Sage UK: London, England; 2011.
2. Ngo A, Gandhi P, Miller WG. Frequency that Laboratory Tests Influence Medical Decisions. The Journal of Applied Laboratory Medicine: An AACC Publication. 2017;1(4):410.
3. Ceriotti F. Prerequisites for use of common reference intervals. The Clinical biochemist Reviews. 2007;28(3):115-21.
4. Solberg HE. Approved recommendation (1987) on the theory of reference values. Part 5. Statistical treatment of collected reference values. Determination of reference limits. Clinica Chimica Acta. 1987;170(2):S13-S32.
5. Pereira P. ISO 15189: 2012 Medical laboratories-Requirements for quality and competence.
6. Horowitz G, Altaie S, Boyd J, Ceriotti F, Garg U, Horn P, et al. Clinical and Laboratory Standards Institute: Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline. Wayne, PA: CLSI; 2008.
7. Harris EK, Boyd JC. Statistical bases of reference values in laboratory medicine: CRC Press; 1995.
8. Wootton ID, King EJ, Smith JM. The quantitative approach to hospital biochemistry; normal values and the use of biochemical determinations for diagnosis and prognosis. British medical bulletin. 1951;7(4):307-11.
9. Wootton ID, King EJ. Normal values for blood constituents; inter-hospital differences. Lancet (London, England). 1953;1(6758):470-1.
10. Reed AH, Henry RJ, Mason WB. Influence of statistical method used on the resulting estimate of normal range. Clinical chemistry. 1971;17(4):275-84.
11. Harris EK, DeMets DL. Estimation of normal ranges and cumulative proportions by transforming observed distributions to gaussian form. Clinical chemistry. 1972;18(7):605-12.
12. Boyd JC, Lacher DA. A multi-stage Gaussian transformation algorithm for clinical laboratory data. Clinical chemistry. 1982;28(8):1735-41.
13. Shultz EK, Willard KE, Rich SS, Connelly DP, Critchfield GC. Improved reference-interval estimation. Clinical chemistry. 1985;31(12):1974-8.

14. Jorgensen LG, Brandslund I, Hyltoft Petersen P. Should we maintain the 95 percent reference intervals in the era of wellness testing? A concept paper. Clinical chemistry and laboratory medicine. 2004;42(7):747-51.
15. Doumas BT. How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline, C28-A2. 2000.
16. Martin H, Hologgitas J, Driscoll J, Fanger H, Gudzinowicz B, Barnett R, et al. Reference values based on populations accessible to hospitals. Reference Values in Laboratory Medicine Chichester: John Wiley. 1981;413.
17. Arzideh F, Wosniok W, Gurr E, Hinsch W, Schumann G, Weinstock N, et al. A plea for intra-laboratory reference limits. Part 2. A bimodal retrospective concept for determining reference limits from intra-laboratory databases demonstrated by catalytic activity concentrations of enzymes. Clinical chemistry and laboratory medicine. 2007;45(8):1043-57.
18. Hoffmann RG. STATISTICS IN THE PRACTICE OF MEDICINE. Jama. 1963;185:864-73.
19. Bhattacharya CG. A simple method of resolution of a distribution into gaussian components. Biometrics. 1967;23(1):115-35.
20. Solberg HE. Using a hospitalized population to establish reference intervals: pros and cons. Clinical chemistry. 1994;40(12):2205-6.
21. Ferre-Masferrer M, Fuentes-Arderiu X, Puchal-Ane R. Indirect reference limits estimated from patients' results by three mathematical procedures. Clinica chimica acta; international journal of clinical chemistry. 1999;279(1-2):97-105.
22. Ceriotti F, Schumann G, Panteghini M. Redefining reference limits needs more attention to the analytical aspects. Liver international : official journal of the International Association for the Study of the Liver. 2006;26(9):1155-6.
23. Virtanen A, Kairisto V, Irjala K, Rajamaki A, Uusipaikka E. Regression-based reference limits and their reliability: example on hemoglobin during the first year of life. Clinical chemistry. 1998;44(2):327-35.
24. Harris EK, Boyd JC. On dividing reference data into subgroups to produce separate reference ranges. Clinical chemistry. 1990;36(2):265-70.
25. Lahti A. Partitioning biochemical reference data intosubgroups: comparison of existing methods. Clinical Chemistry and Laboratory Medicine (CCLM). 2004;42(7):725-33.

26. Lahti A, Petersen PH, Boyd JC, Rustad P, Laake P, Solberg HE. Partitioning of nongaussian-distributed biochemical reference data into subgroups. Clinical chemistry. 2004;50(5):891-900.

27. Gowans EM, Hyltoft Petersen P, Blaabjerg O, Horder M. Analytical goals for the acceptance of common reference intervals for laboratories throughout a geographical area. Scandinavian journal of clinical and laboratory investigation. 1988;48(8):757-64.

28. Y. O. Reference intervals: current status, recent developments and future considerations. Biochemia Medica. 2016;26(1):12.

29. Dixon W. Processing data for outliers. Biometrics. 1953;9(1):74-89.

30. Horn PS, Feng L, Li Y, Pesce AJ. Effect of outliers and nonhealthy individuals on reference interval estimation. Clinical chemistry. 2001;47(12):2137-45.

31. Box GE, Cox DR. An analysis of transformations. Journal of the Royal Statistical Society Series B (Methodological). 1964:211-52.

32. Tukey J. Exploratory data analysis Addison-Wesley USA. 1977.

33. Adetifa IM, Hill PC, Jeffries DJ, Jackson-Sillah D, Ibanga HB, Bah G, et al. Haematological values from a Gambian cohort--possible reference range for a West African population. International journal of laboratory hematology. 2009;31(6):615-22.

