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Determination of Common Hematological Parameters Reference Intervals among Apparently Healthy Pregnant and Non-Pregnant women of South Wollo Zone, Amhara region, Northeast Ethiopia.

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This is to certify that the thesis prepared by **Mesfin Fiseha**, entitled: **Determination of Hematological Parameters Reference Intervals among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia** and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Hematology and Immunohematology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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Abbreviations

AAU	Addis Ababa University
Ab	Antibody
Ag	Antigen
CDC	Center for Diseases Control and Prevention
CLSI	Clinical Laboratory Standard Institute
CRP	C-reactive protein
EDTA	Ethylene diamine tetra acetic acid
ETB	Ethiopian birr
Gran#	Absolute granulocyte count
Gran%	Granulocyte percentage
HBsAg	Hepatitis B surface antigen
HCG	Human Chorionic Gonadotropin
HCT	Hematocrit
HCV	Hepatitis C virus
Hgb	Hemoglobin
HIV	Human immunodeficiency virus
IFCC	International Federation for Clinical Chemistry and Laboratory Medicine
Lymph#	Absolute lymphocyte count
Lymph%	Lymphocyte percentage
MCH	Mean Cell Hemoglobin
MCHC	Mean Cell Hemoglobin Concentration
MCV	Mean Cell Volume
MID#	Absolute Mixed cells count

MID%	Mixed cells percentage
MPV	Mean Platelet Volume
NCCLS	National Committee for Clinical Laboratories
OOR	Out of Range
PCT	Plateletcrit
PDW	Platelet Distribution Width
PI	Principal Investigator
PLT	Platelet
RBC	Red blood cell
RDW-CV	Red cell distribution width coefficient of variation
RDW-SD	Red cell distribution width standard deviation
RIs	Reference intervals
SOP	Standard operating procedure
SPSS	Statistical package for social science
TP	*Treponema pallidum*
WBC	White blood cell

Abstract

Background: The physiology of a normal pregnancy involves major alterations in the hematological parameters. However, hematological parameters reference intervals currently being used in Ethiopia is derived from western populations, even though significant variations are reported previously.

Objective: To determine hematological parameters reference intervals among apparently healthy pregnant and non-pregnant women of South wollo zone, Amhara region, Northeast Ethiopia.

Method: A community based cross-sectional study was conducted among 600 pregnant and non-pregnant women in South Wollo Zone, Amhara Region, Northeast Ethiopia from June to August 2019. Medical history, physical examination and laboratory screening were performed for each study participant. Socio-demographic and other important data were collected using a structured questionnaire. 4ml of Whole blood was collected and Mindary BC-3000 plus hematology analyzer was used to measure hematological parameters following standard procedures. Data were entered and analyzed using SPSS version 23. The mean, median, and 2.5th percentile and 97.5th reference intervals limits with 90% CI were determined for the hematological parameters. P-value <0.05 was considered statistically significant.

Result: The established reference intervals (2.5th–97.5th percentile) for pregnant women were: WBC: 4.0-13.2x10^3/ul; Lymph %: 12.9-38.13 %; Gran %: 50.45-81.54 %; MID %: 4.24-11.64; RBC: 3.45-4.67x10^6/ul; Hgb: 10.1-13.67 g/dl; HCT: 33.49-46.52 %; MCV: 84.76-103.52 fl; MCH: 27.5-33.0 pg; Platelet count: 131.7-373.15x10^3/ul; MPV: 7.24- 10.16 fl. A statistical significant difference between pregnant and non-pregnant was noted in all hematological parameters except MCHC. The mean and median value of WBC count, absolute granulocyte count and percentage, MCV, MPV and PDW increased, whereas mean values of HCT and Platelet count decreased as gestational period advance.

Conclusion: The findings of this study indicated that pregnant women hematological parameters reference intervals differ from other studies in Ethiopian and other countries pregnant women reference intervals. Hence, this would signify the necessity for such determinations of local reference intervals for different populations and the health facilities found in south wollo zone should utilized the currently established reference intervals for pregnant women for better care.

Keywords: Reference interval, Hematological parameter, pregnant women, trimester, South Wollo

1. Introduction

1.1Background

Laboratory information assists health care professionals to make suitable evidence based diagnostic and therapeutic judgment for their patient, and it produces a great impact on patient management(1).

Hematological parameters are one of the most commonly requested laboratory tests. They offer information about the production of all blood cells and detect the patient's oxygen carrying capacity through the assessment of red blood cell count (RBC), Hemoglobin (Hgb), Hematocrit (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC). They also indicate about the immune system and white blood cell disorders such as leukemia through the evaluation of the white blood cell count with differential. These tests are valuable in diagnosing anemia, certain cancers, infection, acute hemorrhagic states, allergies and immunodeficiency. Moreover, the tests are also important for monitoring of side effects of certain drugs, response to treatment, and to determine the effects of chemotherapy and radiation therapy on blood cell production and clinical trials including vaccines (2, 3, 4).

For accurate interpretation of hematological tests, one of the most critical elements is the reference interval. It is the common clinical decision support tool. As laboratory results may be interpreted by comparison with reference intervals (RIs), RIs should be established with greater accuracy and quality (5,6,7).

Reference intervals are also named as reference value, reference range and biological reference intervals (8, 9). Clinical laboratory standard institute (CLSI) defines RIs as the interval between, and including, two reference limits designated as the interval of values from the lower reference limit to the upper reference limit (10). The interval is specified in the distribution of values obtained from population of healthy subjects as an interval corresponding to 95% of the population, centered on the median (11).

The values of the hematological parameters can alter due to several pre-analytical, analytical, pathological and physiological factors such as the technique and time of blood collection, transport and storage of the specimens, posture, exercise and methodology and instrument used to obtain the result. Likewise, inherent variable like age, sex, genetic background, environment

(especially altitude), diet and even certain circumstances such as pregnancy affect RIs. Gender differences in RI have been reported for many of the hematological parameters, and among females differences by pregnancy status as well (8, 12, 13, 14).

Pregnancy is a state of carrying one or more developing embryo or fetus within the female womb. It usually lasts about 40 weeks, or just over nine months, as measured from the date of the women's last menstrual period to delivery. It is conventionally divided in to three trimesters; first trimester: week 1-week 12; second trimester: week 13 to week 28 week; third trimester: 29 week-40 weeks and above (15).

Normal pregnancy is accompanying with profound anatomical, physiological, biochemical and endocrine changes that disturb various organs and systems. These modifications are indispensable in serving women to familiarize the state of pregnancy and to assist nurturing and subsistence of the fetus. The physiology of a normal pregnancy involves major alterations in the hematological parameters and biochemical coagulation system. These changes appear to be linked to the development of uteroplacental circulation and offer a protective mechanism in the course of delivery (16, 17).

Hematological parameters manifest the adaptive changes that become important baseline parameters to assess the forthcoming complications during pregnancy; it is considered as one of the factors affecting pregnancy, and its outcome (18, 19). The most common hematological changes perceived in normal pregnancies are physiological anemia, gestational thrombocytopenia and leukocytosis (neutrophilia) (20).

Physiological anemia is the term often used to describe the drop in hemoglobin concentration, HCT and RBC that happens during normal pregnancy due to independent and uneven variations of plasmatic volume and corpuscular volume. Hgb varies with gestational age due to hemodilution and the corresponding compensatory mechanisms. The high Hgb levels in the 1sttrimester are reduced by hemodilution in the second trimester while compensatory mechanisms (maternal plasma volume reduction and increased atrial-natriuretic peptides) raise Hgb in the last trimester. Comparable trends are seen in RBC count and HCT (20, 21).

Gestational thrombocytopenia is encountered in 7-8% of all pregnancies. Platelet (PLT) counts are somewhat lower during pregnancy and with gestational age due to enhanced destruction of PLT leading to younger and larger PLT. Most thrombocytopenia in pregnancy is due to increased destruction of PLT (the Pathophysiological process is not known but is thought to represent an acceleration of platelet consumption via an exaggeration of the physiological process across the placenta, or possibly via a mild immune process). Hemodilution as well contribute to gestational platelet reduction (22, 23).

The other noticeable change in the hematological parameters during pregnancy is leukocytosis, which is due to physiological stress, predominantly related to increased circulation of neutrophils. These elevations arise early in pregnancy and remain elevated throughout pregnancy (16, 24).

Maternal hematological parameters may decide both the outcome of pregnancy and the hematological parameters of the newborn because pregnancy put extreme stress on the hematological system. Therefore, it is necessary to cognize the physiological changes observed in normal pregnant women during the establishment of RI in order to interpret hematological test result correctly (19, 23, 25).

1.2 Statement of the problem

Maternal mortality and morbidity continue to be a substantial problem in low-income countries, in spite of a worldwide focus on the need to improve maternal health (26). One of the utmost underlying causes of maternal mortality is hematological complications like anemia and thrombocytopenia (21, 27, 28). This complications and physiological changes observed in pregnant women need accurate and reliable hematological tests with appropriate RIs. Because it has a critical role in basic management of care, detection of disease earlier, helping of medical providers to make diagnoses, prescribing therapies, monitoring and detection of a medical condition, and recruitment of pregnant women in clinical trial for proper interpretation and decision-making (29, 30, 31).

These measured parameters RIs are influenced not only by individual's factors such as age, sex, life style, trimester etc. but they vary by population and ecological factors such as ethnic background, climate, and altitude as well. They also differ between populations (8, 10, 14). Because of these factors, each laboratory must establish its particular reference intervals based on its instrumentation, methodology and demographics of the population it serves (32). Several studies in different part of the world revealed that there was a variation of hematological parameter reference intervals within and/or between individuals and populations (31, 33, 34, 35, 36, 37, 38).

Furthermore, a study conducted in west Kenya showed that there was 96.3%, 10.6% prevalence of out of range of hemoglobin values when using the new hemoglobin reference intervals of west Kenya pregnant women with using United state and West Kenya non-pregnant reference values, respectively (39). The highest misclassification of pregnant women occurred in WBC count (49.3%), HCT (53.0%) and Hgb (32.4%) when using adult females RIs , as also demonstrated in biochemical, haemostatic and hematological reference interval study, that can lead to unnecessary and potentially dangerous therapeutic actions without determining the real cause of the abnormality. It also increases the risk of overlooking important physiologic alterations resulting from pathological conditions and of misinterpreting normal changes as pathological events (40).

Even if there is a common pregnancy-induced laboratories abnormality (anemia and thrombocytopenia) and significant variations are well documented, very few laboratories routinely provide normal values for pregnant women (41, 42). Indeed, many laboratories in Ethiopia do not use locally derived RIs rather they have been using RIs established from non-Ethiopian participants, mostly from western countries and available in textbooks, inserted leaflet or manufacturer manual) population (42). Failure to use their own locally derived reference intervals leads to misinterpretation of the laboratory test results, incorrect clinical care for the peoples, unnecessary exclusion of eligible participants and over reporting of adverse events in clinical trials (8, 43,44).

However, currently in the present study area there was no any published research on the determination of hematological parameters among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia.

Thus, the aim of this research was to establish locally derived hematological parameters RIs for apparently healthy pregnant and non-pregnant women that would be utilized in south wollo zone, Amhara region, Northeast Ethiopia.

1.3 Significance of the study

The finding of this study will benefit the community of pregnant and non-pregnant women by increasing the accuracy of disease diagnosis that may potentially improve the quality of clinical care and lowering health care cost.

This paper also benefits physician and other health professional to interpret a set of test results for pregnant and non-pregnant women by using this reference interval as a base for comparison and better defining of hematological abnormalities. In addition, it helps to assess patient response to treatment and prescribing drugs more selectively.

The finding of this study will worth researcher to interpret their clinical research findings, selection of eligible participants and identification of adverse events in clinical trials (Vaccine development) related to pregnant and non-pregnant women. Besides, it will be helpful for other researchers who will be interested in doing similar work in other part of the countries by serving as reference material.

2. Literature review

Several studies have demonstrated differences between women and men particularly for red cell counts and their derivatives where women have lower RBC, Hgb and HCT (8, 34, 35, 38). Moreover, because of pregnancy places intense stress on the hematological system, several quantitative hematological changes happen including blood cell counts (RBC, WBC and PLT), HGB level, and HCT, RBC and PLT indices (Mean platelet volume (MPV), platelet distribution width (PDW) and Plateletcrit (PCT)). Therefore, awarance of physiological changes is required while interpreting test results (18, 19).

Ethnic based difference among pregnant women of Caucasian and non-Caucasian were observed for various hematologic parameter in a retrospective review of a large maternal database, study conducted in Pennsylvania. The study illustrated significantly higher values for Hgb concentration among Caucasian than non-Caucasian from the 27 weeks gestation until delivery. At different gestational age, significantly lower for parameters for HCT, MCV, MCH, and MCHC among non-Caucasians than Caucasians but only RDW-CV was higher among non-Caucasians than Caucasians in the study (45).

A reference interval study conducted in Chinese population among normal pregnant women in early and late pregnancy indicated that there is a significant increase in WBC, MPV and decrease in RBC, Hgb and PLT counts RIs during late pregnancy. Moreover, pregnant women in the 1sttrimester had lower medians and reference intervals for WBC, MCV, RDW and MPV and higher medians and reference intervals for RBC, Hgb, HCT, PLT and PCT than pregnant women in the third trimester ($P<0.05$) (40). A similar study done by Abbassi-Ghanavati *et al* demonstrated a difference in established hematological parameter reference interval in the 1st and 3rd trimester of pregnant women. The greatest misclassification was observed for WBC and HCT, which resulted in the exclusion of more than half of participants in the third trimester while using Abbassi-Ghanavati et al RIs. This RIs study did not include 2nd trimester and it recommend that the RIs for most blood parameters be revised to account for the gestational period (40).

Reference intervals determined by parametric method in a prospective, sequential and longitudinal study done in normal pregnancy in Chinese women showed that the Hgb

concentration was higher in the 1st trimester, reached its lower point in the 2nd trimester and began to rise again in the 3rd trimester. In contrary, WBC count had the lowest value in the 1st, rose to its highest value in the 2nd trimester and then start to decline in the 3rd trimester. MCHC and platelet count decreased but MCH and MCV remained relatively constant throughout pregnancy. The study also indicated that the lower reference value for Hgb and platelet count during pregnancy were different from those of white and black women (46).

A study conducted in Beijing on establishing reference intervals for complete blood count parameters in normal pregnant women revealed that there was a dynamic change during trimesters. RBC, HCT, Hgb declined at 1st trimester, reaching their lowest point at 2nd trimester, and began to rise again at 3rdtrimester. WBC, Gran#, RDW-CV and PDW went up from trimester 1 to trimester 3. On contrary, MCHC, Lymph#, PLT and MPV gradually descended during pregnancy. There were statistically significant differences in all hematological parameters between pregnant women and non-pregnant women, regardless of the trimesters (47).

A comparative cross sectional study conducted in India with a study population comprised of 400 normal pregnant women and 400 healthy non-pregnant women (control group). The study showed that there was a statistical significance difference between pregnant and non-pregnant women in WBC, Lymph%, Gran%, RBC, Hgb, HCT, MCV, MCH, MCHC and PLT count. The mean values of the above parameters were 9.881 $x10^3$/ul, 21.62%, 73.06%, 4.23 $x10^6$/ul, 10.27 g/dl, 32.52 %, 77.33 fl, 25.26 pg, 32.6 g/dl and 199$x10^3$/ul respectively for pregnant women, whereas, 8.729 $x10^3$/ul, 27.54 %, 64.86 %, 4.29 $x10^6$/ul, 12.64 g/dl, 34.6 %, 76.78 fl, 26.99 pg, 34.24 g/dl and 293 $x10^3$/ul respectively for non-pregnant women. Gran% were significantly increased in pregnant women compared to non-pregnant women and Platelet count was significantly decreased in pregnant women compared to non-pregnant women (22).

A large cross sectional study done in Northwest Morocco among healthy population of pregnant women noted that there was a statistically significant difference between pregnant women and non-pregnant women in all hematological parameters. The mean value of WBC, Lymph#, RBC, Hgb, HCT, MCV, MCH, MCHC, Platelet count and MPV were 8.18$x10^3$/ul, 2.17$x10^3$/ul, 4.07$x10^6$/ul,11.80 g/dl, 34.73%, 85.28 fl, 29.05 pg, 34.05 g/dl, 234.89$x10^3$/ul and 10.89 fl respectively for pregnant women. Whereas, 7.12$x10^3$/ul, 2.33$x10^3$/ul, 4.51$x10^6$/ul, 13.01 g/dl,

38.61%, 85.82 fl, 28.93 pg, 33.69 g/dl, 243.50x10^3/ul and 11.20 fl respectively for non-pregnant women. The mean values of RBC, Hgb, HCT, MCV, PLT count and MPV were higher in pregnant women than in non-pregnant women. Whereas, MCH, MCHC, WBC count and Gran# were higher in non-pregnant women than pregnant women. In comparison of the average established reference intervals between the 1st, 2nd and 3rd trimester of pregnancy, the existence of a significant variation was noted with regard to all the parameters of the hematological parameters. The mean value of the RBC, Hgb, HCT and MCHC showed a progressive decrease whereas total number of WBC and Gran# showed a progressive increase with gestational age, particularly in the 3rd trimester of pregnancy (37).

A cross sectional survey was carried out to evaluate the values of hematological parameters in non-pregnant women and pregnant women at different trimesters of pregnancy who attended antenatal care at sabratha teaching hospital, Northwest, Libya. The study revealed that there was a statistical significant difference between pregnant and non-pregnant women in the values of WBC, Gran%, Hgb, HCT, MCH and platelet count. The mean values of WBC, MCV and MCH were high during 1st trimester, then decline in the 2nd trimester and increased in the 3rd trimester. Whereas, RBC and MCHC declined in the 1st trimester, then increased in the 2nd trimester and finally decreased in the 3rd trimester of pregnancy. A progressive decline observed in the mean value of Hgb, HCT, Lymph % and MID% as the gestational period progressed from 1st to 3rd trimester, but Gran% increased as gestational period progress. The study concluded a significant change in the Hematological parameters at different trimesters of pregnancy in pregnant women. Therefore, it is essential to monitor and manage these parameters during pregnancy (48).

A cross sectional study conducted in Nigeria, among productive age group of pregnant and non-pregnant study participants found that the normal hematological parameters of pregnant women by gestational age for Hgb concentration decreases when the gestational age of study participants increase from 11.68-12.25 g/dl to 10.89-11.14 g/dl then 10.80-11.07 g/dl during1st, 2nd then 3nd trimesters, respectively. The PLT count decreased from 241.27-285.49x10^3/ul; 229.91-251.38x10^3/ul and 182.33-202.13x10^3/ul during the 1st, 2nd and 3rd trimester of pregnancy, respectively. According to the study, WBC count increases from 5.82-6.73x10^3/ul to 6.30-6.85x10^3/ul then 6.73-7.22x10^3/ul as the gestational age increases from 1st to 2nd and 3rdtrimester respectively but the Gran# were largely increased as the gestational age increases (49). In

contrary, a crossectional study conducted in sudanese healthy pregnant women showed that there was no satistically significant difference in RBC, HGB and platelet count between pregnanat women in the three different trimesters (17).