34. Greer JP, Foerster J, Lukens JN. Wintrobe's Clinical Hematology (2 Vol. Set): Lippincott Williams & Wilkins; 2003.

35. BM HVDRDMA. Postgraduate Haematology. 7 edition ed. UK: JohnWiley & Sons Ltd; 2016 949 p.

36. McKenzie SB. Clinical Laboratory Hematology. Second Edition ed. USA: Pearson Education Limited 2014. 1097 p.

37. AH AAL. Cellular and Molecular Immunology. 5th edition ed: Saunders. 288 p.

38. Solberg HE, Stamm D. International Federation of Clinical Chemistry, Scientific Division: approved recommendation on the theory of reference values. Part 4. Control of analytical variation in the production, transfer and application of reference values. European journal of clinical chemistry and clinical biochemistry : journal of the Forum of European Clinical Chemistry Societies. 1991;29(8):531-5.

39. Wakeman L, Al-Ismail S, Benton A, Beddall A, Gibbs A, Hartnell S, et al. Robust, routine haematology reference ranges for healthy adults. International journal of laboratory hematology. 2007;29(4):279-83.

40. Karita E, Ketter N, Price MA, Kayitenkore K, Kaleebu P, Nanvubya A, et al. CLSI-derived hematology and biochemistry reference intervals for healthy adults in eastern and southern Africa. PloS one. 2009;4(2):e4401.

41. Tsegaye A, Messele T, Tilahun T, Hailu E, Sahlu T, Doorly R, et al. Immunohematological reference ranges for adult Ethiopians. Clinical and diagnostic laboratory immunology. 1999;6(3):410-4.

42. Yalew A, Terefe B, Alem M, Enawgaw B. Hematological reference intervals determination in adults at Gondar university hospital, Northwest Ethiopia. BMC research notes. 2016;9(1):483.

43. Genetu M, Damtie D, Workineh M, Mathewos Tebeje B, Enawgaw B, Deressa T. Immunological and hematological reference intervals among HIV-seronegative pregnant women in northwest Ethiopia. International journal of women's health. 2017;9:145-50.

44. Miri-Dashe T, Osawe S, Tokdung M, Daniel N, Choji RP, Mamman I, et al. Comprehensive reference ranges for hematology and clinical chemistry laboratory parameters derived from normal Nigerian adults. PloS one. 2014;9(5):e93919.

45. Grasbeck R. Establishment and use of normal values. Scand J Clin Invest. 1969;110:1-15.

46. Sunderman FW. Current concepts of" normal values,"" reference values," and" discrimination values" in clinical chemistry. Clinical Chemistry; 1975.

47. Horowitz GL. Establishment and use of reference values. Tietz textbook of clinical chemistry and molecular diagnostics Fifth ed St Louis, Missouri: Elsevier Inc. 2012:95-118.

48. Henny J, Petersen PH. Reference values: from philosophy to a tool for laboratory medicine. Clinical Chemistry and Laboratory Medicine (CCLM). 2004;42(7):686-91.

49. Solberg HE. International Federation of Clinical Chemistry (IFCC), Scientific Committee, Clinical Section, Expert Panel on Theory of Reference Values, and International Committee for Standardization in Haematology (ICSH), Standing Committee on Reference Values. Approved Recommendation (1986) on the theory of reference values. Part 1. The concept of reference values. Journal of clinical chemistry and clinical biochemistry Zeitschrift fur klinische Chemie und klinische Biochemie. 1987;25(5):337-42.

50. PetitClerc C, Solberg H. International Federation of Clinical Chemistry, expert panel on theory of reference values: approved recommendation (1987) on the theory of reference values. Part 2. Selection of individuals for the production of reference values. Journal of clinical chemistry and clinical biochemistry Zeitschrift fur klinische Chemie und klinische Biochemie. 1987;25:639-44.

51. Solberg HE, PetitClerc C. International Federation of Clinical Chemistry (IFCC), Scientific Committee, Clinical Section, Expert Panel on Theory of Reference Values. Approved recommendation (1988) on the theory of reference values. Part 3. Preparation of individuals and collection of specimens for the production of reference values. Journal of clinical chemistry and clinical biochemistry Zeitschrift fur klinische Chemie und klinische Biochemie. 1988;26(9):593-8.

52. Dybkaer R, Solberg H. Approved recommendation (1987) on the theory of reference values. J Clin Chem Clin Biochem. 1987;25(9).

53. Henny J, Petitclerc C, Fuentes-Arderiu X, Petersen PH, Queraltó JM, Schiele F, et al. Need for revisiting the concept of reference values. Clinical chemistry and laboratory medicine. 2000;38(7):589-95.

54. Horn PS, Pesce AJ. Reference intervals: an update. Clinica chimica acta; international journal of clinical chemistry. 2003;334(1-2):5-23.

55. Grad FP. The preamble of the constitution of the World Health Organization. Bulletin of the World Health Organization. 2002;80:981-.

56. Alstrom T, Grasbeck R, Hjelm M, Skandsen S. Recommendations concerning the collection of reference values in clinical chemistry and activity report by the Committee on Reference Values of the Scandinavian Society for Clinical Chemistry and Clinical Physiology. Scandinavian journal of clinical and laboratory investigation Supplementum. 1975;144:1-44.

57. Berg B, Solberg H, Nilsson J, Tryding N. Practical experience in the selection and preparation of reference individuals: empirical testing of the provisional Scandinavian recommendations. John Wiley & Sons, Chichester (UK); 1981. p. 55-64.

58. Grasbeck R. The evolution of the reference value concept. Clinical chemistry and laboratory medicine. 2004;42(7):692-7.

59. Dosoo DK, Kayan K, Adu-Gyasi D, Kwara E, Ocran J, Osei-Kwakye K, et al. Haematological and biochemical reference values for healthy adults in the middle belt of Ghana. PloS one. 2012;7(4):e36308.

60. Saathoff E, Schneider P, Kleinfeldt V, Geis S, Haule D, Maboko L, et al. Laboratory reference values for healthy adults from southern Tanzania. Tropical Medicine & International Health. 2008;13(5):612-25.

61. Lugada ES, Mermin J, Kaharuza F, Ulvestad E, Were W, Langeland N, et al. Population-based hematologic and immunologic reference values for a healthy Ugandan population. Clinical and diagnostic laboratory immunology. 2004;11(1):29-34.

62. Zeh C, Amornkul PN, Inzaule S, Ondoa P, Oyaro B, Mwaengo DM, et al. Population-based biochemistry, immunologic and hematological reference values for adolescents and young adults in a rural population in Western Kenya. PloS one. 2011;6(6):e21040.

63. Tembe N, Joaquim O, Alfai E, Sitoe N, Viegas E, Macovela E, et al. Reference values for clinical laboratory parameters in young adults in Maputo, Mozambique. PloS one. 2014;9(5):e97391.

64. Abera B, Alem A, Cherenet A, Kibret M. Immunological and hematological reference values for apparently healthy HIV-negative adults in Bahir Dar Town, Ethiopia. Ethiopian Journal of Health Development. 2012;26(3):152-9.

65. Mulu W, Abera B, Mekonnen Z, Adem Y, Yimer M, Zenebe Y, et al. Haematological and CD4+ T cells reference ranges in healthy adult populations in Gojjam zones in Amhara region, Ethiopia. PloS one. 2017;12(7):e0181268.

66. Eshete EA, Weldemariam TZ. Hematological and lipid profiles of blood donors at red cross center in Addis Ababa. Ethiopian medical journal. 2016;54(1).

67. Bimerew LG, Demie T, Eskinder K, Getachew A, Bekele S, Cheneke W, et al. Reference intervals for hematology test parameters from apparently healthy individuals in southwest Ethiopia. SAGE open medicine. 2018;6:2050312118807626.

68. Yeshanew AG, GebreSilasie YM, Mengesha HT. Establishment of Immunohematological Reference Values among HIV Sero-negative Pregnant Women at St. Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia. Ethiopian journal of health sciences. 2017;27(6):641-50.

69. Commission FDRoEPC. Results for Oromia Region Summary and statistical report of 2007 population and housing census 2008.

70. CORPORATION S. Sysmex KX-21 Operator's Manual. KOBE, JAPAN: SYSMEX CORPORATION; 2000, January 306 p.

71. Accuracy O. Oneworld Accuracy User Guide: Oneworld Accuracy; March 2013. 39 p.

72. Siraj N, Issac J, Anwar M, Mehari Y, Russom S, Kahsay S, et al. Establishment of hematological reference intervals for healthy adults in Asmara. BMC research notes. 2018;11(1):55.

11. ANNEXES

11.1. Annex I: Participants' Information sheet (≥18 years)

Project Title: Establishment of hematological reference intervals for apparently healthy adult residents in Asella town southeast, Ethiopia

Project PI: Solomon Tadesse (M.Sc. candidate in hematology and Immuno-hematology specialty at Addis Ababa University College of Health Science)

Organization: AAU

Sponsor: AAU and Arsi University, Ethiopia

Introduction:

Hello! My name is _____ and I am working with researchers from the various Medical Laboratory Science teaching Universities, Regional Laboratories, National Blood Bank of Ethiopia and EMLA. We are conducting a study to Establish Hematological Reference Intervals for Ethiopians aged from18 to 60 years in Asella town of Arsi zone.

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 and local quality control materials. Therefore, the purpose of this proposed study is to Establish Hematological Reference Intervals for Ethiopians aged from 18 to 60 years in Asella town, Arsi zone of Ethiopia. You have been chosen for this study. Therefore, we 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 Ethiopia.

Procedures: After agreeing that you can take part, one or more of our research staff ask you some questions which are take up to 15 minutes. Your weight, height and vital signs are measured. You are asked to provide fresh urine and stool on a particular container we provide. We are also collect 7 ml venous blood (about 1 table spoon) from you by sterile-disposable vacutainer tube and needle (4ml in plane tube and 3 ml in tube containing EDTA). We will conduct laboratory examination

to determine different hematological, serological, parasitological and clinical chemistry parameters.

Benefits

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

Risks and Discomfort

There are minimal discomfort in giving urine and stool samples. 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 are taken by experienced laboratory professionals.

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 permitted. The results of the research study may be published, but participants' names or identities will not 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 coded to prevent identification of the volunteers. Access to data entered into computerized files will permitted only for authorized personnel directly involved with the study and will password protected. Individual-specific information may be provided to responsible local medical personnel only with your permission. Urine, stool and blood collected will not use for other purposes. The leftover samples will stored at the Department of Medical Laboratory Sciences of AAU in a secure place for additional tests as needed. Finally, all the biological wastes, after analysis will safely disposed in an environmentally friendly manner.

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 discriminated in any form of service like health.

Code No._____

11.2. Annex II. Consent Form

I have read the information above, or it has been read to me. 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 give my stool

To give my urine

To collect my blood and be a participant in this study and understand that I have the right to withdraw from the study at any time.

Print name of participant, date and signature or thumb impression of participant

_____ ____ /____ /____ (dd/mm/yy) _____

If illiterate;

Print name of independent literate witness, date and signature of witness (if possible, this person should be selected by the participant and should have no connection to the research team)

_____ ____/____/_____ (dd/mm/yy) _____

Phone number_____

Print name of researcher, date and signature of researcher

_____ _____/____/_____ (dd/mm/yy) _____

11.3. Annex III. Questionnaire

Questionnaires to be filled by health professionals

Part I. General information

Code Number_____ Region _____ Zone _____

Woreda_____ / city /_sub city _____ Kebele _____

Part II. Personal information

1. Age (in years)_____
2. Sex _____
3. Place of Birth_____
4. For how long (years) did you live in the birth place? _____
5. How long do you live in this specific area? (If different from the birth place) _____years

No.	Questions	Responses
	Part III. SOCIO-DEMOGRAPHIC INFORMATION	
6.	Educational status	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above
7.	Occupation	1. Student 2. House wife 3. Government employee 4. Private employee 5. Farmer 6. Others (specify) _____
8.	Marital status	1. Single 2. Married 3. Divorced 4. Widowed 5. Not applicable (children)
9.	Religion	1. Orthodox Christian 2. Muslim 3. Protestant 4. Catholic 5. Others (Specify) _____
10.	Ethnicity	_____If mixed, specify_____ _____
11.	Residence	1. Rural 2. Urban
	Part IV. Clinical information	
	Questions 24-28 for female participant who are pregnant specify	
12	Gestation _____(weeks)	
13	Parity _____	
14	Iron supplementation:	1. Yes 2. No