A study done in Nigeria on 40 pregnant women and 40 non-pregnant women with average age of 27.3 years ranging from 19-34 years showed a statistical significant changes between pregnant and non-pregnant women in the values of Gran%, Lymph%, RBC, HCT, MCV, MCH and PLT. The mean values of WBC, Lymph%, Gran%, RBC, Hgb, HCT, MCV, MCH, MCHC, PLT count and MPV were $6.1x10^3$/ul, 30.2%,62.6%, $3.45x10^6$/ul, 12.8 g/dl, 34.1 %, 95.6 fl, 34.7 pg, 36.2 g/dl,$122x10^3$/ul and 6.3 fl respectively for pregnant women, whereas, $4.2x10^3$/ul, 49.1 %, 47.3 %, $5.2x10^6$/ul, 13.3 g/dl, 39.2 %, 102.2 fl, 36.4 pg, 37.8 g/dl, $198.5x10^3$/ul and 7.2 fl respectively for non-pregnant women. There were significant changes between the trimesters in most of the parameters showing carefulness with pregnant women at any stage of the pregnancy. This study have been used a smaller sample size (50).

A cross sectional study involving pregnant women was conducted from the month of May to August in Central Uganda. The study found that RBCs and Hgb concentration gradually decrease during the 1st two trimesters, but showed a slight increase in the 3rd trimester. There was no significant change in MCV. The total WBC count did not show a statistical change from 1st to the 2nd trimesters, while there was a statistical rise during the 3rd trimester. The PLT count decreased during the 1st to the 3rd trimesters. The mean cell counts for the 1st, 2nd and 3rd trimesters were: WBCs count: $6.66x10^3$/ul, $6.72x10^3$/ul and $7.0x10^3$/ul; Gran#: $4.19x10^3$/ul, $4.36x10^3$/ul and $4.19x10^3$/ul; RBCs count: $4.55x10^6$/ul, $4.22x10^6$/ul and $4.31x10^6$/ul; and PLT count were $209.81x10^3$/ul, $185x10^3$/ul and $174.71x10^3$/ul, respectively. The mean values of Hgb concentration varied across the three trimesters as; 12.46 g/dl, 11.50 g/dl and 11.75 g/dl, respectively. The RBC indices of MCV (fl) and MCH (pg) showed a decrease for the different trimesters as: 90.95, 90.40 and 89.56; and 27.48, 27.40 and 26.38, respectively. The MCHC (g/dl) showed an increase in the second trimester, and the mean values for the first, second and third trimesters were; 30.22, 30.31and 30.15, respectively. The MPV (fl) decreased in the second trimester, and the mean values for the different gestation stages were 7.04, 7.03 and 7.09, respectively (51)

According to a longitudinal cohort sub-study of 120 clinically healthy, HIV-uninfected, self-selected pregnant women seeking antenatal care services at either of two public hospital in western Kenya some hematological parameters, including Hgb and Gran# showed significant variations compared to RIs for non-pregnant women. This study recommend the need to have locally established RIs for this population because use of united state or other western countries RIs markedly increase likelihood of out of range values. However, this study had a limitation of; it did not include a concurrent sample of non-pregnant women from the sample reference population and unable to include first trimester pregnant women (39, 52).

A study investigating Hematological parameters of Sudanese pregnant women attending at Omdurman Al Saudi Maternity Hospital indicated that there was a statistical significant difference between pregnant and non-pregnant women in some the hematological parameters. The mean values of WBC, RBC, Hgb, HCT, MCV, MCH, MCHC and PLT count were $8.3x10^3$/ul,$3.7x10^6$/ul, 9.0 g/dl, 27.7 %, 73.8 fl, 24.4 pg, 33.0 g/dl and $260x10^3$/ul respectively for pregnant women. Whereas, the respective values for non-pregnant women were $7.5x10^3$/ul,$4.2x10^6$/ul, 12.0 g/dl, 35.0 %, 83.5 fl, 28.6 pg, 34.1 g/dl and $260x10^3$/ul. The study revealed that there were significant decreased in RBCs count, Hgb and HCT of pregnant women compared to non-pregnant women and significant decreased in MCV, MCH and MCHC of pregnant women. In contrast, PLT count in pregnant women significantly lower than the non-pregnant women (53).

Changes related to trimester pattern and reference ranges of hematological profile among Sudanese women with normal pregnancy were noted. Accordingly, the mean values of WBC, Gran#, MID#, RBC, MCH, and MCHC were lower in the 1st, then start to increase in 2nd and finally decrease in the 3rd trimester of pregnancy. The respective values were as follows: WBC count (7.69 $x10^3$/ul, 8.45 $x10^3$/ul and 8.36 $x10^3$/ul),Gran# (6.54 $x10^3$/ul, 7.07 $x10^3$/ul and 5.89 $x10^3$/ul), MID# (0.58 $x10^3$/ul, 0.65 $x10^3$/ul and 0.61 $x10^3$/ul), RBC counts (4.30 $x10^6$/ul, 4.35 $x10^6$/ul and 4.08 $x10^6$/ul), MCH (25.16 pg, 26.68 pg and 26.57 pg) and MCHC (30.45 g/dl, 30.80 g/dl and 30.73 g/dl). But the reverse was true for MPV (8.13 fl, 8.10 fl and 8.19 fl) and HCT (35.38 %, 34.43% and 35.17%). The mean values of MCV (82.68 fl, 85.16 fl and 86.40 fl) and PDW (15.57, 15.68 and 16.90) increase steadily throughout pregnancy while Lymph # ($2.20x10^3$/ul, $1.98x10^3$/ul and $1.87x10^3$/ul) and PLT count ($278.02x10^3$/ul, 251.96 $x10^3$/ul and

238.36x10^3/ul) decrease as pregnancy advance. The study also indicated that trimester based reference ranges of RBC, WBC and PLT counts and other hematological indices were mostly parallel to international records (54).

There are limited published studies involving both pregnant and non-pregnant women in the same study in Ethiopia. For example, a cross sectional study conducted in Gondar for developing reference intervals among Human immunodeficiency virus (HIV)-sero negative pregnant women found that platelet count and HCT values were changed as pregnancy advances. There was no statistically significant difference in PLT count, RBC count, Hgb level, MCV, MCH, and MCHC of the pregnant women with their gestation age. With respect to HCT values, a statistically significant difference was observed between different trimesters, with the lowest value observed among the women in the 1st trimester of pregnancy. The mean values of WBC (9.46x10^3/ul, 9.29x10^3/ul and 9.10x10^3/ul) and PLT count (243.87 x10^3/ul, 230.78 x10^3/ul and 223.78 x10^3/ul) decrease as gestational period move from 1st to 3rd trimester. Whereas, mean value of Hgb (12.94 g/dl, 13.07 g/dl and 13.38 g/dl) , HCT (39.18%, 40.53% and 41.96%) and MCV (93.18 fl, 94.19 fl and 94.23 fl) increased as gestational period advances (42).

Institution based cross sectional study carried out at St. Paul's Hospital Millennium Medical college, Addis Ababa with a total of 400 pregnant women to establish immunohematological reference range found that a slight increments in the mean value of WBC and MCV, where as a gradual decrement in platelet count. The mean values of Gran%, Lymph%, MID% and HCT were high in the 1st, then lower in the 2nd and increased in 3rd trimester. RBC, Hgb, MCH and MCHC showed almost constant values (55).

3. Objectives

3.1 General objective

- To determine hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

3.2 Specific objectives

- To establish hematological parameters RIs for apparently healthy pregnant women of South Wollo zone, Amhara region, Northeast Ethiopia from June to August,2019.
- To establish hematological parameters RIs for apparently healthy non-pregnant women of South Wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.
- To analyze trimester related differences of hematological parameters among apparently healthy pregnant women of South Wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.
- To determine the proportion of out of range (OOR) values based on the currently available RIs provided by the company.

4. Hypothesis Testing

1. Ho: All hematological parameter RIs is the same across categories of pregnant and non-pregnant women.
2. Ho: All hematological parameters are the same across categories of trimester of pregnancy (first, second and third).

5. Materials and Methods

5.1 Study Area

The study was conducted in South Wollo zone among pregnant and non-pregnant women with reproductive age (15-49). South Wollo Zone is one of the 11 zones of the Amhara region subdivided administratively in to 20 Woredas (18 rural Woredas and 2-administrative Town).The capital of the zone is Dessie Town, which is situated 400 km and 480km away from the National and regional capitals Addis Ababa and Bahirdar, respectively. South Wollo is bordered on the South by North Shewa and Oromia Region, on the West by West Gojjam, on the Northwest by South Gondar, on the North by North Wollo, on the Northeast by Afar Region, and on the East by the Oromia special zone and the Argobba special woreda. Its highest point is Mount Amba Ferit.

According to the national population and housing census of Ethiopia, the projected population of the zone for 2017 was estimated to be 3,087,132 (525,771 in urban and 2,561,373 in rural). The largest ethnic group reported in South Wollo was the Amhara (94.33%); all other ethnic groups made up 5.67% of the population (56).

South wollo geographically located between latitudes from 10°10‘ N to 38° 28'E longitudes and has a total landmass of 17730.823 km. Dessie, Kombolcha, Kalu and Kutaber were the selected study sites in south wollo zone. The total population of Dessie, Kombolcha, Kalu and Kutaber projected for 2016 were estimated to be 233,927 (Male=115,667 and Female=118,304), 127,499 (Male=63,897 and Female=63,602), 223,013 (Male=112,618 and Female= 110,395) and 111,639 (Male=55,191 and Female=56,448), respectively (56). Moreover, the altitude of Dessie, Kombolcha, Kalu and Kutaber were ranging from 2,450-2,550 meters, 1842-1915 meters, 800-1750 meters and 800-3200 meters above sea level, respectively (57).

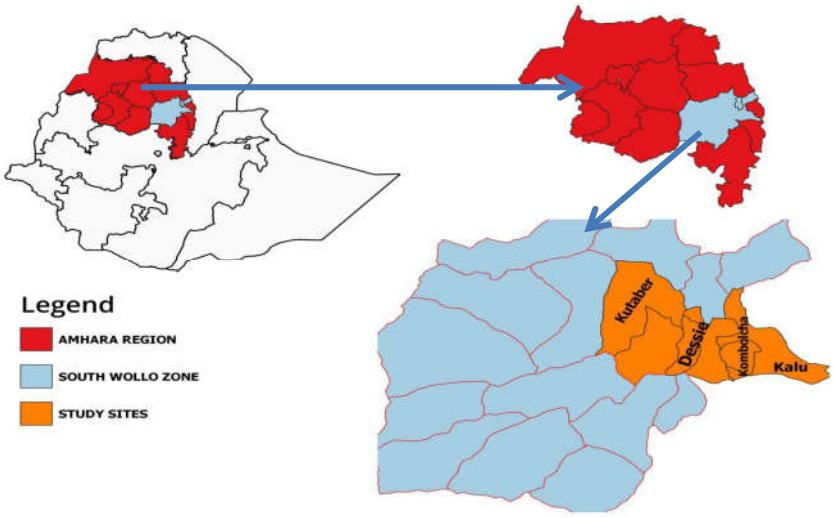

Figure 1: Map of the study area, South Wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

5.2 Study Design and Period

A community based cross sectional study was conducted from June 2019 to August 2019 in South Wollo zone, Amhara region, Northeast Ethiopia.

5.3 Population

5.3.1 Source Population

All pregnant and non-pregnant women who are living in South Wollo zone, Amhara region, Ethiopia, were the source population.

5.3.2 Study Population

The study population were apparently healthy pregnant and non-pregnant women with a reproductive age group (15 – 49) who are living in South Wollo zone during the study period and fulfilling the eligibility criteria.

5.4 Inclusion and Exclusion criteria

5.4.1 Inclusion criteria

Apparently healthy pregnant and non-pregnant women with reproductive age group whom they lived at least for five years in the study area were included. Moreover, willingness of individuals

demonstrated by completion and signing/Thumb printing of the consent form and willingness to provide the sample required.

5.4.2 Exclusion criteria

Individuals were excluded before data collection. Moreover, exclusion was made during the data analysis stage based on questionnaire and screening of viral and parasitic infections. Accordingly, participants were excluded from the study if one of the following criteria is fulfilled:

- ✓ Individuals with history of diabetes mellitus, hypertension, Kidney disease and history of chronic disease in the family, blood transfusion for the last one year, blood donation in the last three month, hospital admission for the last 1 year, hospital surgical procedure for the last three years, chronic gastritis, malaria for the last 6 month, tuberculosis for the last two years, cancer, cardiac illness, bleeding disorder, allergy, wheezing, liver disease and thyroid disease.
- ✓ Individuals who had febrile symptoms, observable mental illness and high blood pressure (> 140 mmHg for diastolic blood pressure and > 90 mmHg for systolic blood pressure).
- ✓ Individuals taking pharmacologically active substances: all prescription drugs (except iron/folic acid for pregnant women since it is routinely given), and had frequent habit of smoking, chewing *Khat* and alcohol consumption.
- ✓ Pregnant women who had active bleeding and obstetrics complications
- ✓ Subjects performing exercise/physical training prior to blood collection.
- ✓ Obese individuals (Body mass index greater than or equal to 30 kg/m^2 according to CDC recommendation (58)).
- ✓ Subjects positive for human immunodeficiency virus (HIV) antigen (Ag) and antibody (Ab), Hepatitis B surface antigen (HBsAg), Hepatitis c virus (HCV), Treponemal (TP) Ab, C-reactive protein (CRP), hemoparasite and intestinal parasite infection like Hookworm.
- ✓ Individuals with frequent exposure for hazardous chemical like gas station and factory workers.
- ✓ Participants with poor quality of Blood specimens (Hemolyed, Lipemic and Clotted)
- ✓ Malnourished and dehydrated individuals
- ✓ In addition, for non-pregnant women, those who were menstruating during data collection and breast-feeding, women taking oral contraceptive were excluded.

5.5 Study variables

5.5.1 Dependent variables

- Common hematological parameters RIs

5.5.2 Independent Variables

- Sociodemographic characteristics, Trimester, pregnancy status (pregnant and non pregnant) and altitude

5.6 Measurement and Data collection

5.6.1 Sample size determination

According to IFCC and CLSI (2000) guideline recommendations, a minimum sample size of 120 observations were needed for determination of reference intervals by using non-parametric method analysis with a power of 90% (10). The research intended to determine the pregnant and non-pregnant women RIs, it needs three partitions (Subjects were divided in to three pregnancy groups, depending on the duration of pregnancy in to the first, second, and third trimester of pregnancy). Furthermore, non-pregnant woman included as a separate partition. Therefore, four partition groups were needed (4x120= 480).

According to previous study conducted in western Kenya to establish reference intervals during normal pregnancy through postpartum, about 20% (30 study subjects exclude from 150 enrolled study participants) of study subjects (39) were not qualifying for the final reference interval determination due to various reasons like HIV infection etc. Considering a 20% exclusion or dropout from data analysis, to reach the CLSI recommended total sample size of 480 for the reference interval determination, the sample size was corrected as follows:

Corrected sample size= Original sample size*(1/ (1-exclusion rate)) (59)

= 480*(1/ (1-0.20))

= 480*(1/0.8) = 600

The total sample sizes that were enrolled in the study were 600 and 150 for each partitions or subgroups were used.

Note: Sample size was allocated proportionally to the total population for selected woredas in eachpartition.

Table 1: Study participants sample size allocation with the respected woredas in each partition, South Wollo zone, Amhara region, Northeast Ethiopia from June to August, 2012.

s/no	Woreda	Total No. of population	Expected number of pregnant	No.of reproductive age women	Number of sample size allocated				Remark
					1st trimester	2nd trimester	3rd trimester	Non-pregnant	
01	Dessie	233,971	9,359	54,474	50	50	50	54	
02	Kombolcha	127,499	5,100	25,500	28	28	28	25	
03	Kalu	223,013	8,921	49,063	48	48	48	49	
04	Kutaber	111,639	4,466	22,328	24	24	24	22	
Total			27,846	151,365	150	150	150	150	

Note: Conversion factor for number of expected pregnancy and reproductive age group in the given population, 4% and 22% conversion factor was used to know the number of expected pregnancy and reproductive age group in the given population, respectively (60). The sample size was calculated and allocated using the following formula.

Number of sample size allocated for each woreda (for pregnant) = (Total number of sample size required for each partition ÷ Total number of expected pregnancy in the selected woredas) X number of expected pregnancy in specific woreda.

Example: Number of sample size allocated for Dessie (**for pregnant**)= (150 X 9359)÷ 27846= 50

Number of sample size required for each woreda (for non-pregnant) = (Total number of sample size required for non-pregnant women partition ÷Total number of reproductive age group in the selected woredas) X number of expected pregnancy in the specific woreda.

Example: Number of sample size allocated for Dessie (**for non-pregnant**)= (150 X 54,474)÷ 151,365= 54

5.6.2 Sampling Method and Techniques

First, by considering altitude (lowland and highland) and residence (urban and rural) difference, four woredas were selected purposively from the study area: Dessie Town (Highland and Urban), Kombolcha Town (Lowland and Urban), Kalu woreda (Lowland and Rural) and Kutaber woreda (Highland and Rural) from the twenty woredas of south wollo zone.

Secondly, the determined sample size was allocated for each selected woredas per partition proportional to their population size. Thirdly, four kebels from Dessie, four kebels from Kombolcha, and two kebeles from Kalu and two kebels from Kutaber were identified conveniently as long as study area was ease to reach and suitable for biological sample transportation. Finally, the reference individuals were recruited by convenient sampling techniques from each selected kebels with the respected partition (first, second and third trimester pregnant women, and non-pregnant women) until the required sample size is attained. While recruiting study subjects, one individual per household per partition was included in the study.

Those selected conveniently were invited to a central location to undertake individual consenting/assenting, demographic and other information, medical history, physical examination, blood sample, urine sample and stool sample collections.

During the selection of reference sample group from the reference population, exclusion takes place until the RIs were established (Before sampling and analyte testing, after sampling and analyte testing, and during data analysis).

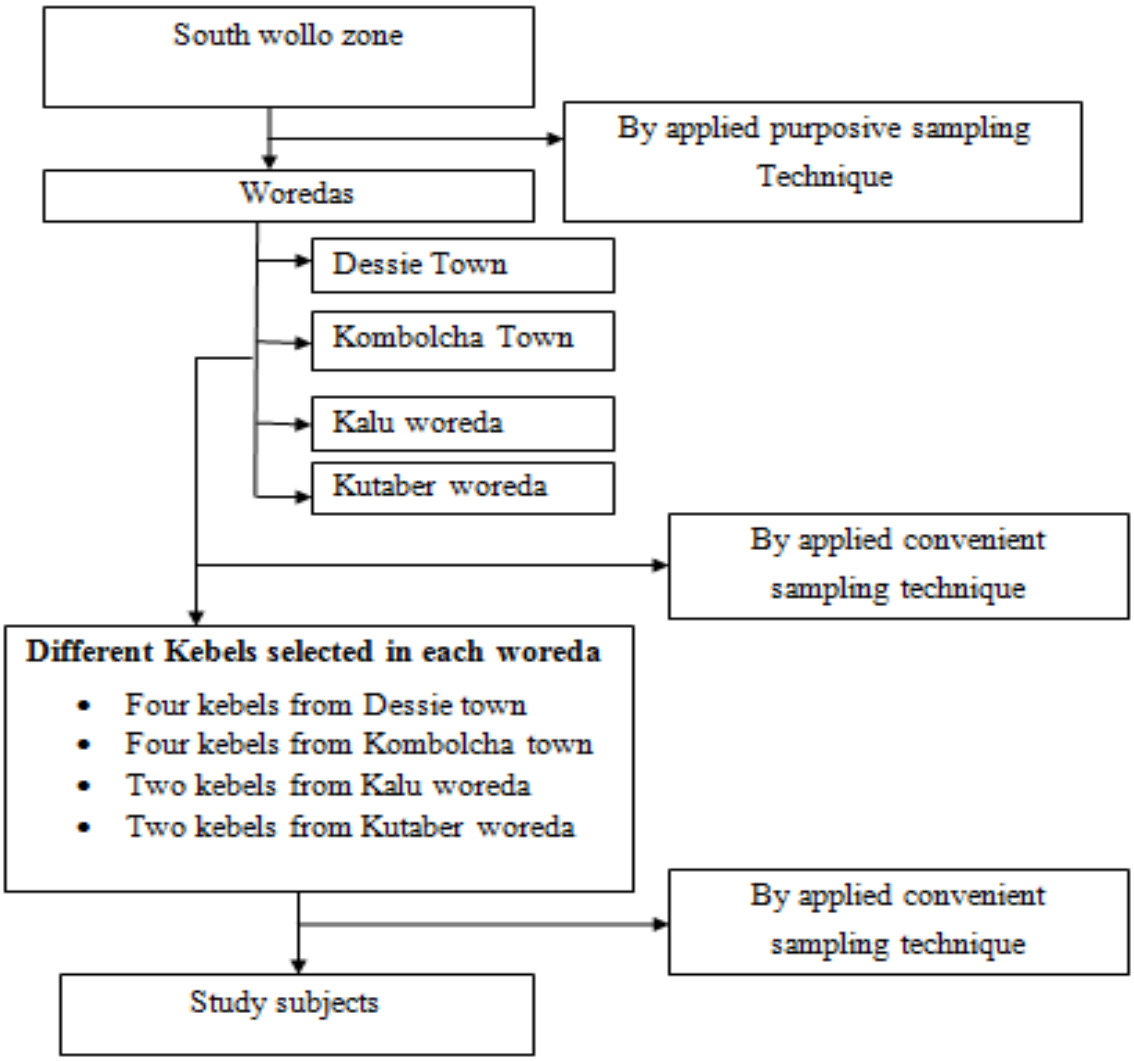

Figure 2: Schematic diagram of sampling procedure in the South Wollo zone, Amhara region, Northeast Ethiopia, 2019.