15	Folate supplementation	1. Yes 2. No
16	Iron and folate combined supplementation	1. Yes 2. No
17	Did you take any type of drug for any illness for the last three month?	1. Yes 2. No
18	If yes to Q29, 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) _____
	History of common diseases	
19	History of diabetes	1. Yes 2. No
20	History of Hypertension	1. Yes 2. No
21	History of Blood transfusion for the last 1 year	1. Yes 2. No
22	Any history of blood transfusion	1. Yes 2. No
23	History of Hospital Admission for the last 1 year	1. Yes 2. No
24.	History of Surgical procedure for the last three years?	1. Yes 2. No
25.	History of chronic gastritis	1. Yes 2. No
26.	History of Malaria for the last 6 month	1. Yes 2. No
27.	History of TB for the last two years	1. Yes 2. No
28.	History of Cancer	1. Yes 2. No
29.	History of Cardiac illness	1. Yes 2. No
30.	History of Bleeding disorders	1. Yes 2. No
31.	History of allergy	1. Yes 2. No
32.	History of Wheezing	1. Yes 2. No

Part V. Nutritional habit and your life style

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

How often do you eat the following food? (put a "√ " mark)							
No .	**Food type**	**A**	**B**	**C**	**D**	**E**	**Remarks**

33	Roots and Tuber (Potato, sweet potato, Enset, Cassava)						
34	Legumes (Beans, peas, chicken pea, etc)						
35	Cereals (Corn, Teff, Wheat, sorghum, etc)						
36	Vegetables (Tomato, cabbage, etc)						
37	Fruits (Orange, banana, etc)						
38	Meat (including poultry, fish, etc)						
39	Milk and Milk products (Butter, yoghurt, cheese, etc)						
40	Egg						
41	Tea and/or coffee						

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

		A	B	C	Once a week	D	E
42	Alcohol						
43	*Khat*						
44	Cigarettes						

45.	Do you have Fasting habit?	1. Yes 2. No
46.	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
47.	Did you eat undercooked/raw meat?	1. Yes 2. No
48.	Do you have the habit of physical Exercise?	1. Yes 2. No
49.	If yes, how many times do you do the exercise per week?	1. Daily 2. 2-3 times/week 3. Weekly
50.	Any sexual contact	1. Yes 2. No 3, Not applicable (children)
51.	If yes to Q45, condom use`	1. Yes 2. No
52.	If yes to Q45, condom use`	2. Yes 2. No

	Part VI. Anthropometric measurement	
53.	Height (in cm)	_____
54.	Weight (in kg)	_____
55.	MUAC	_____in cm (will be interpreted later)
56.	Blood pressure (mm Hg)	_____
57.	Body temperature	

❖ We thank you for your cooperation!

NB: anyone who answer 'Yes' to History of common diseases will be excluded from the study

Interview Date: _____

Interviewer's Name: _____ Signature: _____

11.4. Annex IV: - Gaaffii fi deebii (Afan Oromo Version)

Gaaffii fi deebii Ogeessa Fayyaatiin Guutamu

Qajeelfama:

Duran dursee gaaffii kana guutuuf yeroo nuuf laattaniif galatoomaa.

Kaayyoon gaaffii kanaa "Laabooratoorii keessati qulqulinaa wantoota sakata'aamu fi dhiiga nama fayyaa bulessa ta'ee tokko keessati kan argamu hemaatolojii fi kilinikaal keemistirii ilaalchisee hanga argama isa giddu-galessan umurii 5 fi isa ol kan ta'aani lammii Itiyoophiyaaf hojjeechuuf daata funaanuuf ta'a.

Yaada rime qorannoo kana kan burqisiisan Yunivarsiitii Finfinneetti dursaa pirofesaraa kan ta'ani Dokiteer Asteer Tsaggayee yeroo ta'aan kan hojii qorannochaa to'atani immoo tokkummaa ogeessota laaboratoorii Itiyoophiyaa tti.

Baasii qorannochaaf barbaachisu hunda immoo kan danda'e/hagugu Ministeera saayinsii fi teekinolojii federalaa tti. Kanaaf iyyuu deebii sirrii isin yeroo isatti nuuf laattan fiixaan ba'insa qo'annoo keenyaaf shoora guddaa qaba. Dhaabbileen 15 taa'an kana jechuun;Yunvarsiitii, Rijinaal laabooraatoorii fi baankiin dhiigaa biyyolessaa qorannocha deggeruuf qopha'aaniru. Kanaaf iyyuu gaaffilee kana itti gaafatamummaa fi amanamumman akka guuttaniif kabajaan isin gaafanna.

Galatooma!!!

Kutaa 1ffa. Odeeffannoo Waliigalaa

LakkofsaaKoodi_____Naannoo_____Godina_____Aanaa_____

__Magaalaa/Kutaa Magaala_____Ganda_____

Kutaa 2ffa. Odeeffannoo Dhuunfaa

1. Umurii(Waggaa dhaan)_____
2. Saala_____
3. Bakka/Iddoo dhalootaa_____
4. Bakka/iddoo dhalootaa yeroo hagamiif turtanii?_____
5. Bakka amma jiraattan hagamiif turtanii?_____(Bakka dhalootaa keessan irraa adda yoo ta'e)_____waggaa dhaan.

Lakk	Gaaffii	Deebii
	Kutaa 3ffaa Haala Odedeeffannoo Ummataa fi dinagdee	
6.	Haala Sadarkaa barumsa	1. Kan hin baranne 2. Dubbisuuf barreessu kan danda'u/ss 3. Sadarkaa 1ffaa (1-8) 4. Sadarkaa 2ffaa (9-12) 5. Dippiloomaa/digirii fi isaa ol
7.	Haala Hojii	1. Barataa 2. Haadha/abba warraa 3. Hojjetaa/tu Mootummaa 4. Hojii dhunfaa 5. Qonnaan bulaa 6. kan biro yoo ta'e ibsi _____
8.	Haala ga'eelaa	1. Kan hin fuune/heerumne 2. Kan fuudhe/heerumte 3. Kan hiike/hiikte 4. Kan abban warraa irra du'e/haati warra jalaa duute 5. Hin ilaallatu(Daa'immaniif)
9.	Haala Amantaa	1. Ortoodooksii 2. Musilima 3. Piroteestaantii 4. Kaatoolikii 5. kan biro yoo ta'e ibsi _____
10.	Saba	_____/Walmakaa yoo ta'e ibsi
11.	Bakka jireenyaa	1. Baadiyaa 2. Magaalaa
	Gaaffiwwaan dabalataa 7-12 baratootaaf qofaaf	
12.	Umurii Abbaa keeti	_____
13.	Umurii Haadha keeti	_____
14.	Sadarkaa Barnoota kan abbaa keeti	1. Hin baranne 2. Barreesu fi dubbisuu kan danda'u 3. Sadarkaa 1ffaa(1-8) 4. Sadarkaa 2ffaa(9-12) 5. Dippiloomaa/digirii fi isaa ol
15.	Sadarkaa barnoota haadha kee '***gaaffi lakk. 14 keessa filadhu'!***	_____
16.	Haala Hojii Abbaa keeti	1. Barataa 2. Abba Warraa 3. Hojetaa mootummaa 4. Hojii dhuunfa 4. Qonnan Bula 5. Kan biro yoo ta'e ibsi _____
17.	Haala Hojii Haadha keeti 'gaaffi lakk. 16 keessa filadhu!	_____
18.	Galii walii gala Ji'aan(Miinda, kiraa fi kan biro(qarshiidhaan)	_____(qarshiidhaan)
19.	Baayi'ina maati kee	_____
20.	Madda Bishaan dhugaati	1. Kan ujummoo 2. Burqituu 3. Bishaan boolla 4. Bishaan Laga 5. Madda bira yoo ta'e ibsi _____
21.	Gosa Mana jireenyaa keessanii	1. Dhoqqee 2. Simmintoo 3. Muka 4. Xuubi 5. Kan biro yoo ta'e ibsi
22.	Jiraachuu/xuxuqaa beeladoota mana (Fkn. Adurree, Saree)	1. Eeyye 2. Lakki
23.	Jiraachuu beeladoota mana	1. Eeyye 2. Lakki
	Kutaa 4ffaa Haala odeeffannoo fayyummaa/Kilinikaa	
	Gaaffiiwwaan 24-28 dubartoota ulfaa qofa ilaallata	
24.	Erga ulfoftee hagamii?	_____(Torbaniin)
25.	Ulfi kee meeqaffadhaa ?	_____

26.	Ayirenii dabalata fudhateettaa?	1. Eeyye 2. Lakki
27.	Fooleeti dabalata fudhateettaa?	1. Eeyye 2. Lakki
28.	Ayirenii fi Fooleeti walitti fudhateettaa ?	1. Eeyye 2. Lakki
29.	Ji'oota 3 darban keessatti si dhukkube dawa fudhattee beektaa ?	1. Eeyye 2. Lakki
30.	Gaaffiin lakk. 29ffaa deebiin kee eeyee yoo ta'e dawa isa kamiin fudhattee?	1. Farra Pirotozowaa(Busaa) 2. Farra rammoo garaa keessa 3. Farra Alarjii 4. Kiniin/piilsi Qusanno maati 5. Farra Baakteeriyaa 6. Farra daranyoo sombaa 7. Kan biroo yoo ta'e ibsi
	Haala Odeeffannoo Dhukkuba Waliigalaa	
31.	Dhukkuba sukkaraa qabdaa?	1. Eeyye 2. Lakki
32.	Dhukkuba Dhiibbaa dhiigaa qabdaa?	1. Eeyye 2. Lakki
33.	Waggaa 1 darbe keessa dhiiga fudhattee beektaa?	1. Eeyye 2. Lakki
34.	Yeroo kami iyyuu hata'u dhiiga fudhattee beektaa?	1. Eeyye 2. Lakki
35.	Waggaa 1 darbe keessa hospitaala ciiftee beektaa?	1. Eeyye 2. Lakki
36.	Waggoota 3 darbaan keessati yaala baqaqsani hodhuu siif godhameeraa?	1. Eeyye 2. Lakki
37.	Dhukkubii garachaa sirra ture qabdaa?	1. Eeyye 2. Lakki
38.	Ji'oota 6 darban keessati busaa dhukkubsattee beektaa?	1. Eeyye 2. Lakki
39.	Waggoota 2 darbaan keessati dhukkuba daranyoo sombaa dhukkubsattee beektaa?	1. Eeyye 2. Lakki
40.	Dhukkuba Kansarii qabdaa?	1. Eeyye 2. Lakki
41.	Dhukkuba Onnee qabdaa?	1. Eeyye 2. Lakki
42.	Dhukkuba dhiigni dhaabbachuu dhabuu qabdaa?	1. Eeyye 2. Lakki
43.	Dhukkuba Alarjiiki qabdaa?	1. Eeyye 2. Lakki
44.	Dhukkuba qillensi si hanqachuu qabdaa?(yeroo hafuura baafattu sir sir si jedhaa?)	1. Eeyye 2. Lakki

Kutaa 5^{ffaa}/Amala nyaataa fi haala jireenyaa

Gosota nyaata armaan gadi akkamiin nyaataa?(mallattoo kana kaa'i ("√ ")							
Lakk.	Gosa nyaata	1 Guyyatti tokko	2 Guyyatti si'a tokkoo ol	3 Torbanitti si'a 2 hanga 3	4 Darbee-darbee (Fkn.Yeroo ayyana fi qophee adda)	5 Fayya-dame hinbeeku	Yaada
45.	Nyaata hidda fi jirmaa (Mosee,Mixaaxisaa,Qaacho, Kassava						
46.	Nyaata Dheedhii(baqeelaa,atara,shimbura,Missira)						
47.	Callaa (Boqqollo, Xaafii,Qamadii,Bisingaa)						
48.	Kuduraa(Timaatimaa, raafuu, kkf)						
49.	Fudura(Burtukaana,Muuza kkf)						
50.	Foon (kan lukkuu, qurxummii kkf)						