5.7 Measurement and Data collection

5.7.1 Data collection procedure

Individuals, who were recruited by health extension workers (all potential participants pregnant and non-pregnant women of reproductive age group feeling subjectively well) from the community in the selected kebeles, came to the central data collection center (health center) and contact the data collectors. Initially, the study participants were agreed to give written consent

and assent (signing or thumb printing) (see annex-III, IV, VII, VIII, XI and XII) after being informed about the purpose of the study and associated risks. Secondly, participants underwent physical examination (measuring height, weight, blood pressure and body temperature.) by experienced clinician. Thirdly, socio-demographic, clinical data and other important information for the study was collected using pre-tested interviewer guided structured questionnaire (see annex-XIII and XIV) adopted from CLSI guideline and other related literatures. Finally, eligible participants provided biological specimens like blood, urine and stool specimen for screening of intestinal parasitosis, HIV, HBsAg (Hepatitis B surface antigen), anti-HCV(Hepatitis C virus), pregnancy, and measurement of hematological parameters and others pathological analysis were carried out by experienced laboratory professionals following standard operating procedures (SOPs).

5.7.2 Laboratory Analysis

5.7.2.1 Specimen collection, handling, transportation and storage and processing

After completion of the interview, 4 ml of venous blood had been taken by an experienced laboratory technologist from each study participant in Ethylenediamine tetra acetic acid (EDTA) Vacutainer tubes (Becton-Dickinson, Franklin Lakes, New Jersey, USA) in the morning between 8.00 am and 11.00 am for hematological and serological tests. Moreover, a peripheral blood film was prepared in order to detect morphological abnormalities of blood cell and detection of hemoparasite. Venous blood specimens were collected, mixed thoroughly by gently inverting eight times to ensure mixing with EDTA anticoagulant to prevent clotting. The test tube was appropriately labelled (with a unique identification number starting from 001-600 and site name), placed in vaccine carrier and transported to Dessie health center laboratory at room temperature within 3 hours of collection for analysis. To minimize diurnal variation, specimens were drawn in the morning and processed within 8 hour of collection.

Stool specimens were collected with a clean, wide mouthed container for the detection and identification of intestinal parasites. Once the specimen was collected, immediately direct stool examination was done at the collection site. All enrolled participants provided about 15ml of urine specimen with a clean and screw-capped container for urine pregnancy test, and processed immediately.

All the above specimen collection and processing procedures adhered to good clinical laboratory practices and follow established standard operating procedures(SOP)[see annex-XV].

5.7.2.2 HIV, Syphilis, Hepatitis B and Hepatitis C screening

By using plasma specimen infectious disease screening was performed for HIV-I and II (HIV 1/2 STAT-PAK: Chembio Diagnostic sysstems, INC.Medford, New York, USA; SD BIOLINE HIV 1/2 3.0: SD standard diagnostics, INC. Republic of Korea; ABON HIV 1/2/0 tri-line: Abon Biopharma Co., Ltd. Hangzhou, China.) , HBsAg (ACON Biotech, INC, Co., Ltd. Hangzhou, China), HCV (ACON Biotech, INC, Co., Ltd. Hangzhou, China) and Syphilis (ACON Biotech, INC, Co., Ltd. Hangzhou, China) by rapid test Kits at Kombolcha 03 health center. Its detailed principle, procedure and safety issues were stated in the SOP.[See annexXVI, XVII, XVIII, IXX, XX, XXI].

5.7.2.3 Urine for Pregnancy testing

HCG tests for pregnancy were performed for all female age group between 15 and 49 study participants by using Laboquick pregnancy test strip (Labex Engineering, Ltd, Bulgaria). This test used for qualitative determination of HCG in urine specimens for early detection of pregnancy. Its SOP is found in the annex-XXII.

5.7.2.4 Stool examination

At the collection site direct stool examination was performed by taking specimen from the container and mixing with one drop saline then examine by low and high power objective microscope to detect and identify intestinal parasites. These laboratory procedures adhere to good clinical laboratory practice and follow established SOP. [See annex-XXIII]

5.7.2.5 Blood film examination

From the whole blood specimen, thick and thin smear was prepared and stained with Giemsa, and examined microscopically for hemoparasite and blood cell abnormalities. Brief description of the principle, procedure and interpretation and reporting of the results were stated in the SOP [See annex- XXIV]

5.7.2.6 Hematological parameter analysis

Hematological parameters were measured by Mindray BC-3000 plus, an automated 3-part differential hematology analyzer (Mindray Corporation, Kobe, china), that able to perform 19 parameters at a time(WBC, Lymph#, Mid#, Gran#, Lymph%, Mid%, Gran%, RBC, Hgb, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW, PC and 3 histogram (WBC, RBC, PLT). Mindray BC-3000 plus hematology analyzer operates whole blood mode for venous blood, and pre-diluted mode for capillary blood.

The machine utilized two basic principles for measurement of the parameter: the impedance method for determining the WBC, RBC, and PLT data and the colorimetric method for determining the Hgb.

WBC, RBC and PLT were counted and sorted by the electrical impedance method, which were based on the measurement of changes in electrical impedance produced by a particle passing through an aperture.

The Hgb is determined by the colorimetric method in which the lyse reagent releases Hgb when RBC is broken down and react with Hgb to generate a mixture for Hgb measurement. The WBC/Hgb dilution was delivered to the WBC bath where it is bubble mixed with a certain amount of lyse, which converts hemoglobin to a hemoglobin complex that is measurable at 525 nm. The value of Hgb expressed in g/dl.

The machine automatically dilutes a whole-blood sample, lyses and counts the cells, and then gives a printout result.

Standardization of the instrument and processing of the specimens were done according to the manufacturer's instructions (61). Detail explanation of the analysis is well stated in the SOP annex-XXV.

5.7.2.7 C-reactive protein test

A rapid AVITEX CRP (Omega diagnostics LTD, Scotland, USA) latex agglutination test kits were used for the detection of CRP in the human serum or plasma. (See annex-XXVI for detail information).

5.8 Data quality assurance

5.8.1 Data collection tool quality assurance

For ensuring the quality of data, the questionnaire was translated to Amharic and checked for its consistency through back translation to English by different individuals, and it was tested on 5% of the total sample in a non-study area before the actual data collection processes begin. Based on the feedback, amendment was done to ensure accuracy and consistency. One-health officer and one laboratory technologist was assigned at each collection site as a data collector, and one-health extension worker nominated for mobilization of the study participants in the community together with the principal investigator. Two day training was provided for the data collectors and community mobilizers about the objective of the study, study participants' rights, confidentiality of patient information, procedure of physical examination, procedure of specimen collection and measurements, and how to approach and interview participants before the actual data collection by the principal investigator and experienced clinician. Furthermore, the principal investigator (PI) gave feedback and correction on daily basis for the data collectors. Before they were re-deployed to the field, completeness, accuracy, and clarity of the collected data was checked carefully. Any error, ambiguity and incompleteness encountered were addressed on the following day before starting the next day activities. Each data in the hardcopy transferred into a structured database and statistical software.

5.8.2 Laboratory quality assurance

Pre-analytical quality assurance

It is all of the steps required to deliver the analyte from the sampled environment or patient to the analytical assay. A protocol for sample collection, processing, transportation and storage was strictly followed to have safe procedures and reliable specimens.

Every specimen and questionnaire were assessed for their acceptability and rejection criteria prior to analysis. The rejection criteria were inappropriate sample quality (Hemolyzed, leaked tube, clotted), inadequate or overfilled sample volume, inappropriate sample transportation (delay time and incorrect temperature), incorrect labelling (mislabelled, miss matched with the questionnaire code), collection of specimen in wrong tube and incomplete questionnaires. About 0.01 % of the study participant's specimens were rejected due to hemolysis and clotting of sample.

Analytical quality assurance

The performance of the instrument and reagents was controlled by running quality control prior to the start of the test. During analysis, quality control protocols were followed. As an internal Quality assurance, commercial or in-house quality control specimens were run daily and in every batch. For hematology tests, the three levels of commercial controls by Mindray BC-3000 plus hematology cell controls (Low, Normal and High) were run daily after mixing well by inverting 8-10 times. Test specimens were run if and only if quality control specimens were within range or pass. For blood film examination, known positive and negative slides were performed before the actual test done.

Post analytical quality assurance

Cross checking of the proper verification, recording and entering the result in laboratory result form and in the software was carried out. In addition, inspections of the test result whether it attached appropriately with the respected study participant questionnaire/request form or not was made. Leftover specimens were stored for further analysis.

5.9 Data analysis, Interpretation and Presentation

Once the data recorded on questionnaire were cleaned, edited and checked for completeness, it was entered into Statistical package for social science version 23(SPSS version 23.0, SPSS Inc. Chicago, IL, USA) for statistical analysis. The data was tested for normality of its distribution by Kolmogorov-Sminro test. The qualitative variables were expressed in frequency and percentages in tables, while quantitative variables were expressed by mean, median and percentiles 2.5^{th} and 97.5^{th}.

To identify outliers within each subgroup and parameters Dixon and Reed method was used. The extreme value (outlier) was excluded when the observed values of D were equal to or greater than one-third of the range R, where D is the absolute difference between the extreme distribution and the next value and R is the range (Maximum minus Minimum). Reference intervals were determined according to the guideline of the CLSI/IFCC using nonparametric test methods, 2.5% and 97.5% in a ranked list of reference value data using the formula: lower limit has the rank number 0.025(n+1) and the upper limit the rank number 0.975(n+1) (10). In

addition to this, Out of range (OOR) values were calculated by comparing the finding of the current study with the company-derived values and see how much misclassification occur.

Because of majority of the hematological parameter were showed a non-normal distribution, trimester specific variations of hematological parameters were evaluated using Krusal-Wallis test and Mann-Whitney U test was employed to evaluate pregnant and non-pregnant hematological parameter variations. A two-sided p-value of <0.05 was considered statistically significant.

5.10 Operational Definitions

Apparently healthy participant: Pregnant and non-pregnant women (age between 15 and 49) with the absence of disease or abnormalities based on medical history, physical examination, clinical sign and symptom, and laboratory investigation.

Hematological parameters: A complete blood count that includes WBC, Lymph, Mid, Gran, Lymph%, Mid%, Gran%, RBC, Hgb, HCT, MCV, MCH, MCHC, RDW-CV, RDW-SD, PLT, MPV, PDW and PCT

Reference individual : It is a person/ individual selected for testing/establishing RIs based on well-defined criteria (10).

Reference population: It is a group consisting of all possible reference individuals (10).

Reference sample group: An adequate number of persons selected to represent the Reference population (10).

Trimester: Any of three periods of approximately three months each into which a human pregnancy is divided.

5.11 Ethical consideration

The protocol was ethically approved by research and ethics review committee of Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University. Letter of permission to conduct the study was obtained from South Wollo Zonal Health department, woreda health office and respective kebels catchment health facilities. Written informed consent from each study participants were sought after the study participants informed about the aim, risk and benefits of the study and prior to involving them in the study. Confidentiality was kept and interruption was possible at any stage of the study. Personal identifiers were not used in the

questionnaire and in the lab analysis rather three digits unique identification number (starting from 001 up to 600) known by the participant was deployed. Individuals positive for infections and other disease conditions were linked to the nearby government health facilities for further counseling, diagnosis and treatment accordingly. Study participants were adequately counseled before HIV testing and done by a trained counselor. Participants were provided their results free. (See annex-I, II, V, VI, IX and X- information sheet for non-pregnant age 15-17 years, pregnant and non-pregnant adults and parents/Guardian).

5.12 Dissemination of the result

The result of the study will be presented to Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University (AAU) at a public defense and it will be disseminated to AAU Department of Medical Laboratory Science, South Wollo zone health department and woredas health offices, and to the targeted health facilities through reports. Findings will be presented at scientific conferences. Further attempt will be made to publish the research paper in peer-reviewed journals to access the information for clinicians, academicians, researchers, policy makers, stakeholders and anyone interested in the subject area.

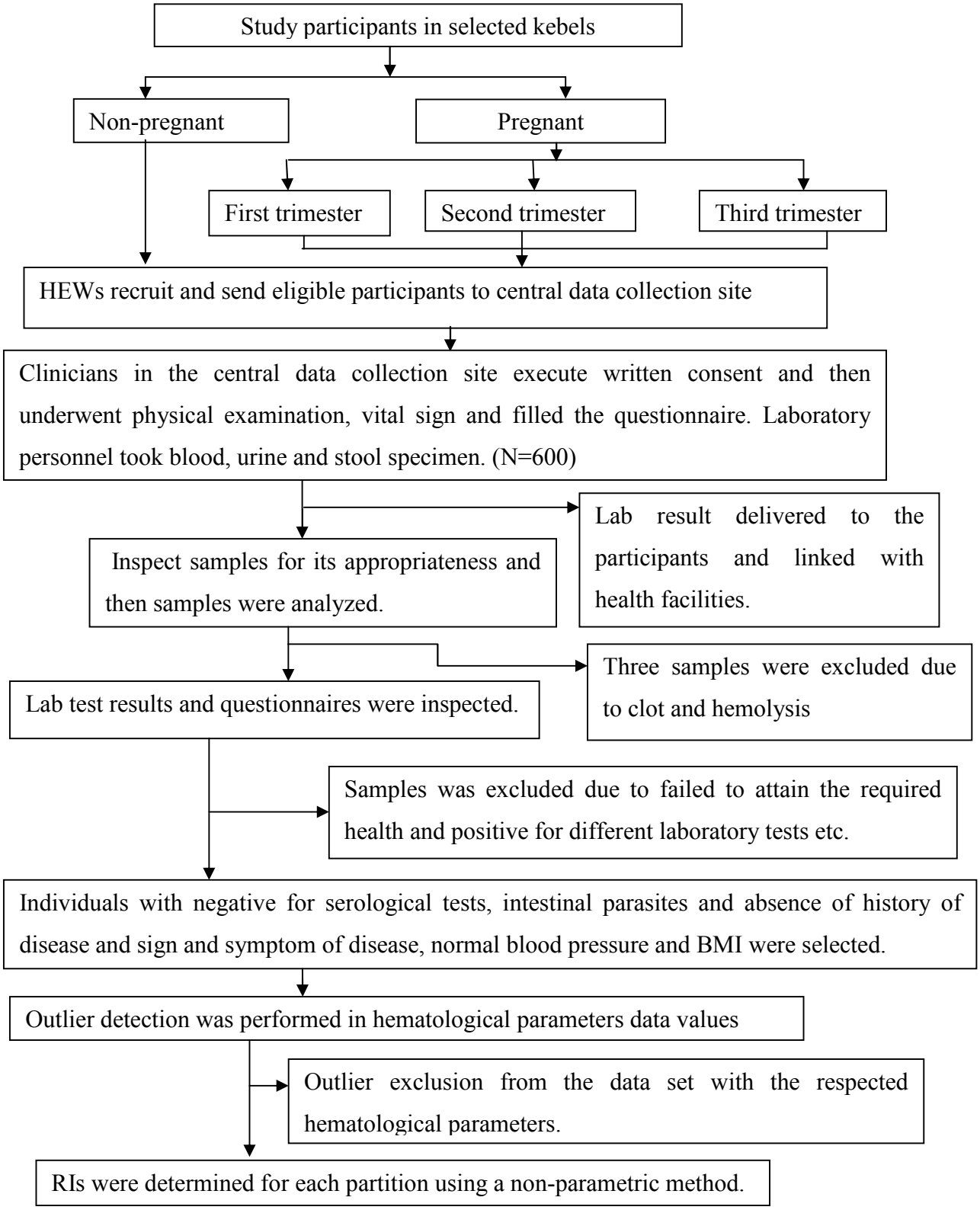

Figure 3: work flow diagram of subject recruitment and determination of reference intervals, South wollo zone, Amhara region, Northeast Ethiopia, 2019.

6. Result

6.1 Socio demographic characteristics of the study subjects

A total of 600 participants were enrolled in the study. Of these, 450 were pregnant and 150 were non-pregnant women. The mean age and monthly income of pregnant women were 26.1 years and 4,430.29 ETB (with ranging between 1,000-25,000 ETB), respectively. Likewise, the mean age and monthly income of non-pregnant women were 25.8 and 3,917.29 ETB (with ranging between 1,300-20,000 ETB). Majority of pregnant women study participants were primary school (38.7%), housewives (69.8%) and married (97.4%) with regard to educational background, occupation and marital status, respectively. However, majority of non-pregnant women study participants were diploma and above (79.3%), government employee (46.7%) and single (54.0%) with respect to educational background, occupation and marital status, respectively. Moreover, majority of the participants both in pregnant and non-pregnant women were urban dweller (52.0%) and lived in at lowland (54.7%). Table 2-depicts the socio demographic characteristics of study subjects in South wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

Of the total 600 subjects who were recruited, 533 (88.8%) fulfilled all of the inclusion criteria were selected in the establishment process of reference intervals and distributed as 136 women in the 1st trimester, 130 women in the 2nd trimester, 131 women in the 3rd trimester of pregnancy and 136 non-pregnant women. The remaining 67 (11.2%) samples were excluded from the study. Of them, 20 (3.33%) in the 2nd trimester, 19 (3.17%) in the 3rd trimester, 14 (2.33% %) in the 1st trimester and 14 (2.33% %) in the non-pregnant women) were excluded due to the following reasons: positive serological tests (HIV, HBsAg, HCV, Syphilis and CRP), history of chronic diseases, recent blood transfusion and donation, obesity, high blood pressure and UTI infection. Additionally, outlier data was also excluded from the final RIs determination but variable numbers of data were excluded for each hematological parameter with the respected partitions.

Table 2: Socio demographic characteristics of study subjects in South wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

Variables	Category	Pregnant women		Non-pregnant women	
		Frequency (*N*= 450)	Percentage (%)	Frequency (*N*= 150)	Percentage (%)
Woredas					
	Dessie	150	33.3	54	36.0
	Kutaber	72	16.0	22	14.6
	Kombolcha	84	18.7	25	16.7
	Kalu	144	32.0	49	32.7
Educational status					
	Illiterate	42	9.3	3	2.0
	Read and write	15	3.3	0	0.0
	Primary	174	38.7	12	8.0
	Secondary	143	31.8	16	10.7
	Diploma and above	76	16.9	119	79.3
Occupation					
	Student	9	2.0	57	38.0
	House wife	314	69.8	15	10.0
	Government employee	55	12.2	70	46.7
	Private employee				
	Farmer	31	6.9	6	4.0
	Merchant	16	3.5	2	1.3
		25	5.6	0	0.0

Marital status	Single	10	2.2	81	54.0
	Married	438	97.4	62	41.3
	Divorced	2	0.3	6	4.0
	Widowed	0	0.0	1	0.7
Religion	Orthodox Christian	143	31.8	74	49.3
	Muslim	303	67.3	76	50.7
	Protestant	4	0.9	0	0
	Others	0	0.0	0	0
Residence	Rural	216	48.0	72	48.0
	Urban	234	52.0	78	52.0
Altitude	Lowland	246	54.7	82	54.7
	Highland	204	45.3	68	45.3

6.2 Anthropometric measurement of study subjects

The mean heights of pregnant and non-pregnant women study participants were similar. However, the mean weight and BMI of pregnant women study participants were higher than its counterpart. Four individuals (two from pregnant and two from non-pregnant) were excluded from the final reference intervals determination as of high blood pressure recorded. Majority of pregnant women (71.56%) BMI were lies within Mean±SD (19.78-25.56). In addition, the majority of non-pregnant women (62.67%) BMI were lies within Mean±SD (19.02-24.06). Three study subjects (from pregnant women only) were excluded from the reference interval

determination due to high BMI. Table 3 showed a descriptive statistics of anthropometric measurements of the study subjects in South wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

Table 3: Descriptive statistics of anthropometric measurements of the study subjects in South wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

Variables	Pregnant women					Non-pregnant women				
	Mean	Median	SD	Range (Min-Max)	Mean±SD	Mean	Median	SD	Range (Min-Max)	Mean±SD
Age in years	26.1	26.0	4.3	18-40	26.1±4.3	25.8	24.0	6.7	17-48	25.8±6.7
Height in centimeter	1.59	1.59	0.06	1.31-1.89	1.59±0.06	1.59	1.58	0.06	1.41-1.79	1.59±0.06
Weight in kilogram	57.03	56.45	8.32	41.0-100.0	57.03±8.32	54.34	53.75	7.67	38.0-75.0	54.34±7.67
BMI in kg/m^2	22.67	22.33	2.89	17.94-40.40	22.67±2.89	21.54	21.02	2.52	18.02-29.29	21.54±2.52
Blood pressure in mmHg										
Systolic blood pressure	106.4	105.0	11.3	78-160	106.4±11.3	108.3	110.0	11.5	80-144	108.3±11.5
Diastolic blood pressure	68.1	70.0	8.2	40-94	68.1±8.2	71.2	70.0	8.7	48-110	71.2±8.7

6.3 Life style and Nutritional habits

Of the total participants of pregnant women, 62.9 % had been taken iron/folate supplementation but 37.1% were not taken iron/folate supplementation. Majority of the study participants both in pregnant and non-pregnant women were never used alcohol (Pregnant=96.7%, Non-pregnant=92.7%),*Khat(*Pregnant=96.7%, Non-pregnant=92.7%) and Cigarette (Pregnant=96.7%, Non-pregnant=92.7%). However, the majority of pregnant and non-pregnant participants were always used legumes (Pregnant=78.2%, Non-pregnant=66.0%), cereals (Pregnant=97.3%, Non-pregnant=94.7%), vegetables (Pregnant=31.3%, Non-pregnant=32.7%), and tea and coffee (Pregnant=86.9%, Non-pregnant=88.7%). Three pregnant women were excluded from RIs determination because they have been used Khat always. Table 4 showed nutritional and others consumption habits of the study subjects in South wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

Table 4: Nutritional and others consumption habits of study subjects in south wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

Type of diet and other consumption habits	Pregnant women		Non-pregnant women	
	Frequency	Percentage (%)	Frequency	Percentage (%)
Roots & tubers				
Always (once a day –regular)	45	10.0	10	6.7
2 or 3 times a week	33	7.3	24	16.0
Once a week	138	30.7	31	20.7
Occasionally	216	48.0	82	54.6
Never	18	4.0	3	2.0
Legumes				
Always (once a day –regular)	352	78.2	99	66.0
2 or 3 times a week	7	1.6	6	4.0
Once a week	15	3.3	7	4.7
Occasionally	64	14.2	35	23.3
Never	12	2.7	3	2.0

Cereals				
Always (once a day –regular)	438	97.3	142	94.7
2 or 3 times a week	3	0.7	2	1.3
Once a week	2	0.4	1	0.7
Occasionally	7	1.6	5	3.3
Never	0	0.0	0	0.0
Vegetable				
Always (once a day –regular)	141	31.3	49	32.7
2 or 3 times a week	63	14.0	36	24.0
Once a week	134	29.8	35	23.3
Occasionally	107	23.8	26	17.3
Never	5	1.1	4	2.7
Fruits				
Always (once a day –regular)	72	16.0	12	8.0
2 or 3 times a week	51	11.3	23	15.3
Once a week	137	30.4	50	33.3
Occasionally	181	40.2	64	42.7
Never	9	2.0	1	0.7
Meat				
Always (once a day –regular)	18	4.0	1	0.7
2 or 3 times a week	36	8.0	12	8.0
Once a week	77	17.1	15	10.0
Occasionally	311	69.1	121	80.7
Never	8	1.8	1	0.7
Milk and milk product				
Always (once a day –regular)	73	16.2	12	8.0
2 or 3 times a week	22	4.9	9	6.0
Once a week	50	11.1	14	9.3
Occasionally	202	44.9	94	62.7
Never	103	22.9	21	14.0

Egg				
Always (once a day –regular)	59	13.1	13	8.7
2 or 3 times a week	38	8.4	20	13.3
Once a week	82	18.2	24	16.0
Occasionally	218	48.4	87	58.0
Never	53	11.8	6	4.0
Tea and coffee				
Always (once a day –regular)	391	86.9	133	88.7
2 or 3 times a week	4	0.9	1	0.7
Once a week	11	2.4	2	1.3
Occasionally	27	6.0	10	6.7
Never	17	3.8	4	2.7
Alcohol				
Always (once a day –regular)	0	0.0	0	0.0
2 or 3 times a week	0	0.0	1	0.7
Once a week	3	0.7	1	0.7
Occasionally	12	2.7	9	6.0
Never	435	96.7	139	92.7
Khat				
Always (once a day –regular)	3	0.7	0	0.0
2 or 3 times a week	0	0.0	0	0.0
Once a week	5	1.1	1	0.7
Occasionally	32	7.1	2	1.3
Never	410	91.1	147	98.0
Cigarette				
Always (once a day –regular)	0	0.0	0	0.0
2 or 3 times a week	0	0.0	0	0.0
Once a week	0	0.0	0	0.0
Occasionally	2	0.4	0	0.0
Never	448	99.6	150	100.0

Iron /Folate supplementation				
Yes	283	62.9	0	0.0
No	167	37.1	150	100.0

6.4 Determination of hematological parameter RIs

Mean, Median, 95 % (2.5th -97.5th) RIs with 90% CI for lower and upper reference limit, P-value of hematological parameters of pregnant and non-pregnant women, pregnant women with the respected trimester partitions are presented in different tables.

6.4.1 Hematological parameter RIs in pregnant women and non-pregnant women

As shown in Table 5, the overall median and 2.5th - 97.5th percentile RIs of WBC, Lymph #, MID #, Gran #, Lymph %, MID % and Gran % were 8.1(4.00-13.21x10^3/µl), 1.9 (1.1-2.71 x10^3/µl), 0.5 (0.2-1.0 x10^3/µl), 5.8 (2.2-9.83 x10^3/µl), 22.5 (12.9-38.14%), 7.4 (4.24-11.64%) and 70.35 (50.45-81.54%) in pregnant women, respectively. Whereas, the respective values in non-pregnant women were: 6.45 (3.64-10.33 x10^3/µl), 2.3 (1.24-3.7 x10^3/µl), 0.5 (0.2-0.8 x10^3/µl), 3.5 (1.28-6.88 x10^3/µl), 35.65 (19.87-57.05%), 6.8 (3.9-10.9%) and 56.1 (33.42-71.84%). There were a statistically significant differences (*P-value*< 0.05) between pregnant and non-pregnant women in all white blood cell parameters (WBC, Lymph #, MID #, Gran #, Lymph %, MID % and Gran %). Pregnant women had a higher median value of WBC (8.1 Vs 6.45x10^3/µl), Gran # (5.8 Vs 3.5x10^3/µl), Gran% (70.35 Vs 56.1), MID% (7.4 Vs 6.8) and MID # (0.5 Vs 0.4x10^3/µl) than their counterpart, whereas non-pregnant women had higher median values for Lymph # (2.3 Vs 1.9x10^3/µl) and Lymph% (35.5 Vs 22.5%) than pregnant women. Likewise, pregnant women had higher mean value of WBC, Gran #, Gran %, MID % and MID # than their counterpart, whereas non-pregnant women had higher mean values for Lymph # and Lymph % than pregnant women.

The median and 2.5th - 97.5th percentile RIs of RBC and the respective indices are also shown in Table 5. There was a statistically significant difference between pregnant and non-pregnant women for RBC count (P=0.000), Hgb (P=0.000), HCT (P=0.000), MCV (P=0.001), MCH (P=0.036), RDW-CV (P=0.000) and RDW-SD (P=0.001) but there was no statistical significant difference between pregnant and non-pregnant in MCHC (P=0.590). Pregnant women had a

lower median value of Hgb (11.9 Vs 13.1 g/dl), HCT (40.4 Vs 44.6%) and RBC count (3.52 Vs $4.76x10^6/\mu l$) than non-pregnant women had. Pregnant women had a higher median value of MCV (94.7 Vs 92.6 fl), MCH (30.2 Vs 29.8 pg), RDW-CV (14.7 Vs 12.4) and RDW-SD (48.3 Vs 45.4) than the non-pregnant women. Similarly, pregnant women had a lower mean value of RBC, Hgb and HCT than non-pregnant women, but pregnant women had a higher mean value of MCV, RDW-SD and RDW-CV than the non-pregnant women.

As indicated in table 5, there was a statistical significant difference between pregnant and non-pregnant women in the values of Platelet count, MPV, PDW and PCT. Pregnant women had a higher median value of MPV (8.7 Vs 8.5 fl) and PDW (15.8 Vs 14.0) than non-pregnant women. On the other hand, the median value of platelet count (304 Vs $253x10^3/\mu l$) and PCT (0.267 Vs 0.217) were higher in non-pregnant women than pregnant women. The median and 2.5th -97.5^{th} percentile RIs of platelet count, MPV, PDW and PCT of pregnant women are summarized in the table 5.

Table 5: Mean, Median, 95% (2.5th- 97.5th) RIs with 90% CI lower and upper reference limit of hematological parameters of pregnant and non-pregnant women of South Wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

Parameter		N	Mean	Median	RI (2.5^{th}-97.5^{th})	90% CI		P-value
						LLC	ULC	
WBC ($x10^3/\mu l$)	Pregnant	395	8.3	8.1	4.00-13.21	3.7-4.4	12.7-15.1	0.000*
	Non- pregnant	136	6.56	6.45	3.64-10.33	3.5-3.9	9.7-11.1	
Lymph# ($x10^3/\mu l$)	Pregnant	395	1.88	1.9	1.1-2.71	1.0-1.1	2.7-3.0	0.000*
	Non-pregnant	136	2.34	2.3	1.24-3.7	1.1-1.4	3.4-3.7	
MID# ($x10^3/\mu l$)	Pregnant	393	0.6	0.5	0.2-1.0	0.2-0.3	1.0-1.1	0.020*
	Non-pregnant	135	0.47	0.4	0.2-0.8	0.1-0.3	0.7-0.8	
GRAN# ($x10^3/\mu l$)	Pregnant	393	5.81	5.8	2.2-9.83	1.9-2.4	9.6-11.6	0.000*
	Non-pregnant	135	3.7	3.5	1.28-6.88	1.0-1.6	6.2-7.2	
Lymph (%)	Pregnant	384	23.4	22.5	12.9-38.13	11.7-13.4	37.2-41.4	0.000*
	Non-pregnant	134	36.6	35.65	19.87-57.05	13.9-23.3	54.1-62.1	
MID (%)	Pregnant	390	7.6	7.4	4.24-11.64	3.9-4.7	11.2-12.9	0.002*
	Non-pregnant	135	6.9	6.8	3.9-10.9	3.7-4.0	10.7-12.0	
GRAN (%)	Pregnant	390	69.3	70.35	50.45-81.54	49.3-52.8	81.2-84.9	0.000*
	Non-pregnant	135	55.5	56.1	33.42-71.84	27.7-38.0	70.1-75.5	
HGB (g/dl)	Pregnant	394	10.9	11.9	10.1-13.67	9.9-10.4	13.31-13.98	0.000*
	Non-pregnant	135	13.8	13.1	12.4-14.3	11.8-12.6	14.1-14.6	

RBC ($x10^6/\mu l$)	Pregnant	396	3.5	3.52	3.45-4.67	3.36-3.52	4.45-4.98	0.000*
	Non-pregnant	134	4.7	4.76	4.44-5.01	4.21-4.83	4.91-5.23	
HCT (%)	Pregnant	396	40.2	40.4	33.49-46.52	32.3-34.0	46.1-49.7	0.000*
	Non-pregnant	134	44.2	44.6	38.44-50.09	37.3-39.1	48.8-50.9	
MCV (fl)	Pregnant	391	94.71	94.7	84.76-103.52	83.7-87.2	103.0-106.6	0.001*
	Non-pregnant	135	92.3	92.6	86.1-101.64	83.5-86.7	99.4-102.8	
MCH (pg)	Pregnant	370	30.3	30.2	27.5-33.00	27.2-27.7	32.8-33.9	0.036*
	Non-pregnant	133	29.7	29.8	27.135-32.36	26.1-27.4	31.9-33.3	
MCHC (g/dl)	Pregnant	389	31.91	31.9	30.30-33.73	30.1-30.3	33.6-34.2	0.590
	Non-pregnant	135	31.97	31.9	30.4-34.06	30.1-30.5	33.7-34.1	
RDW-CV	Pregnant	381	14.9	14.7	12.5-16.145	12.4-12.6	15.9-16.7	0.000*
	Non-pregnant	127	12.31	12.4	12.14-14.68	12.0-12.4	14.5-15.0	
RDW-SD	Pregnant	389	48.98	48.3	42.1-58.2	41.1-42.1	57.3-60.9	0.001*
	Non-pregnant	133	45.5	45.4	39.72-57.3	38.4-41.1	55.5-58.2	
Platelet ($x10^3/\mu l$)	Pregnant	393	255.5	253	131.7-373.15	125-155	369-403	0.000*
	Non-pregnant	136	310.4	304	173.28-456.30	171-206	429-463	
MPV (fl)	Pregnant	395	8.8	8.7	7.24-10.16	7.0-7.6	10.0-10.4	0.024*
	Non-pregnant	136	8.6	8.5	7.09-10.12	6.8-7.3	10.1-10.8	
PDW	Pregnant	393	15.81	15.8	15.2-16.4	15.1-15.3	16.4-16.6	0.000*
	Non-pregnant	133	14.52	14.0	14.1-15.5	14.0-14.4	15.1-16.1	
PCT	Pregnant	384	0.216	0.217	0.121-0.316	0.110-0.136	0.306-0.336	0.000*
	Non-pregnant	136	0.269	0.267	0.168-0.382	0.161-0.193	0.349-0.390	

LLC-Lower limit confidence interval, ULC- Upper limit confidence interval
WBC-white blood cell count, Lymph# - Absolute lymphocyte count, MID# - Absolute mixed cell count, Gran# -Absolute granulocyte count, Lymph% - lymphocyte percentage, MID% - mixed cell percentage, Gran% - granulocyte percentage, Hgb- hemoglobin, RBC- red blood cell count, HCT- hematocrit, MCV- mean cell volume, MCH- mean cell hemoglobin, MCHC- mean cell hemoglobin concentration, RDW-CV- red cell distribution width coefficient of variation, RDW-SD- red cell distribution width standard deviation, MPV- mean platelet volume, PDW- platelet distribution width, PCT- Plateletcrit
Mann-Whitney U-test for non-normally distributed parameters was done between pregnant and non-pregnant women: all hematological parameters except MCHC showed significant differences between pregnant and non-pregnant women.
$P < 0.05$ was considered as statistically significant.
*Statistically significant
Note: The number of reference subjects varied for each parameter because of exclusion of variable number of outliers.

6.4.2 Hematological parameter RIs in pregnant women according to trimester

Table 6 presented that the mean, median, RI (2.5^{th}-97.5^{th}), 90% upper and lower reference limit and P-values between trimester, 1^{st} Vs 2^{nd}, 1^{st} Vs 3^{rd}, and 2^{nd} Vs 3^{rd} trimester of pregnant women. Total WBC count and Lymph % showed a statistically significant difference between trimesters, 1^{st} Vs 2^{nd}, 1^{st} Vs 3^{rd} but not between 2^{nd} Vs 3^{rd}. The median values of total WBC count (7.7 $x10^3/\mu l$ in the 1^{st}, 8.2 $x10^3/\mu l$ in the 2^{nd}, 8.6 $x10^3/\mu l$ in the 3^{rd} trimester) increased as gestational period progress. The median values of Lymph # and Lymph % had the increased in the 1^{st} (2.0 $x10^3/\mu l$ and 24.9%), decreased in the 2^{nd} trimester ($1.8x10^3/\mu l$ and 20.8%) and then increased in the 3^{rd} trimester ($1.9x10^3/\mu l$ and 22.02%). Gran # and Gran % showed a statistical significant difference between trimesters, 1^{st} Vs 2^{nd}, 1^{st} Vs 3^{rd}, and 2^{nd} Vs 3^{rd} trimester of pregnant women. The median and mean values of WBC, Gran # and Gran % increased as gestational age advances. The mean values of Lymph # and Lymph % had the higher in the 1^{st}, lower in the 2^{nd} trimester and then higher in the 3^{rd} trimester.

RBC count, Hgb and HCT showed a statistically significant difference between trimesters, 1^{st} Vs 2^{nd}, 1^{st} Vs 3^{rd}, and 2^{nd} Vs 3^{rd} trimester of pregnant women. The median values of RBC count and Hgb were greater in 1^{st} trimester ($3.86x10^6/\mu l$ and 12.3 g/dl), low in 2^{nd} trimester ($3.49x10^6/\mu l$ and 11.6 g/dl) then increased in the 3^{rd} trimester ($3.78x10^6/\mu l$ and 12.7 g/dl). However, the mean and median values of HCT decreased as gestational age increases. There was no a statistically significant difference in MCH, MCHC, RDW-CV and RDW-SD between trimesters, 1^{st} Vs 2^{nd}, 1^{st} Vs 3^{rd}, and 2^{nd} Vs 3^{rd}. On the other hand, MCV showed statistically significant variations between trimester and its mean and median values increases as gestational period advances.

As shown in table 6, platelet count revealed that there was a statistically significant difference between trimesters, 1^{st} Vs 2^{nd}, 1^{st} Vs 3^{rd}, and 2^{nd} Vs 3^{rd} trimester of pregnant women. MPV and PDW showed statistically significant variations between trimesters. The mean and median value of platelet count was decreased as gestational period goes up. In the contrary, MPV and PDW increased as gestational period advance.