51.	Annaanii fi bu'aawwan annaanii (Itittuu, Dhadhaa,dhama kkf)						
52.	Killee						
53.	Shaayee fi/ykn/ buna						

kan armaan gaditti argamu yeroo hagamiif sorratuu/fayyadamtuu? (mallattoo kana kaa'i ("√ ")

		Guyyaatti si'a 1 (yeroo hunda)	Guyyaatti si'a 1 ol	Torbeeti si'a 2 hanga 3	Torbeetti guyyaa 1	Darbe-darbe(Fakk.Yeroo ayyana fi qophe adda)	Fayyaadame hin beeku
54.	Dhugaatii Alkoolii						
55.	Caatii						
56.	Sigaaraa/Tamboo aarsuu						

	Kutaa 5ffaa tti kan itti….fufe "Amala nyaataa fi haala jireenyaa"	
57.	Amala nyaata lagachuu/tsoomuu qabduu?	1. Eeyye 2. Lakki
58.	Deebiin kee lakk 57 eeyye yoo ta'e haala akkamiin nyaata lagattaa?	1. Kuduraa qofa nyaachuun 2. Nyaata hunda lagachuun isa booda immoo hunda isa nyaachuun 3. Nyaata hunda lagachuun isa booda immoo kuduraa qofa nyaachun
59.	Foon Sirritti hin bilchanne/dheedhii isa ni sorrataa?	1. Eeyye 2. Lakki
60.	Jabina qaama amala shaakalu qabdaa?	1. Eeyye 2. Lakki
61.	Deebin 60ffaa eeyyee yoo ta'e torbanitti hagami shaakala gootaa?	_____
62.	Wal Qunnamtii saalaa rawwattee beektaa?	1. Eeyye 2. Lakki 3.nan hin ilaallatu(Ijoollee)
63.	Deebin kee lakk 62 eeyye yoo ta'e kondomii ni fayyaadamtaa?`	1. Eeyye 2. Lakki
	Kutaa 6ffaa lakkoftu ulfaatina, dheerina, irree fi dhiibba dhiigaa	
64.	Dheerina	_____(Seentimeetiriin)
65.	Ulfaatina qaama	_____(Kiiloo giramaan)
66.	Naannoo irree gidu-galessa isa lakka'u (MUAC)	_____(Seentimeetiriin)
67.	Dhiibba dhiiga (miili meetir meerkurin)	_____(mm Hg)

❖ Waan nu gargaartaniif guddaa galatoomaa!

Guyyaa gaaffii fi deebin itti raawwatame:_____

Maqaa nama gaaffi gaafatee_____Mallattoo:_____

11.5. Annex V: Questionnaire Amharic version (ቃለ መጠይቅ)

በጤና ባለሞያዎች የሚሞላ ቃለ መጠይቅ

መመሪያ፡

በቅድሚያ ይህንን ቃለ መጠይቅ ለመሙላት ለሰጡን ጊዜና ትብብር አድናቆቴን እገልፃለሁ። የዚህ ቃለ መጠይቅ አላማ "በላቦራቶሪ ውስጥ የጥራት መመርመሪያ ንጥረ ነገር እና የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂና የክሊኒካል ኬሚሰትሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው አምስት ዓመትና ከዚያ በላይ ለሆኑ ኢትዬጵያውያን ለመስራት" መረጃ ለመሰብሰብ ነው። የዚህ ጥናት ሃሳቡን ያመጡት የጥናቱ ዋና ተመራማሪ በአዲስ አበባ ዩኒቨርስቲ የህክምና ላቦራቶሪ ትምህርት ክፍል ተባባሪ ፕሮፌሰር የሆኑት ዶ/ር አስቴር ፀጋዬ ሲሆኑ የኢትዮጵያ ህክምና ላቦራቶሪ ማህበር ያስተዳድረዋል። የጥናቱን ወጪ የሸፈነው የፌደራል ሳይንስና ቴክኖሎጂ ሚኒስቴር ነው። ስለሆነም የእርስዎ ቅን ትክክለኛ መልስ በሰዓቱ መስጠት የዚህን ጥናት ስኬት ይወስናል። አስራ አምስት የሚሆኑ ተቋማት ማለትም ዩኒቨርስቲዎች፣ ሪጅናል ላቦራቶሪዎች፣ እና ብሄራዊ የደም ባንክ አገልግሎት ጥናቱን ለመደገፍ ዝግጁነታቸውን ገልፀዋል። ስለሆነም ይህንን ቃለ መጠይቅ ሃቀኝነትና ሃላፊነት በተሞላው መንገድ እንዲሞሉ በትህትና እጠይቃለሁ።

አመሰግናለሁ!!!

ክፍል 1. አጠቃላይ መረጃ

ኮድ_____ ክልል _____ ዞን_____

ወረዳ_____ ከተማ/ክፍለከተማ _____ቀበሌ _____

ክፍል 2. የግል መረጃ

1. እድሜ _____
2. ጾታ_____
3. የትውልድ ቦታ_____
4. በትውልድ ቦታዎ ለምን ያህል ጊዜ ኖረዋል?_____
5. አሁን ያሉበት ቦታ ለምን ያህል ጊዜ ኖረዋል? (ከትውልድ ቦታዎ የተለየ ከሆነ) _____ዓመት