Table 6: Mean, Median, 95%(2.5th-97.5th) RIs with 90% CI lower and upper reference limit of hematological parameters stratified by gestational period (trimester) of South Wollo zone, Amhara region, Northeast Ethiopia, From June to August, 2019.

Parameter	Trimester	N	Mean	Median	RI (2.5^{th}-97.5^{th})	90% CI		P-value			
						LLC	ULC	1^{st}& 2^{nd}	1^{st}& 3^{rd}	2^{nd}& 3^{rd}	1^{st}, 2^{nd}& 3^{rd}
WBC ($x10^3/\mu l$)	1^{st}	135	7.9	7.7	3.64-13.24	3.3-4.3	12.1-15.4	0.019*	0.006*	0.724	0.001*
	2^{nd}	130	8.5	8.2	4.56-13.59	4.0-5.0	13.1-15.2				
	3^{rd}	131	8.7	8.6	4.56-13.62	3.5-5.1	12.2-14.1				
Lymph# ($x10^3/\mu l$)	1^{st}	135	2.0	2.0	1.1-2.8	0.9-1.2	2.7-2.9	0.004*	0.020*	0.562	0.001*
	2^{nd}	130	1.79	1.8	1.03-2.6	0.7-1.2	2.6-2.8				
	3^{rd}	130	1.92	1.9	1.13-2.77	1.0-1.3	2.7-3.0				
MID# ($x10^3/\mu l$)	1^{st}	132	0.50	0.5	0.2-0.9	0.2-0.3	0.8-0.9	0.066	0.059	0.923	0.198
	2^{nd}	129	0.56	0.5	0.2-1.08	0.2-0.3	0.9-1.1				
	3^{rd}	129	0.57	0.5	0.2-1.08	0.2-0.3	1.0-1.1				
GRAN# ($x10^3/\mu l$)	1^{st}	130	5.5	5.3	2.23-8.62	2.1-2.4	7.8-10.2	0.002*	0.004*	0.012*	0.002*
	2^{nd}	128	6.1	5.9	2.42-9.78	2.2-2.7	9.5-10.6				
	3^{rd}	131	6.3	6.2	2.61-10.23	2.3-2.9	9.6-10.8				
Lymph (%)	1^{st}	135	26.48	24.9	12.78-45.60	11.4-14.0	42.1-49.8	0.001*	0.002*	0.106	0.001*
	2^{nd}	126	21.41	20.8	10.96-32.96	9.0-13.4	30.5-35.4				
	3^{rd}	128	23.03	22.2	13.53-45.68	12.3-14.9	44.3-48.1				
MID (%)	1^{st}	135	6.8	6.8	3.94-12.00	2.8-4.5	11.9-12.9	0.451	0.990	0.548	0.769
	2^{nd}	126	6.7	6.65	3.74-9.60	3.0-4.4	9.4-9.7				
	3^{rd}	130	7.0	6.8	3.83-12.33	3.2-4.4	10.8-12.7				
GRAN (%)	1^{st}	135	67.8	65.8	45.86-80.42	44.3-48.7	80.1-82.2	0.001*	0.007*	0.003*	0.001*
	2^{nd}	127	71.7	70.4	58.62-81.50	55.0-61.2	81.3-84.9				
	3^{rd}	131	72.1	71.9	60.53-82.55	58.7-62.1	81.6-85.3				
HGB	1^{st}	136	11.1	12.3	10.37-13.53	10.0-10.8	13.2-14.4	0.005	0.050	0.006*	0.016*

(g/dl)	2nd	130	10.4	11.6	9.99-12.90	9.5-10.4	12.3-13.2	*	*		
	3rd	131	11.9	12.7	10.68-13.71	10.3-10.9	13.34-14.1				
RBC ($x10^6/\mu l$)	1st	135	3.94	3.86	3.58-4.90	3.52-3.68	4.35-5.01	0.001*	0.004*	0.009*	0.001*
	2nd	128	3.45	3.49	3.35-4.01	3.31-3.40	3.87-4.31				
	3rd	130	3.79	3.78	3.76-4.99	3.32-3.98	4.89-5.15				
HCT (%)	1st	136	41.71	41.05	34.86-47.80	31.6-35.7	46.1-49.7	0.007*	0.048*	0.008*	0.024*
	2nd	129	40.56	40.3	33.93-46.19	32.5-34.9	45.3-49.6				
	3rd	131	39.42	39.9	32.33-45.98	30.9-33.6	45.3-47.8				
MCV (fl)	1st	133	94.55	94.2	86.67-103.03	84.7-89.3	100.9-104.8	0.067	0.030*	0.927	0.02*
	2nd	126	94.72	94.4	86.10-103.58	83.9-88.8	102.5-106.6				
	3rd	129	95.96	95.0	87.62-105.77	84.0-89.8	103.0-107.8				
MCH (pg)	1st	130	29.94	30.0	26.40-32.94	26.3-27.2	32.3-33.8	0.121	0.204	0.167	0.055
	2nd	124	30.20	30.1	26.89-33.20	26.4-28.0	33.0-34.3				
	3rd	124	30.51	30.4	27.51-33.99	26.8-28.0	33.6-34.4				
MCHC (g/dl)	1st	135	32.16	32.5	30.30-33.66	29.7-30.8	33.7-34.6	0.321	0.559	0.270	0.234
	2nd	124	31.86	31.9	30.13-33.2	30.0-30.5	33.1-33.5				
	3rd	130	31.69	32.6	30.31-33.86	29.9-30.5	33.2-34.1				
RDW-CV	1st	134	14.07	14.1	12.44-15.99	12.1-12.5	15.7-16.6	0.075	0.458	0.140	0.064
	2nd	127	14.03	14.2	12.52-17.00	12.0-12.7	16.2-17.2				
	3rd	125	14.05	13.9	12.62-16.20	12.5-12.9	15.9-16.7				
RDW-SD	1st	134	47.49	46.5	40.60-55.50	38.4-42.1	54.6-56.4	0.120	0.071	0.894	0.23
	2nd	128	49.78	49.6	42.28-57.99	41.1-43.9	56.4-61.9				
	3rd	127	49.66	49.2	41.30-59.84	40.3-43.9	58.2-60.0				
Platelet ($x10^3/\mu l$)	1st	133	258.5	273	167.05-390.00	155-182	369-400	0.005*	0.001*	0.001*	0.005*
	2nd	130	253.3	253	149.58-373.32	140-164	365-390				
	3rd	131	251.6	245	124.60-356.90	90-132	369-386				
MPV (fl)	1st	136	8.25	8.6	6.73-9.80	6.3-7.1	9.4-10.3	0.003*	0.001*	0.677	0.001*
	2nd	129	8.54	8.78	7.05-10.25	7.1-7.4	9.9-10.4				
	3rd	130	8.71	8.85	7.40-10.30	7.6-7.8	10.0-10.5				
PDW	1st	136	15.74	15.7	15.10-16.36	15.1-15.3	16.2-16.4	0.624	0.000*	0.000*	0.001*
	2nd	128	15.77	15.8	15.22-16.48	15.0-15.3	16.4-16.5				

	3rd	130	15.92	15.9	15.16-16.57	15.1-15.3	16.5-16.8				
PCT	1st	131	0.225	0.22	0.152-0.316	0.113-0.161	0.305-0.337	0.161	0.209	0.351	0.054
	2nd	127	0.216	0.217	0.110-0.321	0.108-0.132	0.309-0.335				
	3rd	127	0.209	0.21	0.118-0.321	0.090-0.136	0.291-0.333				

LLC-Lower limit confidence interval, ULC- Upper limit confidence interval

WBC-white blood cell count, Lymph#- Absolute lymphocyte count, MID#- Absolute mixed cell count, Gran#-Absolute granulocyte count, Lymph%- lymphocyte percentage, MID%- mixed cell percentage, Gran%- granulocyte percentage, Hgb-hemoglobin, RBC- red blood cell count, HCT- hematocrit, MCV- mean cell volume, MCH- mean cell hemoglobin, MCHC- mean cell hemoglobin concentration, RDW-CV- red cell distribution width coefficient of variation, RDW-SD- red cell distribution width standard deviation, MPV- mean platelet volume, PDW- platelet distribution width, PCT- Plateletcrit

Mann-Whitney U-test for non-normally distributed parameters was done between trimesters (1st Vs 2nd, 1st Vs 3rd, and 2nd Vs 3rd) and Krusal-Wallis test for non-normally distributed parameters was done between inter-trimesters (1st Vs 2nd Vs 3rd).

$P < 0.05$ was considered as statistically significant.

***** Statistically significant

Note: The number of reference subjects varied for each parameter because of the variable number of outliers excluded.

6.4.3 Hematological parameters RIs for pregnant women according to Altitude

As shown in Table 7, the overall median and 2.5th - 97.5th percentile RIs of WBC, Lymph #, MID #, Gran #, Lymph %, MID % ,Gran %, PLT count, MPV, PDW and PCT were 8.1(4.1-13.3 x10^3/µl), 1.9 (1.1-2.7x10^3/µl), 0.115-0.2900.6 (0.3-1.0 x10^3/µl), 5.8 (2.1-9.8x10^3/µl), 22.4 (13.3-38.1%), 7.3 (4.4-11.9 %) , 70.2 (54.5-80.9 %), 250 (126-363 x10^3/µl), 8.6 (6.9-10.1 fl), 15.8 (15.2-16.5) and 0.211 (0.124-0.326) in lowland dwellers of pregnant women, respectively. Whereas, the respective values in highland dwellers of pregnant women were: 8.1 (3.9-14.1x10^3/µl), 1.9 (1.1-2.8 x10^3/µl), 0.5 (0.2-0.9 x10^3/µl), 5.8 (2.2-10.2x10^3/µl), 22.8 (12.6-41.7%), 6.3 (3.79-9.7%), 70.5 (50.38-82.5%), 257(139-378 x10^3/µl), 8.7 (7.2-10.1 fl), 15.8 (15.19-16.4) and 0.211 (0.115-0.290). There were no a statistically significant differences between lowland and highland dwellers of pregnant women in all white blood cell parameters (WBC, Lymph #, , Gran #, Lymph %, and Gran %) except MID # and MID %. Similarly, there were no a statistically significant between lowland and highland dwellers of pregnant women in all platelet parameters.

The median and 2.5th - 97.5th percentile RIs of RBC and the respective indices are also shown in Table 7. There was a statistically significant difference between lowland and highland dwellers of pregnant women for RBC count (P=0.000), Hgb (P=0.000), HCT (P=0.000), MCV (P=0.002), MCH (P=0.038), MCHC (P=0.000), RDW-CV (P=0.000) and RDW-SD (P=0.000). Highland dwellers of pregnant women had a higher median value of Hgb (13.3Vs 10.4 g/dl), HCT (41.5 Vs 38.5 %) , RBC count (4.39 Vs 4.16 x10^6/µl), MCV (95.6Vs 93.7 fl), MCH (30.4 Vs 30.0 pg), MCHC (32.3 Vs 31.5 g/dl), RDW-CV (14.3 Vs 13.7) and RDW-SD (50.0 Vs 46.5).

Table 7: Mean, Median, 95% (2.5th-97.5th) RIs with 90% CI lower and upper reference limits of hematological parameters of pregnant women according to Altitude of south wollo zone, Amhara region, Northeast Ethiopia, From June to August , 2019.

Parameter		N	Mean	Median	RI (2.5^{th}-97.5^{th})	90% CI		P-value
						LLC	ULC	
WBC ($x10^3/\mu l$)	Lowland	200	8.3	8.1	4.1-13.3	3.6-4.7	12.7-15.4	0.977
	Highland	196	8.3	8.1	3.9-14.1	3.4-4.7	12.1-15.1	
Lymph# ($x10^3/\mu l$)	Lowland	199	1.8	1.9	1.1-2.7	1.0-1.2	2.6-3.0	0.432
	Highland	196	1.8	1.9	1.1-2.8	0.9-1.2	2.6-2.9	
MID# ($x10^3/\mu l$)	Lowland	196	0.6	0.6	0.3-1.0	0.2-0.3	0.9-1.1	0.000*
	Highland	191	0.5	0.5	0.2-0.9	0.2-0.3	0.7-0.9	
GRAN# ($x10^3/\mu l$)	Lowland	199	5.9	5.8	2.1-9.8	1.9-2.6	9.4-10.7	0.873
	Highland	195	5.9	5.8	2.2-10.2	1.8-2.6	9.5-11.6	
Lymph (%)	Lowland	194	23.1	22.4	13.3-38.1	12.3-14.8	35.6-39.2	0.322
	Highland	194	24.1	22.8	12.575-41.7	10.1-13.5	41.1-42.2	
MID (%)	Lowland	195	7.4	7.3	4.4-11.9	2.8-4.7	10.9-12.0	0.000*
	Highland	195	6.4	6.3	3.79-9.7	3.2-4.0	9.5-10.9	
GRAN (%)	Lowland	192	69.7	70.2	54.5-80.9	51.3-56.5	79.8-81.7	0.576
	Highland	193	69.6	70.5	50.38-82.5	48.8-54.8	81.3-84.0	
HGB	Lowland	199	10.4	10.4	9.7-12.4	9.0-10.5	12.1-12.9	0.000*

(g/dl)	Highland	196	13.4	13.3	10.4-13.4	10.2-11.1	13.21-14.1	
RBC ($x10^6/\mu l$)	Lowland	199	4.14	4.16	3.43-4.45	3.36-3.52	4.39-4.79	0.000*
	Highland	196	4.39	4.39	3.87-5.01	3.75-4.02	4.99-5.23	
HCT (%)	Lowland	198	38.3	38.5	32.5-44.7	31.4-33.1	43.7-46.5	0.000*
	Highland	194	41.3	41.5	34.0-47.1	32.7-35.3	46.4-48.6	
MCV (fl)	Lowland	195	94.1	93.7	87.2-101.83	84.5-87.8	101.0-103.6	0.002*
	Highland	188	95.4	95.6	85.9-103.8	84.0-87.4	103.0-105.5	
MCH (pg)	Lowland	193	30.1	30.0	26.4-33.2	26.3-27.4	33.0-33.9	0.038*
	Highland	185	30.4	30.4	27.6-32.9	26.8-28.1	32.6-34.1	
MCHC (g/dl)	Lowland	193	31.5	31.5	30.1-33.2	29.7-30.3	32.7-33.3	0.000*
	Highland	194	32.3	32.3	30.5-33.8	30.3-31.0	33.7-34.1	
RDW-CV	Lowland	193	13.8	13.7	12.4-15.7	12.0-12.5	15.7-15.9	0.000*
	Highland	187	14.4	14.3	12.6-16.8	12.4-13.0	16.2-17.0	
RDW-SD	Lowland	195	47.4	46.5	41.1-55.5	40.3-42.1	53.8-56.4	0.000*
	Highland	195	50.7	50.0	42.9-60.0	42.1-43.9	58.2-61.9	
Platelet ($x10^3/\mu l$)	Lowland	199	250	250	126-363	124-159	351-374	0.290
	Highland	193	260	257	139-378	124-169	372-395	
MPV (fl)	Lowland	198	8.6	8.6	6.9-10.1	6.5-7.4	9.7-10.4	0.127
	Highland	196	8.7	8.7	7.2-10.1	6.8-7.4	10.0-10.5	

PDW	Lowland	198	15.8	15.8	15.2-16.5	15.1-15.3	16.3-16.6	0.140
	Highland	195	15.8	15.8	15.19-16.4	15.1-15.3	16.3-16.5	
PCT	Lowland	196	0.221	0.221	0.124-0.326	0.110-0.155	0.315-0.333	0.385
	Highland	187	0.211	0.211	0.115-0.290	0.107-0.136	0.284-0.305	

LLC-Lower limit confidence interval, ULC- Upper limit confidence interval

WBC-white blood cell count, Lymph# - Absolute lymphocyte count, MID# - Absolute mixed cell count, Gran# -Absolute granulocyte count, Lymph% - lymphocyte percentage, MID% - mixed cell percentage, Gran% - granulocyte percentage, Hgb- hemoglobin, RBC- red blood cell count, HCT- hematocrit, MCV- mean cell volume, MCH- mean cell hemoglobin, MCHC- mean cell hemoglobin concentration, RDW-CV- red cell distribution width coefficient of variation, RDW-SD- red cell distribution width standard deviation, MPV- mean platelet volume, PDW- platelet distribution width, PCT- Plateletcrit

Mann-Whitney U-test for non-normally distributed parameters was done between pregnant and non-pregnant women: all hematological parameters except MCHC showed significant differences between pregnant and non-pregnant women.

$P < 0.05$ was considered as statistically significant.

*Statistically significant

Note: The number of reference subjects varied for each parameter because of exclusion of variable number of outliers.

6.4.4Comparison of hematological parameters of Non-pregnant women with each trimester

As presented in table 8, all hematological parameters showed a statistical significant difference between non-pregnant women and 3rd trimester. The study also indicated that there were a statistically significant difference in all hematological parameters except MCHC and MPV between non-pregnant women and 2nd trimester. However, Absolute MID count and percentage, MCV, MCH, MCHC, RDW-CV, MPV, PDW and PCT did not showed a statistically significant difference beween non-pregnant women and 1st trimester.

Table 8: Comparison of hematological parameters between non-pregnant women and with each trimester of pregnant women of South wollo zone, Amhara region, Northeast Ethiopia from June to August, 2019.

Parameters	Category	Median	P-value		
			Non-pregnant Vs 1st	Non-pregnant Vs 2nd	Non-pregnant Vs 3rd
WBC ($x10^3/\mu l$)	Non-pregnant	6.45	0.000*	0.000*	0.000*
	1st trimester	7.7			
	2nd trimester	8.2			
	3rd trimester	8.6			
Lymph# ($x10^3/\mu l$)	Non-pregnant	2.3	0.000*	0.000*	0.000*
	1st trimester	2.0			
	2nd trimester	1.8			
	3rd trimester	1.9			
MID# ($x10^3/\mu l$)	Non-pregnant	0.4	0.917	0.022*	0.018*
	1st trimester	0.5			
	2nd trimester	0.5			
	3rd trimester	0.5			

Gran # ($x10^3/\mu l$)	Non-pregnant	3.5	0.000*	0.000*	0.000*
	1st trimester	5.3			
	2nd trimester	5.9			
	3rd trimester	6.2			
Lymph (%)	Non-pregnant	35.65	0.000*	0.000*	0.000*
	1st trimester	24.9			
	2nd trimester	20.8			
	3rd trimester	22.2			
MID (%)	Non-pregnant	6.8	0.119	0.001*	0.022*
	1st trimester	6.8			
	2nd trimester	6.67			
	3rd trimester	6.8			
Gran (%)	Non-pregnant	56.1	0.000*	0.000*	0.000*
	1st trimester	65.8			
	2nd trimester	70.4			
	3rd trimester	71.9			
HGB (g/dl)	Non-pregnant	13.1	0.000*	0.000*	0.000*
	1st trimester	12.3			
	2nd trimester	11.6			
	3rd trimester	12.7			
RBC ($x10^6/\mu l$)	Non-pregnant	4.76	0.000*	0.000*	0.000*
	1st trimester	3.86			
	2nd trimester	3.49			

	3rd trimester	3.78			
HCT (%)	Non-pregnant	44.6	0.000*	0.000*	0.000*
	1st trimester	41.05			
	2nd trimester	40.3			
	3rd trimester	39.9			
MCV (fl)	Non-pregnant	92.6	0.613	0.000*	0.000*
	1st trimester	94.2			
	2nd trimester	94.4			
	3rd trimester	95.0			
MCH (pg)	Non-pregnant	29.8	0.517	0.001*	0.043*
	1st trimester	30.0			
	2nd trimester	30.1			
	3rd trimester	30.4			
MCHC (g/dl)	Non-pregnant	31.9	0.104	0.649	0.008*
	1st trimester	32.5			
	2nd trimester	31.9			
	3rd trimester	32.6			
RDW-CV	Non-pregnant	12.4	0.450	0.000*	0.000*
	1st trimester	14.1			
	2nd trimester	14.2			
	3rd trimester	13.9			
RDW-SD	Non-pregnant	45.4	0.083	0.000*	0.000*
	1st trimester	46.5			

	2nd trimester	49.6			
	3rd trimester	49.2			
PLT count ($x10^3/\mu l$)	Non-pregnant	304	0.000*	0.000*	0.000*
	1st trimester	273			
	2nd trimester	253			
	3rd trimester	245			
MPV (fl)	Non-pregnant	8.5	0.097	0.144	0.042*
	1st trimester	8.6			
	2nd trimester	8.78			
	3rd trimester	8.85			
PDW	Non-pregnant	14.0	0.154	0.000*	0.000*
	1st trimester	15.7			
	2nd trimester	15.8			
	3rd trimester	15.9			
PCT	Non-pregnant	0.217	0.254	0.000*	0.000*
	1st trimester	0.220			
	2nd trimester	0.217			
	3rd trimester	0.210			

6.4.5 Proportion of out of range values by comparing established RIs of pregnant women with currently utilized manufacturer provided RIs

Table 7 depicts the proportions of out of range (OOR) values in the pregnant women by comparing established hematological parameters RIs with currently utilized manufacturer provided RIs. The result showed that 19.1%, 19.3%, 23.8%, 26.4%, 27.8%, 43.3% and 44.9% out of range values were observed in WBC count, PLT count, Hgb, Gran#, Lymph%, Gran% and MCHC, respectively. The lowest proportion of out of range value was found in MID# (1.3%) and RDW-CV (1.6%).

Table 9: Proportions of out of range values in pregnant women by comparison of established hematological parameters RIs with currently utilized manufacturer provided RIs.

Parameters	Manufacturer RIs	Currently established RIs	Out of range			
			Lower limit (N)	Upper limit (N)	Total	
					N	%
WBC($x10^3/\mu l$)	4.0-10.0	4.0-13.2	0	86	86	19.1
Lymph#($x10^3/\mu l$)	0.8-4.0	1.1-2.7	5	11	16	3.6
MID#($x10^3/\mu l$)	0.1-1.5	0.2-1.0	0	6	6	1.3
GRAN#($x10^3/\mu l$)	2.0-7.0	2.2-9.8	3	104	107	23.8
Lymph (%)	20.0-40.0	12.9-38.1	120	5	125	27.8
MID (%)	3.0-15.0	3.9-10.9	6	10	16	3.6
GRAN (%)	50.0-70	50.5-81.5	1	194	195	43.3
HGB(g/dl)	11.0-15.0	10.1-13.7	22	97	119	26.4
RBC($x10^6/\mu l$)	3.50-5.00	3.45-4.67	3	56	56	12.4
HCT (%)	37.0-47.0	33.5-46.5	53	3	55	12.2
MCV(fl)	80.0-100.0	84.8-103.5	9	40	49	10.9
MCH(pg)	27.0-34.0	27.5-33.0	4	8	12	2.7
MCHC(g/dl)	32.0-36.0	30.3-33.7	190	12	202	44.9
RDW-CV	11.0-16.0	12.5-16.1	7	0	7	1.6
RDW-SD	35.0-56.0	42.1-58.2	8	15	23	5.1
Platelet($x10^3/\mu l$)	100-300	131.7-373.2	9	78	87	19.3
MPV(fl)	6.5-12.0	7.1-10.1	9	2	11	2.4
PDW	9.0-17.0	15.2-16.4	8	10	18	4.0
PCT	0.108-.282	0.121-0.316	6	21	27	6.0

7. Discussion

Several factors including sex affect RIs hence necessitating sex specific RIs for most of hematological parameters; this fact is well documented by several studies (7, 8, 30, 31). Among women, physiological change during pregnancy result a substantial increase of metabolic demands and hormonal disparity. These phenomena affect the hematological parameters. Moreover, due to its impact on the outcome of pregnancy, it increases the attention on the management of the health of pregnant women. One of the tests frequently requested to monitor the health status of pregnant women is hematological parameters. The diagnostic accuracy of this lab test is based on the evaluation of result in relation to reference values of the local population. Even though changes in normal laboratory values induced by pregnancy are well known (20, 62, 63), very few studies have been conducted in Ethiopia to establish reference intervals for pregnant women and never been established hematological parameters RIs for pregnant women in the present study area. Therefore, this study determined RIs of 19 hematological parameters among apparently healthy pregnant and non-pregnant women of South Wollo zone, and may be a baseline for future national reference range establishment.

The pregnant women hematological parameters RI derived in this study varied from those reported from Gondar (42) and Addis Ababa (55), Sudan (54) Central Uganda (51), Northwest Morocco (37), China (46) and textbooks (63) as would be expected for populations in other geographical locations, with ethnic and dietary diversities. In addition, study period seasonal variations, methodology and instrument used also contribute to the varied RI (8, 14, 63).

As summarized in a table and annexed for clarity (annex XXVIII), the lower RI of total WBC count of pregnant women in this study was lower than previously reported from different parts of Ethiopia (42, 55), Central Uganda (51) and Northwest Morocco (37). The upper RI limit of total WBC count in this study was higher than a study conducted in Ethiopia (42, 55) and central Uganda (51); but it was comparable with a study done in Northwest morocco (37). The lower reference limit of Lymph # in this study was lower than Gondar and Northwest Morocco, but the upper reference limit was higher than central Uganda. The upper reference limit of MID # in the present study was higher than a study conducted in central Uganda, but the lower reference limit of current study was lower than Central Uganda. The reference interval of Gran # in this study

was comparable with a study done in Northwest Morocco, but different in a study conducted in Central Uganda. The lower reference limit of Lymph%, MID% and Gran% among pregnant women of this study was lower than Addis Ababa and central Uganda, but the upper reference limit was higher than a study done in Addis Ababa and central Uganda (37, 51, 55). These variations are usually associated with well-known differences in diet and ethnic origin or genetic diversity. Nevertheless, factors such as study design and general nutritional status might also contribute to such discrepancies (8, 14, 63).

In the current study there was a statistical significant difference between pregnant and non-pregnant women in which pregnant women had a higher mean value of total WBC count ($8.3x10^3/\mu l$) than non-pregnant women($6.56x10^3/\mu l$), confirming results of other studies from India (22), North Morocco (37), Nigeria (50) and Sudan (54). This is due to the physiological stress induced by pregnancy state (16). According to gestational age, the mean value of total WBC count showed parallel trend with a study conducted in Addis Ababa (55) and Central Uganda (51). The trend indicated that the mean values of total WBC count slightly increased as gestational period progress. This may be because of a complex physiological process (serial endocrine system and metabolic changes) that increases WBC by accepting stimulatory signals as pregnancy progress. This helps to build the immunity of the fetus that is achieved by a state of selective immune tolerance, in the presence of a strong antimicrobial immunity (64).

This study also demonstrated that the mean values of Lymph # in this study had the highest value in the 1st, decline in the 2nd trimester and then increased in the 3rd trimester. This finding is consistent with previous study (42). However, the mean values of Lymph # showed a different pattern of changes as goes from 1st to 3rd trimester compared to other study (51, 54) in which Lymph # decline as gestational age advance. Gran # and Gran % showed a statistical significant difference between trimesters, 1st Vs 2nd, 1st Vs 3rd, and 2nd Vs 3rd trimester of pregnant women. Moreover, the mean value of Gran# increased as gestational age advances. This finding is in agreement with previous studies (54, 64). There were statistically significant differences between pregnant and non-pregnant women in all WBC parameters, which are consistent with other findings (37, 47, 54). Pregnant women had higher mean values in Gran # and Gran %, than the non-pregnant counterpart, whereas non-pregnant women had higher mean values in Lymph # and Lymph % than pregnant women. The highest value of Gran # and % in pregnant women is

likely due to impaired neutrophilic apoptosis in pregnancy. Neutrophil cytoplasm shows toxic granulation. Neutrophil chemotaxis and phagocytic activities are depressed, especially due to the inhibitory factors present in the serum of pregnant female (65). On the other hand, the reduction of lymphocyte levels in a pregnant woman is a natural consequence of conception and it is a normal body process. When conception occurs and the embryo is awaiting implantation into the uterus, the body makes adjustments within itself to allow this to happen without any hurdles. For the human body, an external embryo within itself is an alien entity. It is quite natural for the immune system to observe it as something harmful and reject it. Therefore, the body ends up suppressing the immune system's response by cutting down the lymphocyte count, which allows the embryo to implant successfully and grow into a fetus. Even during this stage, the body still keeps the mother protected. Other entities such as granulocytes are activated, which temporarily take on the duties of protecting the body from external attacks. Moreover, low value of Lymphocyte may be attributed to monocytosis which help in preventing fetal allograft rejection by infiltrating the decidua tissue (7th – 20th week of gestation) possibly, through Prostaglandin E2 mediated immunosuppression (16, 66).

In the current study, RIs of Hgb (10.1-13.7g/dl) and HCT (33.49-45.98%) in the pregnant women was higher than a study done in Lagos (Hgb= 9.08-12.80 g/dl; HCT=24.61-35.71%) (67). In the contrary, the RIs of Hgb and RBC (3.45 $x10^6$/ul) in the present study was lower than RI determined earlier in Addis Ababa (Hgb=13.3-14.7 g/dl) (55). The varied RIs observed in the above result probably could be due to altitude difference, in which the altitude of the current study (2132 meters above sea level) was higher than Lagos (41m above sea level), but lower than Addis Ababa (2355 meters above sea level). These increases appear to be the result of both increased erythropoiesis which is secondary to the hypoxic stimulus and the decreased in plasma volume that occurs at high altitude. Seasonal variations, diet, ethnic background, method and instrument used for analysis may also contribute for the variations (8, 10, 14, and 63).

In the current study, the value of RBC count, HCT and Hgb showed a statistical significant different between pregnant and non-pregnant women in which pregnant women had a lower mean value (RBC=3.4x10^6/ul Vs 4.7x10^6/ul; HCT= 40.2% Vs 44.2%; Hgb= 10.9 g/dl Vs 13.8g/dl) than their counterpart. This result was expected and in line with other studies (37, 47, 51). This might be due to hemodilution. In normal pregnancy, there is an increase in

erythropoietic activity. However, at the same time an increase in plasma volume occurs 40% to 50% over the non-pregnant state. This increases in plasma volume is more as compared to red cell mass leading to ‘‘hemodilution’’ and results a progressive decrease in hemoglobin level, HCT and RBC count which creates a sort of artificial anemia called ‘‘the physiological anemia of pregnancy’’(8, 20, 68).

RBC count, Hgb and HCT in this study showed a statistical significant difference between trimesters, 1st Vs 2nd, 1st Vs 3rd, and 2nd Vs 3rd trimester of pregnant women. The mean and RIs of RBC count and Hgb were higher in 1st trimester (Hgb=11.1 g/dl; RBC count= $3.94x10^6$/ul), low in 2nd trimester (Hgb=10.4 g/dl; RBC count= $3.45x10^6$/ul) then higher in the 3rd trimester (Hgb=11.9 g/dl; RBC count= $3.79x10^6$/ul) and similar trend was observed with a study conducted in Sudan (54). However, in this study, the mean value of HCT decreased as gestational age increases and this finding is consistent with other studies (42, 47, 48). The value of Hgb varied with gestation age due to hemodilution. The high Hgb levels in the first trimester are subsequently lowered by hemodilution in the second trimester while compensatory mechanisms (maternal plasma volume reduction and increased atrial-natriuretic peptides) raise Hgb in the last trimester. The increased plasma volume, hormonal changes and conditions that promote fluid retention contribute for reduction of HCT values as gestational period advances (20).

The pregnant women RIs of MCV in the current study was higher than study conducted in different countries (37, 45, 55). The upper and lower limit of MCH RIs in this study was lower than a study reported from Gondar (42) and Addis Ababa (55), but higher than central Uganda (51). In addition, the upper and lower limit of MCHC RI in this study higher than central Uganda(53), and lower than Addis Ababa (55) and Northwest Morocco (37), China (40). These discrepancies might be due to Altitude, nutritional factor, method and instrument used (10, 63).

The current study also demonstrated a statistically significant difference between pregnant and non-pregnant women in the value of MCV, MCH, RDW-CV and RDW-SD, and similar results are documented in Northwest Morocco (37) and Beijing (47). Pregnant women had higher mean values of MCV (94.71 Vs 92.3 fl), MCH (30.3Vs 29.7 pg), RDW-CV (14.9 Vs 12.31) and RDW-SD (48.98 Vs 45.5) than their counterpart. When analyzed by trimester, the value of MCH

and MCHC showed no statistical significant difference between trimesters, but MCV, RDW-CV and RDW-SD showed a statistically significant difference between trimesters. The mean value of MCV increased as gestational period advances that is consistent with other study done in Gondar (42). Higher mean value of MCV in pregnant women and increment as gestational period advance may be due to increased production of RBCs to meet the demands of pregnancy, and result a higher proportion of young RBCs outpouring from the hemopoietic organ, which are large in size (21).

The lower limit of platelet count RIs in the current study is lower than the limits reported from Gondar (42), Addis Ababa (55) and Central Uganda (51). However, the upper limit of platelet count RIs in the current study is higher than in other areas (42, 51, 55). Similarly, the upper and lower limit RI of MPV is higher than a study done in central Uganda (51), but lower than Northwest Morocco (37). Among the plausible explanation for these findings is that the variation of geographical location, genetic background and nutritional factors contribute for the different RIs documented (69, 70).

The observed statistically significant difference between pregnant and non-pregnant women in the values of Platelet count is in agreement with the result of Abi-state Nigeria (50), Libya (48), Northwest Morocco (37), Beijing (47) and China (46). In addition to this, the value of PCT and PDW showed a statistical significant difference between pregnant and non-pregnant women. This finding is consistent with the results of past studies in Northwest Morocco (37) and Beijing (47). Pregnant women had higher mean value of MPV and PDW than non-pregnant women. However, the mean value of platelet count (255.3 $x10^3$/ul Vs 310.4 $x10^3$/ul) and PCT (0.216 Vs 0.269) were higher in non-pregnant women than pregnant women. Low mean value of platelet count in pregnant women may be due to multiple physiological changes. Dilution of platelets by the increased plasma volume that occur during pregnancy is an apparent mechanism (71). In healthy, non-pregnant adult women, one third of all circulating platelets are transiently pooled within the low flow-rate circulation of the splenic sinusoids; an increased spleen size results in more pooling of the platelet and a lower platelet count. Therefore, the 50% increase in spleen size that occurs during pregnancy (72) would also contribute to a lower platelet count.

As summarized in the annexed table (annex-XXVIII), the RIs of platelet count in the current study showed similar trend with study conducted in Gondar (42), Sudan (54), central Uganda (51) and china (46) in which platelet count RI decline as gestational age advances. The mean value of platelets decreased with gestational age. This may be due to the continuous expansion of the uterine wall during pregnancy to accommodate fetal growth. This causes laceration of blood vessels at the uterus leading to massive hemorrhage. The primary haemostatic plug where these tears occur is formed by platelets. Gestational thrombocytopenia is due to increased platelet destruction by their activation and increased clearance. Hemodilution also contributes to gestational platelet reduction (20, 21). Although the average platelet count decreases monotonically in pregnancy, there is an increase in platelet aggregation especially during last 8 weeks of gestation. It has been reported that there can occur significant fall in platelet count from 32 weeks gestation onwards. Increased consumption of platelets as well as decreased life span in the uteroplacental circulation has been suggested to be the explanation of the reduction in the number of circulating platelets during pregnancy (73).

The proportion of out of range values in the current study showed that 19.1%, 19.3%, 23.8%, 26.4%, 27.8%, 43.3% and 44.9% values out of range values were observed in WBC count, PLT count, Hgb, Gran#, Lymph%, Gran% and MCHC, respectively. The lowest proportion of out of range value was found in MID# (1.3%) and RDW-CV (1.6%). Misclassification of pregnant women in the value of WBC and Hgb out of range values consistent with earlier study in China (40). This could be increase the risk of overlooking important physiologic alterations resulting from pathological conditions. In addition, it increases unnecessary and potentially dangerous therapeutic actions without determining the real cause of the abnormality.

8. Strength and limitations of the study

8.1 Strength of the study

The strength of the study includes:

- Recruitment of the study participants from highland and lowland dwellers in order to accommodate the effect of altitude on some of the hematological parameters.
- The use of sufficient sample size for each trimester as per CLSI recommendations, which is a limitation of most of the earlier studies.
- The use of multiple parameters to screen and recruit the reference individuals for the final RIs determinations.

8.2 Limitations of the study

Although this study meets the minimum CLSI requirements for establishing valid RIs, few limitations were existed:

- Lack of screening of multiple pregnancies due to resource limitations. It is, therefore, likely that some participants with multiple pregnancies were included in the study, which may influenced the results to some extent.
- Unequal study subjects were included in each trimester (1^{st}, 2^{nd} and 3^{rd}) with regard to iron/ folate supplementation usage.
- Our research design did not allow to follow up any pregnant woman through all the three-trimesters to establish the physiological changes.
- Rapid test was employed to screen HIV, syphilis, Hepatitis and inflammation, which is limited to screen early-acquired infectious agent.
- This study is limited to one group of population i.e, women (pregnant and non-pregnant) in one locality and cannot be used for other populations in Ethiopia.

9. Conclusion and Recommendation

9.1 Conclusion

The present work constitutes a first attempt for determination of hematological parameters RIs among apparently health pregnant and non-pregnant women living in South Wollo Zone, Amhara region, Northeast Ethiopia. The findings of this study highlight the pregnant women hematological parameters RIs differ from Ethiopia and other countries. Furthermore, variation of hematological parameters between pregnant and non-pregnant women and significant changes in some of the hematological parameters at different trimesters of pregnancy in pregnant women was confirmed. Therefore, this suggesting the need for such establishments of local reference intervals for different populations and clinicians familiarity with this pregnancy related physiological changes in the hematologic system would encourage an optimal management of maternal and fetal medical care. The finding of this study also could be good resources to prepare and update guidelines, and for interpretation of laboratory data in further studies.

9.2. Recommendation

- Patient management and interpretation of laboratory findings of pregnant and non-pregnant women should be based on the locally derived hematological parameters RIs and suggested to be utilized by all health facilities of south wollo zone.
- Changes in maternal hematological parameters during pregnancy were confirmed. We recommend that the reference intervals for most hematological parameters be revised to account for the gestational period.
- We recommend conducting similar nationwide study to determine the hematological parameter RIs of pregnant women in Ethiopian population as a whole.
- Further research is needed on other hematological tests including erythrocyte sedimentation rate, reticulocyte count and coagulation tests.

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10. Annex

Annex-I: Information sheet for Non-pregnant women aged 15-17 years

Project Title: Determination of hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia.

Project PI: Mesfin Fiseha (Bsc, Department of Medical Laboratory Science)

Organization: Health centers, Woreda health office, South wollo health department and Amhara public health institute Dessie branch

Introduction:

Hello! My name is _____ and I am a final year master student in Addis Ababa University, college of health science, Department of medical laboratory science in hematology and immunohematology Track. I am conducting a study to determine hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population. Therefore, the purpose of this proposed study is to determine hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia.