ቁጥር.	ጥያቄ	ምላሽ
	ክፍል 3. ማህበራዊና ኢኮኖሚያዊ መረጃ	
24.	የትምህርት ደረጃ	1. ያልተማሩ 2. ማበብና መፃፍ 3. አንደኛ ደረጃ (1-8) 4. ሁለተኛ ደረጃ (9-12) 5. ኮሌጅ ዲፕሎማ/ዲግሪ እና ከዚያ በላይ
25.	ሥራ	2. ተማሪ 3. የቤት እመቤት 4. የመንግስት ሠራተኛ 5. የግል ተቀጣሪ 6. ገበሬ 7. ሌላ ካለ ይግለፁ _____
26.	የጋብቻ ሁኔታ	1. ያላገቡ 2. ያገቡ 3. የተፋቱ 4. ባል/ሚስት የሞተባቸው 5. አይመለከታቸውም (ህፃናት)
27.	ሃይማኖት	2. ኦርቶዶክስ ክርስቲያን 3. ሙስሊም 4. ፕሮቴስታንት 5. ካቶሊክ 6. ሌላ ካለ ይግለፁ _____
28.	ዘር	_____ድብልቅ ከሆኑ ይግለፁ
29.	መኖሪያ ቦታ	2. ገጠር 2. ከተማ
	ጥያቄ 7-12 ለተማሪዎች ተጨማሪ ጥያቄዎች	
30.	የአባት እድሜ	_____
31.	የእናት እድሜ	_____
32.	የአባት የትምህርት ደረጃ	2. ያልተማሩ 3. ማንበብና መፃፍ 4. አንደኛ ደረጃ (1-8) 5. ሁለተኛ ደረጃ (9-12) 6. ኮሌጅ ዲፕሎማ/ዲግሪ እና ከዚያ በላይ
33.	የእናት የትምህርት ደረጃ (ከተ/ቁ 14 ይምረጡ)	_____
34.	የአባት ሥራ	2. ተማሪ 3. የቤት እመቤት 4. የመንግስት ሠራተኛ

		5. የግል ተቀጣሪ 6. ገበሬ 7. ሌላ ካለ ይግለፁ _____
35.	የእናት ሥራ (ከተ/ቁ 16 ይምረጡ)	_____
36.	ወሃዊ ገቢ (በብር ከደሞዝ፣ ኪራይ፣ እና ሌሎች ገቢዎች)	_____ብር
37.	የቤተሰብ ብዛት	_____
38.	የውሃ ምንጭ	2. ቧንቧ 3. የምንጭ 4. የጉድጓድ 5. የወንዝ 6. ሌላ ካለ ይግለፁ _____
39.	የቤት አይነት	2. ጭቃ 2. ሲሚንቶ 3. እንጨት 4. ጡብ/ሸክላ 5. ሌላ ካለ ይግለፁ _____
40.	የቤት ውስጥ ለማዳ እንስሳ መኖር ወይም ንክኪ (ለምሳሌ ድመት፣ ውሻ)	2. አለ 2. የለም
41.	የቤት እንስሳት መኖር	2. አለ 2. የለም
	ክፍል 4. የጤና መረጃ	
	ከ 24-28 ያሉት ጥያቄዎች ለነፍሰጡር ሴቶች ብቻ ነው	
45.	ከፀነሱ ስንት ጊዜዎ ነው?	_____(ሳምንት)
46.	ለስንተኛ ጊዜ ነው የፀነሱት?	_____
47.	ተጨማሪ ብረት ንጥረነገር	2. አዎን 2. የለም
48.	ተጨማሪ ፎሌት ንጥረነገር	2. አዎን 2. የለም
49.	ተጨማሪ የብረት ንጥረነገር ና ፎሌት	2. አዎን 2. የለም
50.	ባፉት ሶስት ወራ ለማንኛውም ዓይነት ህመም ማንኛውንም ዓይነት መድሃኒት ወስደኋል?	2. አዎን 2. የለም
51.	ለተራ ቁጥር 29 መልስዎ ወስጃለሁ ከሆነ የትኛውን ዓይነት መድሃኒት ነው ወሰዱት? (ከአንድ በላይ መልስ ይቻላል)	2. ፀረ-ፕሮቶዞአ 3. ፀረ-ሄልሚንትስ 4. ፀረ-አለርጂ 5. የወሊድ መከላከያ ኪኒን 6. ፀረ-ባክቴሪያ 7. ፀረ-ቲቢ 8. ሌላ ካለ ይግለፁ _____
	የሚከተሉት የህመም ዓይነቶች አሞዎት ያውቃል?	
52.	የስኳር ህመም?	2. አዎን 2. የለም
53.	የደም ግፊት ከፍ ማለት?	2. አዎን 2. የለም
54.	ባለፈው 1 ዓመት ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም

55.	ማንኛውም ጊዜ ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም
56.	ባለፈው 1 ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1. አዎን 2. የለም
57.	ባለፉት 3 ዓመታት የቀዶ ህክምና ተደርጎልዎ ያውቃል?	2. አዎን 2. የለም
58.	የቆየ የጨጓራ ህመም አለብዎት?	1. አዎን 2. የለም
59.	ባፉት 6 ወራት የወባ ህመም አጋጥሞዎት ያውቃል?	1. አዎን 2. የለም
60.	ባለፉት 2 ዓመታት የቲቢ ህመም ኖሮዎት ያውቃል?	1. አዎን 2. የለም
61.	ካንሰር ህመም	1. አዎን 2. የለም
62.	የልብ ህመም	1. አዎን 2. የለም
63.	የመድማት ችግር/ህመም	1. አዎን 2. የለም
64.	አለርጂ (የሰውነት መቆጣት)	1. አዎን 2. የለም
65.	የመተንፈስ ችግር (ሲተነፍሱ ሲር ሲር የሚል ድምፅ)	1. አዎን 2. የለም

ክፍል 5. የአመጋገብ እና የህይወት ልምድዎ

የሚከተሉትን የምግብ ዓይነቶች ምን ያህል ጊዜ ይመገቧቸዋል ? (“√ “ ይህን ምልክት ያስቀምጡ)							
ተ/ቁ	**የምግብ ዓይነት**	**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**	**ማብራሪያ**
64.	ሥራ ሥር (ድንች፤ ስኳር ድንች፣ እንሰት፣ ካሳቫ ወዘተ)						
65.	አባዝርት (Legumes፡ ባቄል፣ አተር፣ ሽንብራ ወዘተ)						
66.	ጥራጥሬ (በቆሎ፣ ጤፍ፣ ስንዴ፣ ማሽላ)						
67.	አትክልት (ቲማቲም፣ ጎመን፣ ወዘተ)						
68.	ፍራፍሬ (ብርትኳን፣ ሙዝ፣ ወዘተ)						
69.	ሥጋ (የዶሮ፣ የ አሳን ጨምሮ)						
70.	ወተትና የወተት ተዋፅዎ (እርጎ፣ ቅቤ፣ አይብ፣ ወዘተ)						
71.	እንቁላል						
72.	ሻይ እና/ወይም ቡና						
የሚከተሉትን ምን ያህል ይበላሉ/ይጠቀማሉ (√ ይህን ምልክት ያስቀምጡ)							