You have been chosen for this study as well your guardian/family has been asked and gave consent for your participation in the study. Therefore, we invite you to take part in this study and contribute to the establishment of indigenous RIs. It is needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of the pregnant and non-pregnant women in south wollo zone.

Procedures:

After agreeing that you can take part, one or more of our research staff will visit your home on a certain day and ask you/your parents some questions that will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide urine and fresh stool on a particular container we provide. We will also collect 4 ml venous blood from you by sterile-disposable Vacutainer tube and needle in tube containing EDTA. We will conduct laboratory examination to determine different serological, parasitological and hematological parameters. Leftover specimens will be stored for further analysis.

Confidentiality:

The information obtained during the study will remain confidential. Disclosure of any of the data to third parties other than those allowed in the informed consent form will not be permitted. The results of the research study may be published, but participants' names or identities will not be revealed. To maintain confidentiality, the investigator will keep records in locked cabinets in a locked room at the office and the results of the tests will be coded to prevent identification of the volunteers. Access to data entered into computerized files will be permitted only for authorized personnel directly involved with the study and will be password protected. Individual-specific information may be provided to responsible local medical personnel only with your permission. Urine, stool and blood collected will not be used for other purposes. The left over specimens will be 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 be safely disposed in an environmentally friendly manner.

Risks and Discomfort:

There will be minimal discomfort in giving urine and stool specimens. 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 specimens will be taken by experienced laboratory professionals.

Safety:

The venous blood specimen will be collected using sterile Vacutainer tube/syringe and needle by experienced health professional after disinfecting the site of puncture by 70% ethanol. Moreover,

leftover stool, urine and blood specimen (that is not stored) will be discarded following the guideline of bio-safety.

Benefits:

By participating in the study, you will directly benefit by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. In the future, the established reference interval will help to improve the health status of pregnant and non-pregnant women in the community.

Incentives:

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

Right to refuse or withdraw:

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

Whom to contact:

If you have any questions, you may ask the person whom you are giving your urine, stool and blood or the PI of the study or the investigators/focal persons using the following addresses:

1. Mesfin Fiseha, PI: 09 12 997130
2. Dr Aster Tsegaye, Focal person 09 11 696085

IRB address: Addis Ababa University, College of Health Science +251 -11-896-13 96

Annex-II: 15—17 ዓመት(ነፍሰጡር ያልሆኑ ሴቶች) ለሆኑ የመረጃ ቅፅ

የፕሮጀክቱ ርዕስ: ‘‘በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መስራት’’

የፕሮጀክቱዋናተመራማሪ:መስፍን ፍስሃ (ቢኤስሲ፣በህክምና ላቦራቶሪ ሳይንስ)

ተቋማት:ጤና ጣቢያ፣ወረዳ ጤና ጽ/ቤት፣ደቡብ ወሎ ዞን ጤና መምሪያ እና አማራ ህብረተሰብ ጤና ተቀም ደሴቅርንጫፍ

መግቢያ:

ጤና ይስጥልኝ! ስሜ_____ነው:: በአዲስ አበባ ዩኒቨርስቲ፣ጤና ሳይንስ ኮሌጅ ህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የመጨረሻ አመት የሁለተኛ ድግሪ ተማሪ ነኝ:: በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል ለመስራት በዞኑ የተለያዩ አካባቢዎች ጥናት እያካሄድኩ ነው::

የምርምር ጥናቱ አላማ:

የህክምና ላቦራቶሪ በጤናው አገልግሎት ውስጥ ከፍተኛ ሚና ይጫወታል::ምርመራን ለማረጋገጥ፣ህሙማን ለመድሃኒቶች ምላሽ መስጠታቸውን ከትትል ለማድረግ፣ የበሽታዎችን ስርጭት ለማጥናት፣በሽታ ለመከላከል እና ስለበሽታዎች ምንጭ ምርምር ለማድረግ አስተዋፅዎ ያደርጋል::በተለይም በአገራችን የጤናማ ሰው የላቦራቶሪ ውጤት ማወዳደሪያ ሪፈረንስ ኢንተርቫል የለም::ስለሆነም የዚህ ጥናት ዓላማ በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል ለመስራት አንቺም በዚህ ጥናት እንድትሳተፊ እየጋበዝን ወላጆችሽ ፈቃዳቸውን ገልፀዋል::ስለዚህ በዚህ ጥናት በመሳተፍ በዞናችን ውስጥ ለሚስራው የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች ውጤት ማወዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንድታደርጊ ተጋብዘሻል::ይህም ጥራት ያለው የላቦራቶሪ አገልግሎት ለመስጠት አስፈላጊ ነው::ስለዚህ የዚህ ጥናት ውጤት በዞኑ ውስጥ ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ጤናን ለማሻሻል ይረዳል::

የጥናቱአካሄድ:

በጥናቱ ለመሳተፍ ከተስማማሽ የጥናቱ አባል/አባላት 15 ደቂቃ የሚወስድ ጥያቄ ይጠይቁሻል::ክብደት፣ቁመት፣የሙቀት እና የደም ግፊት ልኬት ይወሰዳል::ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጭን እንጠይቃለን:::፣በተጨማሪም 4 ሚሊ ሊትር በንፁህ ቫኩቴይነር ብልቃጥ፣መርፌ እና ደም እንዳይረጋ የሚያደርግ ንጥረ ነገር ባለበት ቲዩብ ደም ይወሰዳል:: የሄማቶሎጂ፣ሴሮሎጂ እና ፓራሲቶሎጂ ምርመራዎችን እናካሂዳለን::

ሚስጥር ስለመጠበቅ:

በዚህ ጥናት የሚሰበሰብ መረጃ በሙሉ በሚስጥር ይጠበቃል::መረጃ በዚህ የስምምነት ቅፅ ከተፈቀደው ውጪ ለሶስተኛ ወገን ተላልፎ አይሰጥም::የዚህ ጥናት ውጤት ሊታተም ይችላል ነገር ግን የጥናቱ ተሳታፊዎች ስምና ማንኛውም መለያ አይገለፅም:: ሚስጥራዊነቱን ለመጠበቅ የዚህ ጥናት አባላት መረጃዎችን በተቆለፈ ክፍል በተቆለፈ ካቢኔት ውስጥ ያስቀምጣሉ፣የፈቃደኛ

ተሳታፊዎችን ማንነትን ላለማሳወቅ ውጤቶችም በኮድ ይቀመጣሉ፡፡በኮምፒዩተር ውስጥ ለተቀመጡ ፋይሎች ለጥናቱ ተመራማሪዎች ብቻ የሚፈቀዱና በሚስጥር ቁልፍ የሚጠበቁ ይሆናል፡፡የተሳታፊ ውጤት ለህክምና ባለሞያ ሊተላለፍ የሚችለው በተሳታፊው ፈቃድ ብቻ ነው፡፡የተሰበሰበው ሽንት፣ዓይነምድርና ደም ለሌላ አገልግሎት አይውልም፡፡የሚተርፉት ናሙናዎች በአዲስ አበባ ዩኒቨርስቲ ህክምና ላቦራቶሪ ትምህርት ክፍል ደህና ቦታ ተቀምጠው ለተጨማሪ ምርመራዎች እንደአስፈላጊታቸው ጥቅም ላይ ይውላሉ፡፡በመጨረሻም ተሰርቶባቸው የተራረፉ የሚደፉ ናሙናዎች አካባቢን በማይበክል መልኩ በጥንቃቄ ይወገዳሉ፡፡

ጥናቱ የሚያስከትላቸው የጤናች ግሮችና አለመመቸት:

ሽንትና ዓይነምድር በመስጠት የሚደርስ መጠነኛ አለመመቸት ሊኖር ይችላል፡፡ሆኖም ደም በሚቀዳበት ጊዜ መጠነኛ መነዳትና የተወሰነ አለመመቸት ሊኖር ይችላል፡፡ይሁን እንጂ በተቻለ መጠን ልምድ ያለው የላቦራቶሪ ባለሞያ በመጠቀም አለመመቸቱን ለመቀነስ እንሞክራለን፡፡

ደህንነት:

የደም ናሙና በሚወሰድበት ጊዜ በንፁህ የደም መቅጃ በመጠቀም የሚቀዳውን ቦታ በ70% አልኮል በማፅዳት ልምድ ባለው ባለሞያ ይከናወናል፡፡በተጨማሪም ጥቅም ላይ ከዋሉ በኋላ ለማስቀመጥ የማይሆኑ የሚደፉ የዓይነምድር፣ሽንት እና ደም ትራፊዎች የላቦራቶሪ ደህንነት መመሪያ በመከተል ይወገዳሉ፡፡

ጥቅማጥቅሞች፡

በዚህ ጥናት በመሳተፍ ለበሽታ አምጪ ተህዋስያን፣ደምና ሽንት ምርመራ በማድረግ የጤንነት ሁኔታ ማወቅ ይቻላል፡፡በዘን ውስጥ ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መሰራቱ የዞኑን የጤና ሁኔታ ለማሻሻል ይረዳል፡፡

በጥናቱለመሳተፍማትጊያ:

ከዓይነምድር፣ ሽንት እና ደም ምርመራ ጤናማ ያልሆነ ውጤት ከተገኘ በአቅራቢው ወደ ሚገኝ ጤና ተቋም ትላኪያለሽ፣ የላቦራቶሪ ውጤቶቹን በነፃ ታገኚያለሽ፡፡ይሁን እንጂ በዚህ ጥናት ለመሳተፍም ሆነ ለመድሃኒት ክፍያ አይሰጥም፡፡ስለተሳትፎሽ ግን እናመሰግናለን፡፡

ያለመሳተፍ መብት:

በዚህ ጥናት ከተሳተፍሽ የቻልነውን ሁሉ እንክብካቤ እናደርጋለን፡፡በማኛውም ሰዓት ከጥናቱ መውጣት እንደሚቻልና ይህም በምታገኘው/ኚው አገልግሎት ላይ (ለምሳሌ የጤና አገልግሎት) ምንም አይነት ልዩነት አይደረግም፡፡

ጥያቄ ካለ ለማነጋገር:

ምንም ዓይነት ጥያቄ ካለ የዓይነምድር፣ሽንት እና የደም ናሙና የሰጠሽውን ሰው መጠየቅ ይቻላል ወይም የፕሮጀክቱ ዋና ተመራማሪን ወይም ተጠሪ በሚከተለው አድራሻ መጠየቅ ይቻላል፡፡

1. መስፍን ፍስሃ መሪ ተመራማሪ 09 12 99 7130

2. ዶ/ር አስቴር ፀጋዬ ተጠሪ 09 11 696085

በአዲስ አበባ ዩኒቨርስቲ የጤና ሳይንስ ኮሌጅ የምርምር ስነምግባር ቢሮ ስልክ፡+251-11-896-13 96

Code number_____

Annex-III: Assent form for Non-pregnant women aged 15-17 years

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 assent that I would participate in this study provided my parents/guardians give their consent.

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 (parents/guardians)_____

Print name of researcher, date and signature of researcher

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

መለያ ቁጥር._____

Annex-IV: 15—17 ዓ መት ነፍሰጡር ያልሆኑ ሴቶች የስምምነት ቅፅ

ከላይ የተገለፀውን መረጃ አንብቤአለሁ /ወይም ተነቦልኛል።ጥያቄ ለመጠየቅ ዕድል ተሰጥቶኝ ጠይቄ በሚያረካ መልኩ ተመልሶልኛል።ወላጆቼ እስከፈቀዱ ድረስ በዚህ ጥናት ለመሳተፍ ተስማምቻለሁ።

የዓይነምድር ናሙና ለመስጠት☐

የሽንት ናሙና ለመስጠት☐

ደም ለመቀዳት ☐

እና በዚህ ጥናት ተሳታፊ ለመሆን፣በማንኛውም ሰዓት ከጥናቱ ለመውጣት መብት እንዳለኝም ተረድቻለሁ☐.

የተሳታፊ ስም፣ቀን እና ፊርማ (ወይም አሻራ) ከዚህ በታች ይፃፉ

_____ _____ /_____ /_____ (ቀን/ወር/ዓመተምህረት)

ያልተማሩ ከሆኑ;

የተማሩ ገለልተኛ እማኝ ሰው ስም፣ ቀንና ፊርማ (ከተቻለ ይህ ሰው በተሳታፊው ቢመረጥና ከተመራማሪ አባላት ግኑኝነት የሌለው ቢሆን)

_____ _____/_____/_____ (ቀን/ወር/ዓመተምህረት) _____

ስልክ ቁጥር (የወላጅ ወይም አሳዳጊ)_____

የተመራማሪው ስም፣ቀንና ፊርማ

_____ _____/_____/_____ (ቀን/ወር/ዓመተምህረት) _____

Annex-V: Information sheet for pregnant and non-pregnant women (≥18 years)

Project Title: Determination of hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia.

Project PI: Mesfin Fiseha (Bsc, Department of Medical Laboratory Science)

Organization: Health centers, Woreda health office, South wollo health department, Amhara public health institute Dessie branch.

Introduction:

Hello! My name is _____ and I am a final year master student in Addis Ababa University, college of health science, Department of medical laboratory science in hematology and immunohematology Track. I am conducting a study to determine the hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population. Therefore, the purpose of this proposed study is to determine the hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast 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. It is needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status of the pregnant and non-pregnant women at large in south wollo zone.

Procedures:

After agreeing that you can take part, one or more of our research staff will ask you some questions that will take up to 15 minutes. Your weight, height and vital signs will be measured. You will be asked to provide urine and fresh stool on a particular container we provide. We will

also collect 4 ml venous blood from you by sterile-disposable Vacutainer tube and needle in tube containing EDTA. We will conduct laboratory examination to determine different serological, parasitological and hematological parameters.

Confidentiality:

The information obtained during the study will remain confidential. Disclosure of any of the data to third parties other than those allowed in the informed consent form will not be permitted. The results of the research study may be published, but participants' names or identities will not be revealed. To maintain confidentiality, the investigator will keep records in locked cabinets in a locked room at the office and the results of the tests will be coded to prevent identification of the volunteers. Access to data entered into computerized files will be permitted only for authorized personnel directly involved with the study and will be password protected. Individual-specific information may be provided to responsible local medical personnel only with your permission. Urine, stool and blood collected will not be used for other purposes. The left over specimens will be 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 be safely disposed in an environmentally friendly manner.

Risks and Discomfort:

There will be minimal discomfort in giving urine and stool specimens. 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 specimens will be taken by experienced laboratory professionals.

Safety:

The venous blood specimen will be collected using sterile Vacutainer tube/syringe and needle by experienced health professional after disinfecting the site of puncture by 70% ethanol. Moreover, leftover stool, urine and blood specimen (that is not stored) will be discarded following the guideline of bio-safety.

Benefits:

By participating in the study, you will directly benefit by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. In the future, the established reference interval will help to improve the general health status of pregnant and non-pregnant women in the community.

Incentives:

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

Right to refuse or withdraw:

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

Whom to contact:

If you have any questions, you may ask the person whom you are giving your urine, stool and blood or the principal investigator (PI) of the study or the investigators/focal persons using the following addresses:

1. Mesfin Fiseha, PI: 09 12 997130
2. Dr Aster Tsegaye, Focal person 09 11 696085

IRB address: Addis Ababa University, College of Health Science +251 -11-896-13 96

Annex-VI: 18-ዓመትና ከዚያ በላይ ለሆኑ ነፍሰጡር እናቶች እና ነፍሰጡር ላልሆኑ ሴቶች የመረጃ ቅፅ

የፕሮጀክቱ ርዕስ: ‘‘በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መስራት’’

የፕሮጀክቱ ዋና ተመራማሪ: መስፍን ፍስሃ (ቢኤ ስ ሲ፣ በህክምና ላቦራቶሪ ሳይንስ)

ተቋማት:ጤና ጣቢያ፣ ወረዳ ጤና ጽ/ቤት፣ደቡብ ወሎ ዞን ጤና መምሪያ፣አማራ ህብረተሰብ ጤና ተቀም ደሴ ቅርንጫፍ::

መግቢያ:

ጤና ይስጥልኝ! ስሜ_____ነው::በአዲስ አበባ ዩኒቨርስቲ፣ጤና ሳይንስ ኮሌጅ ህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የመጨረሻ አመት የሁለተኛ ድግሪ ተማሪ ነኝ:: ‘‘በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መስራት በዞኑ የተለያዩ አካባቢዎች ጥናት እያካሄድኩ ነው::

የምርምር ጥናቱ አላማ:

የህክምና ላቦራቶሪ በጤናው አገልግሎት ውስጥ ከፍተኛ ሚና ይጫወታል:: ምርመራን ለማረጋገጥ፣ ህሙማን ለመድሃኒቶች ምላሽ መስጠታቸውን ክትትል ለማድረግ፣ የበሽታዎችን ስርጭት ለማጥናት፣ በሽታ ለመከላከል እና ስለበሽታዎች ምንጭ ምርምር ለማድረግ አስተዋፅዎ ያደርጋል:: በተለይም በአገራችን የጤናማ ሰው የላቦራቶሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል የለም:: ስለሆነም የዚህ ጥናት ዓላማ በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መስራት እርስዎም ለዚህ ጥናት ተመርጠዋል:: ስለዚህ በዚህ ጥናት በመሳተፍ በዞናችን ውስጥ ለሚስራው ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንድታደርጊ ተጋብዘሻል:: ይህም ጥራት ያለው የላቦራቶሪ አገልግሎት ለመስጠት አስፈላጊ ነው:: ስለዚህ የዚህ ጥናት ውጤት በዞኑ ውስጥ ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ጤናን ለማሻሻል ይረዳል::

የጥናቱ አካሄድ:

በጥናቱ ለመሳተፍ ከተስማማሽ የጥናቱ አባል/አባላት 15 ደቂቃ የሚወስድ ጥያቄ ይጠይቁሻል:: ክብደት፣ ቁመት፣ የሙቀት እና የደም ግፊት ልኬት ይወሰዳል:: ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጠን/ጭን እንጠይቃለን:::፣ በተጨማሪም 4 ሚሊ ሊትር በንፁህ ቫኩቴይነር ብልቃጥ፣መርፌ እና ደም እንዳይረጋ የሚያደርግ ንጥረ ነገር ባለበት ቲዩብ ደም ይቀዳሉ:: የሄማቶሎጂ፣ ሴሮሎጂ እና ፓራሲቶሎጂ ምርመራዎችን እናካሂዳለን::

ሚስጥር ስለመጠበቅ:

በዚህ ጥናት የሚሰበሰብ መረጃ በሙሉ በሚስጥር ይጠበቃል:: መረጃ በዚህ የስምምነት ቅፅ ከተፈቀደው ውጪ ለሶስተኛ ወገን ተላልፎ አይሰጥም:: የዚህ ጥናት ውጤት ሊታተም ይችላል ነገር ግን የጥናቱ ተሳታፊዎች ስምና ማንኛውም መለያ አይገለፅም:: ሚስጥራዊነቱን ለመጠበቅ የዚህ ጥናት አባላት መረጃዎችን በተቆለፈ ክፍል በተቆለፈ ካቢኔት ውስጥ