		በቀን አንድ ጊዜ (ሁልጊዜ)	በቀን ከ1 ጊዜ በላይ	በሳምንት ከ 2 እስከ 3 ጊዜ	በሳምንት 1 ቀን	አልፎ አልፎ (ለምሳሌ፣ ለበዓል፣ ልዩ ዝግጅቶች ሲኖሩ)	ተጠቅሜ አላውቅም
73.	አልኮል						
74.	ጫት						
75.	ሲጋራ						

	ከ ክፍል 5 የቀጠለ. የህይወት አመራርና ልምዶች	
76.	የመፅም ልምድ አለዎት?	2. አዎን 2. የለም
77.	መልስዎ አዎን ከሆነ፣ የመፅም ልምድዎ እንዴት ነው?	4. አትክልቶችን ብቻ መመገብ 5. በአጠቃላይ ከምግብ መታቀብ ከዚያም ያገኙትን መመገብ 6. በአጠቃላይ ከምግብ መታቀብ ከዚያም አትክልቶችን መመገብ
78.	በደንብ ያልበሰለ ወይም ጥሬ ሥጋ ይመገባሉ?	2. አዎን 2. የለም
79.	የሰውነት እንቅስቃሴ የማድረግ ልምድ አለዎት?	2. አዎን 2. የለም
80.	መልስዎ አለኝ ከሆነ በሳምንት ለምን ያህል ጊዜ ይንቀሳቀሳሉ?	
81.	የግብረ ሥጋ ግኑኝነት አድርገው ያውቃሉ	1. አዎን 2. የለም 3, አይመለከትም (ለህፃናት)
82.	ለ ተ/ቁ 66 መልስዎ አዎን ከሆነ፣ ኮንዶም ይጠቀማሉ?`	2. አዎን 2. የለም
	ክፍል 6. ክብደት፣ ቁመት፣ የክንድና የደም ግፊት ልኬት	
68.	ቁመት	_____ሴንቲ ሜትር
69.	ክብደት	_____ኪሎ ግራም
70.	የክንድ መሃለኛው ክፍል ዙሪያው (MUAC)	_____ሴንቲ ሜትር
71.	የደም ግፊት (በሚሊሜትር ሜርኩሪ)	_____(mm Hg)

❖ ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን:_____

ቃለ መጠይቁን ያካሄደው ስም_____ ፊርማ_____

Koodii_____

11.6. Annex VI: Unkaa walgaltee ga'eesotaa umriin isaanii waggaa 18 fi isaa ol ta'aniif

Oddeeffannoon olitti ibsaman dubbiseera ykn naa dubbifameera. Gaaffii gaafadhe haala quubsa ta'en naaf deebi'eera. qorannotti hirmaachuuf itti waligalera.

Sagaraa kennuuf ☐

Fincaan kennuuf ☐

Dhiiga kennuuf ☐ Akkasumaas qoranichatti hirmaachuuf yeroo kamiiyyuu keessas ba'uf/addan kutuuf mirgaa akkan qabu hubadheera ☐

Maqaa hirmaata, Guyyaa fi mallattoo (Ykn mallattoo qubaa) kana gaditti barreessa.

_____ ____ /____ /____ (guyyaa/Ji'a/Bara)

Kan hinbarannee yoo ta'an

Nama baratee garuu qoranicha keessa hingalle ragaaf maqaan isa, guyyaan fi mallattoon isa ni taa'a (haga danda'ametti nami akkasi hirmaatotaan kan filatamuu fi qo'aata walin walitti dhufeenya kan hinqabne yoo ta'e filatamadha.

_____ ____/____/_____ (guyyaa/Ji'a/Bara)

_____

Lakk bilbilaa_____

Maqaa qo'aataa, guyyaa fi Mallattoo _____

_____/____/_____ (guyyaa/Ji'a/Bara) _____

11.7. Annex VII. Laboratory result format

Code _____Region _____

Woreda_____/ city /_sub city _____Kebele _____

Stool examination

Consistency_____

Direct: - _____

Formol ether concentration: -

_____

_____

Urine analysis

Dipstick: Protein___Glucose___Bilu____Spg____Ketone___Leuco___Urob ___Blood ___Nitrite ___PH ___

Microscopy: - _____

_____

HCG (for female): - _____

Blood film _____

Hematology (CBC attach print out)

ABO/RH_____

11.8. Annex VIII:

11.8.1. Operating Procedure

(1) Press [SELECT] key in the Ready status. The Select Menu screen appears.

(2) Using up key or down key, move the cursor to select "7: Maintenance."

(3) Press [ENTER] key. The Maintenance Menu screen appears.

(4) Using up key or down key, move the cursor to select "5: Status Display."

(5) Press [ENTER] key. The Status Display screen 1 appears.

(6) Using left side key or right side key, turn over the page to display the Status Display screen 2.

11.8.2. HGB determination

1. Blood is aspirated from the sample probe into the sample rotor valve.
2. 6 mL of blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.994 mL of diluent. At the same time, 1.0 mL of WBC/HGB lyse is added to prepare 1:500 dilution sample. When the solution is made to react in this status for approximately 10 seconds, RBC is hemolyzed and platelets shrink, with WBC membrane held as they are. At the same time, hemoglobin is converted into red colored methemoglobin.
3. Of the diluted/hemolyzed sample in the WBC transducer chamber, approximately 1 mL is transferred to the HGB flow cell.
4. 500 mL of sample in the WBC transducer is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method.
5. In the HGB flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluent alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin value).

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.

MSc candidate: Solomon Tadesse (B.Sc.)

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Jemal Alemu (MSc, PhD candidate)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.