ያስቀምጣሉ፣ የፈቃደኛ ተሳታፊዎችን ማንነትን ላለማሳወቅ ውጤቶችም በኮድ ይቀመጣሉ፡፡ በኮምፒዩተር ውስጥ ለተቀመጡ ፋይሎች ለጥናቱ ተመራማሪዎች ብቻ የሚፈቀዱና በሚስጥር ቁልፍ የሚጠበቁ ይሆናል፡፡ የተሳታፊ ውጤት ለህክምና ባለሞያ ሊተላለፍ የሚችለው በተሳታፊው ፈቃድ ብቻ ነው፡፡ የተሰበሰበው ሽንት፣ ዓይነምድርና ደም ለሌላ አገልግሎት አይውልም፡፡ የሚተርፉት ናሙናዎች በአዲስ አበባ ዩኒቨርስቲ ህክምና ላቦራቶሪ ትምህርት ክፍል ደህና ቦታ ተቀምጠው ለተጨማሪ ምርመራዎች እንደ አስፈላጊታቸው ጥቅም ላይ ይውላሉ፡፡ በመጨረሻም ተሰርቶባቸው የተራረፉ የሚደፉ ናሙናዎች አካባቢን በማይበክል መልኩ በጥንቃቄ ይወገዳሉ፡፡

ጥናቱ የሚያስከትላቸው የጤና ችግሮችና አለመመቸት:

ሽንትና ዓይነምድር በመስጠት የሚደርስ መጠነኛ አለመመቸት ሊኖር ይችላል፡፡ ሆኖም ደም በሚቀዳበት ጊዜ መጠነኛ መነዳትና የተወሰነ አለመመቸት ሊኖር ይችላል፡፡ ይሁን እንጂ በተቻለ መጠን ልምድ ያለው የላቦራቶሪ ባለሞያ በመጠቀም አለመመቸቱን ለመቀነስ እንሞክራለን፡፡

ደህንነት:

የደም ናሙና በሚወሰድበት ጊዜ በንፁህ የደም መቅጃ በመጠቀም የሚቀዳውን ቦታ በ70% አልኮል በማፅዳት ልምድ ባለው ባለሞያ ይከናወናል፡፡ በተጨማሪም ጥቅም ላይ ከዋሉ በኋላ ለማስቀመጥ የማይሆኑ የሚደፉ የዓይነምድር፣ ሽንት እና ደም ትራፊዎች የላቦራቶሪ ደህንነት መመሪያ በመከተል ይወገዳሉ፡፡

ጥቅማ ጥቅሞች፡

በዚህ ጥናት በመሳተፍ ለበሽታ አምጪ ተህዋስያን፣ ደምና ሽንት ምርመራ በማድረግ የጤንነት ሁኔታ ማወቅ ይቻላል ::በዞን ውስጥ ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መሰራቱ የዞኑን የጤና ሁኔታ ለማሻሻል ይረዳል፡፡

በጥናቱ ለመሳተፍ ማትጊያ:

ከዓይነምድር፣ ሽንት እና ደም ምርመራ ጤናማ ያልሆነ ውጤት ከተገኘ በአቅራቢው ወደ ሚገኝ ጤና ተቋም ትላካለህ/ትላኪያለሽ፣ የላቦራቶሪ ውጤቶቹን በነፃታገኛለህ/ታገኚያለሽ፡፡ ይሁን እንጂ በዚህ ጥናት ለመሳተፍም ሆነ ለመድሃኒት ክፍያ አይሰጥም፡፡ ስለተሳትፎሽ ግን እናመሰግናለን፡፡

ያለመሳተፍ መብት:

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

ጥያቄ ካለ ለማነጋገር:

ምንም ዓይነት ጥያቄ ካለ የዓይነምድር፣ ሽንት እና የ ደም ናሙና የሰጠኸውን/የሰጠሽውን ሰው መጠየቅ ይቻላል ወይም የፕሮጀክቱ ዋና ተመራማሪን ወይም ተባባሪዎችና በየተቋሙ የሚገኙ ተወካዮችን በሚከተለው አድራሻ መጠየቅ ይቻላል፡፡

1. መስፍን ፍስሃ መሪ ተመራማሪ፣ 09 12 99 7130

2. ዶ/ር አስቴር ፀጋዬ ተጠሪ 09 11 696085

በአዲስ አበባ ዩኒቨርስቲ የጤና ሳይንስ ኮሌጅ የምርምር ስነምግባር ቢሮ ስልክ፡ +251 -11-896-13 96

Code number_____

Annex-VII: Consent form for pregnant and non-pregnant women (≥18 years)

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) _____

መለያ ቁጥር._____

Annex-VIII: 18 ዓመት እና ከዚያ በላይ ለሆኑ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች የስምምነት ቅፅ

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

የዓይነምድር ናሙና ለመስጠት☐

የሽንት ናሙና ለመስጠት☐

ደም ለመቀዳት ☐እና በዚህ ጥናት ተሳታፊ ለመሆን፣ በማንኛውም ሰዓት ከጥናቱ ለመውጣት መብት እንዳለኝም ተረድቻለሁ ☐.

የተሳታፊ ስም፣ ቀን እና ፊርማ (ወይም አሻራ) ከዚህ በታች ይፃፉ

_____ _____ /_____ /_____ (ቀን/ወር/ዓመተ ምህረት)

ያልተማሩ ከሆኑ;

የተማሩ ገለልተኛ እማኝ ሰው ስም፣ ቀንና ፊርማ (ከተቻለ ይህ ሰው በተሳታፊው ቢመረጥና ከተመራማሪ አባላት ግኑኝነት የሌለው ቢሆን)

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

ስልክ ቁጥር_____

የተመራማሪው ስም፣ ቀንና ፊርማ

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

Annex-IX: Information sheet for Parents/ Guardians

Project Title: Determination of hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia.

Project PI: Mesfin Fiseha (Bsc, Department of Medical Laboratory Science)

Organization: Health centers, Woreda health office, South wollo health department, Amhara public health institute Dessie branch.

Introduction:

Hello! My name is _____ and I am a final year master student in Addis Ababa University, college of health science, Department of medical laboratory science in hematology and immunohematology Track. I am conducting a study to determine the hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia.

Purpose of the research:

The health laboratory plays an indispensable role in the health care system. It supports diagnosis (to rule in or rule out a diagnosis), monitoring of response to treatment, epidemiological surveillance, prevention as well as Research (to understand the pathophysiology of a particular disease process). Especially there is lack of local reference interval for indigenous population. Therefore, the purpose of this proposed study is to determine the hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia. Your child has been chosen for this study. Therefore, we invite you and your child to take part in this study and contribute to the establishment of indigenous reference values. It is needed for providing quality laboratory service. Thus, result from this study is anticipated to improve the health status pregnant women and non-pregnant women at large in south wollo zone.

Procedures:

After agreeing that your child can take part, one or more of our research staff will ask you some questions that will take up to 15 minutes. Your child's weight, temperature, height and vital signs will be measured. Your child will be asked to provide urine and fresh stool on a particular container we provide. We will also collect 4 ml venous blood from your children by sterile-

disposable Vacutainer tube and needle in tube containing EDTA. We will conduct laboratory examination to determine different serological, parasitological and hematological parameters.

Confidentiality:

The information obtained during the study will remain confidential. Disclosure of any of the data to third parties other than those allowed in the informed consent form will not be permitted. The results of the research study may be published, but participants' names or identities will not be revealed. To maintain confidentiality, the investigator will keep records in locked cabinets in a locked room at the office and the results of the tests will be coded to prevent identification of the volunteers. Access to data entered into computerized files will be permitted only for authorized personnel directly involved with the study and will be password protected. Individual-specific information may be provided to responsible local medical personnel only with your permission. Urine, stool and blood collected will not be used for other purposes. The left over specimens will be 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 be safely disposed in an environmentally friendly manner.

Risks and Discomfort:

There will be minimal discomfort in giving urine and stool specimens. 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 specimens will be taken by experienced laboratory professionals.

Safety:

The venous blood specimen will be collected using sterile Vacutainer tube/syringe and needle by experienced health professional after disinfecting the site of puncture by 70% ethanol. Moreover, leftover stool, urine and blood specimen (that is not stored) will be discarded following the guideline of bio-safety.

Benefits:

By participating in the study, your child will directly benefit by being investigated for any pathogenic organisms and other clinical and hematological abnormalities. To determine the hematological parameters RIs among apparently healthy pregnant and non-pregnant women of

south wollo zone will be used in the future to improve the general health status of the pregnant and non-pregnant women in the zone.

Incentives:

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

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/your child are free to withdraw from the study at any time and that you/your child will not be discriminated in any form of service like health.

Whom to contact:

If you have any questions, you may ask the person whom you are giving your urine, stool and blood or the principal investigator (PI) of the study or the investigators/focal persons using the following addresses:

1. Mesfin Fiseha, PI: 09 12 997130
2. Dr Aster Tsegaye, Focal person 09 11 696085

IRB address: Addis Ababa University, College of Health Science +251 -11-896-13 96

Annex-X: ለወላጆች/አሳዳጊዎች መረጃ

የፕሮጀክቱ ርዕስ: ‘‘በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መስራት’’

የፕሮጀክቱ ዋና ተመራማሪ: መስፍን ፍስሃ (ቢኤ ስ ሲ፣ በህክምና ላቦራቶሪ ሳይንስ)

ተቋማት: ጤና ጣቢያ፣ ወረዳ ጤና ጽ/ቤት፣ደቡብ ወሎ ዞን ጤና መምሪያ እና አማራ ህብረተሰብ ጤና ተቀም ደሴ ቅርንጫፍ

መግቢያ:

ጤና ይስጥልኝ! ስሜ _____ ነው፡፡ በአዲስ አበባ ዩኒቨርስቲ፣ጤና ሳይንስ ኮሌጅ፣ህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል የመጨረሻ አመት የሁለተኛ ድግሪ ተማሪ ነኝ፡፡ በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል ለመስራት በዞኑ የተለያዩ አካባቢዎች ጥናት እያካሄድን ነው፡፡

የምርምር ጥናቱ አላማ:

የህክምና ላቦራቶሪ በጤናው አገልግሎት ውስጥ ከፍተኛ ሚና ይጫወታል፡፡ ምርመራን ለማረጋገጥ፣ ህሙማን ለመድሃኒቶች ምላሽ መስጠታቸውን ክትትል ለማድረግ፣ የበሽታዎችን ስርጭት ለማጥናት፣ በሽታ ለመከላከል እና ስለበሽታዎች ምንጭ ምርምር ለማድረግ አስተዋፅዎ ያደርጋል፡፡ በተለይም በአገራችን የጤናማ ሰው የላቦራቶሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል የለም፡፡ ስለሆነም የዚህ ጥናት ዓላማ በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል ለመስራት ነው፡፡

ልጅዎ ለዚህ ጥናት ተመርጣለች፡፡ ስለዚህ በዚህ ጥናት በመሳተፍ በዞናችን ውስጥ ለሚስራው የነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንድታደርጊ ተጋብዘሻል፡፡ ይህም ጥራት ያለው የላቦራቶሪ አገልግሎት ለመስጠት አስፈላጊ ነው፡፡ ስለዚህ የዚህ ጥናት ውጤት በዞኑ ውስጥ ለሚገኙ ነፍሰጡርእናቶች እና ነፍሰጡር ያልሆኑ ጤናን ለማሻሻል ይረዳል፡፡

የጥናቱ አካሄድ:

በጥናቱ ልጅዎ እንዲሳተፍ ከተስማሙ የጥናቱ አባል/አባላት 15 ደቂቃ የሚወስድ ጥያቄ ይጠይቁዎታል፡፡ ክብደት፣ ሙቀት፣ቁመት፣ የክንድ እና የደም ግፊት ልኬት ይወሰዳል፡፡ ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጭን እንጠይቃለን፡፡፣ በተጨማሪም 4 ሚሊ ሊትር በንፁህ ቫኩቴይነር ብልቃጥ፣መርፌ እና ደም እንዳይረጋ የሚያደርግ ንጥረ ነገር ባለበት ቲዩብ ደም ይቀዳሉ፡፡የሄማቶሎጂ፣ ሴሮሎጂ እና ፓራሲቶሎጂ ምርመራዎችን እናካሂዳለን፡፡

ሚስጥር ስለመጠበቅ:

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

ጥናቱ የሚያስከትላቸው የጤና ችግሮችና አለመመቸት:

ሽንትና ዓይነምድር በመስጠት የሚደርስ መጠነኛ አለመመቸት ሊኖር ይችላል፡፡ ሆኖም ደም በሚቀዳበት ጊዜ መጠነኛ መነዳትና የተወሰነ አለመመቸት ሊኖር ይችላል፡፡ ይሁን እንጂ በተቻለ መጠን ልምድ ያለው የላቦራቶሪ ባለሞያ በመጠቀም አለመመቸቱን ለመቀነስ እንሞክራለን፡፡

ደህንነት:

የደም ናሙና በሚወሰድበት ጊዜ በንፁህ የደም መቅጃ በመጠቀም የሚቀዳውን ቦታ በ70%አልኮል በማፅዳት ልምድ ባለው ባለሞያ ይከናወናል፡፡ በተጨማሪም ጥቅም ላይ ከዋሉ በኃላ ለማስቀመጥ የማይሆኑ የሚደፉ የዓይነምድር፣ ሽንት እና ደም ትራፊዎች የላቦራቶሪ ደህንነት መመሪያ በመከተል ይወገዳሉ፡፡

ጥቅማ ጥቅሞች፡

በዚህ ጥናት በመሳተፍ ለበሽታ አምጪ ተህዋስያን፣ ደምና ሽንት ምርመራ በማድረግ የጤንነት ሁኔታ ማወቅ ይቻላል፡፡በዞን ውስጥ ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል መሰራቱ የዞኑን የጤና ሁኔታ ለማሻሻል ይረዳል፡፡

በጥናቱ ለመሳተፍ ማትጊያ:

ከዓይነምድር፣ ሽንት እና ደም ምርመራ ጤናማ ያልሆነ ውጤት ከተገኘ በአቅራቢው ወደ ሚገኝ ጤና ተቋም ትላካለህ/ትላኪያለሽ፣ የላቦራቶሪ ውጤቶቹን በነፃታገኚያለሽ፡፡ ይሁን እንጂ በዚህ ጥናት ለመሳተፍም ሆነ ለመድሃኒት ክፍያ አይሰጥም፡፡ ስለተሳትፎሽ ግን እናመሰግናለን፡፡

ያለመሳተፍ መብት:

በዚህ ጥናት ከተሳተፍሽ የቻልነውን ሁሉ እንክብካቤ እናደርጋለን፡፡ በማኛውም ሰዓት ከጥናቱ መውጣት እንደሚቻልና ይህም በምታገኘው/ኚው አገልግሎት ላይ (ለምሳሌ የጤና አገልግሎት) ምንም አይነት ልዩነት አይደረግም፡፡

ጥያቄ ካለ ለማነጋገር:

ምንም ዓይነት ጥያቄ ካለ የዓይነምድር፣ ሽንት እና የ ደም ናሙና የሰጠሽውን/የሰጠሽውን ሰው መጠየቅ ይቻላል ወይም የፕሮጀክቱ ዋና ተመራማሪን ወይም ተባባሪዎችና በየተቋሙ የሚገኙ ተወካዮችን በሚከተለው አድራሻ መጠየቅ ይቻላል፡፡

1. መስፍን ፍስሃ መሪ ተመራማሪ፣ 09 12 99 7130
2. ዶ/ር አስቴር ፀጋዬ ተጠሪ 09 11 696085

በአዲስ አበባ ዩኒቨርስቲ የጤና ሳይንስ ኮሌጅ የምርምር ስነምግባር ቢሮ ስልክ፡+251-11-896-13 96

Code No._____

Annex-XI: Consent form for parents/guardians

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 my child participates in this study (provided he/she gives assent for children 15-17 years).

To give his/her stool ☐

To give his/her urine ☐

To collect her/his blood ☐ and be a participant in this study and understand that I have the right to withdraw my child 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) _____

Print name of researcher, date and signature of researcher

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

መለያ ቁጥር._____

Annex-XII: ለወላጆች/አሳዳጊዎች የስምምነት ቅፅ

ከላይ የተገለፀውን መረጃ አንብቤአለሁ /ወይም ተነቦልኛል፡፡ ጥያቄ ለመጠየቅ ዕድል ተሰጥቶኝ ጠይቄ በሚያረካ መልኩ ተመልሶልኛል፡፡ ልጄ እንድትሳተፍ ተስማምቻለሁ፡፡ ከ 15-17 ዓመት በታች ለሆኑ ልጄ ከተስማማች በዚህ ጥናት እንድትሳተፍ/እንዲሳተፍ ፈቃደኝነቴን ገልጫለሁ፡፡

የዓይነምድር ናሙና ለመስጠት☐

የሽንት ናሙና ለመስጠት ☐

ደም ለመቀዳት ☐እና በዚህ ጥናት ተሳታፊ ለመሆን፣ በማንኛውም ሰዓት ልጄን ከጥናቱ ለማስወጣት መብት እንዳለኝም ተረድቻለሁ ☐.

የተሳታፊ ስም፣ ቀን እና ፊርማ (ወይም አሻራ) ከዚህ በታች ይፃፉ

_____ ____ /____ /____ (ቀን/ወር/ዓመተ ምህረት)

ያልተማሩ ከሆኑ;

የተማሩ ገለልተኛ እማኝ ሰው ስም፣ ቀንና ፊርማ (ከተቻለ ይህ ሰው በተሳታፊው ቢመረጥና ከተመራማሪ አባላት ግኑኝነት የሌለው ቢሆን)

_____ ____/____/_____ (ቀን/ወር/ዓመተ ምህረት) _____

ስልክ ቁጥር_____

የተመራማሪው ስም፣ ቀንና ፊርማ

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

Annex-XIII: Questionnaire

Questionnaires to be filled by health professionals

INSTRUCTION:

First, I would like to express my appreciation for your time and cooperation to fill this questionnaire. The aim of this questionnaire is to gather information for Determination of hematological parameters RIs among apparently healthy pregnant and non-pregnant women of south wollo zone, Amhara region, Northeast Ethiopia. The idea of this research generated by principal investigator Mesfin Fiseha and it is under the guidance of Addis Ababa University, college of health science, department of medical laboratory science. Therefore, the success of this research relies on you by providing timely and accurate information upon request. Finally you are kindly requested to answer the questionnaire honestly and responsibly.

Part I. General information

Code Number_____ Region _____ Zone _____

Woreda_____ / city /_sub city _____ Kebel _____

Part II. Personal information

1. Age (in years)_____
2. Sex _____
3. Place of Birth_____
4. How long do you live in this specific area? _____years

No.	Questions	Responses
	Part III. SOCIO-DEMOGRAPHIC INFORMATION	
5	Educational status	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above

6	Occupation	1. Student 2. House wife 3. Government employee 4. Private employee 5. Farmer 6. Others (specify) _____
7	Marital status	1. Single 2. Married 3. Divorced 4. Widowed 5. Not applicable (children)
8	Religion	1. Orthodox Christian 2. Muslim 3. Protestant 4. Catholic 5. Others (Specify) _____
9	Ethnicity	_____If mixed, specify_ _____
10	Residence	1. Rural 2. Urban
11	Family monthly income (in birr collected from salary, rent, and other income)	_____ ETB
	Part IV. Clinical information	
	Questions 12-17 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	Is there any vaginal bleeding?	1. Yes 2.No
18	Are you a lactating mother?	1. Yes 2. No
Medication related questions		
19	Did you take any type of drug for any illness for the last three month?	1. Yes 2. No
20	If yes to Q19, 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	
21	History of diabetes	1. Yes 2. No
22	History of Hypertension	1. Yes 2. No
23	History of Blood transfusion for the last 1 year	1. Yes 2. No
24	History of blood donation within the last 3 month	1. Yes 2. No
25	History of Hospital Admission for the last 1 year	1. Yes 2. No
26	History of Surgical procedure for the last three years	1. Yes 2. No
27	History of chronic gastritis	1. Yes 2. No
28	History of Malaria for the last 6 month	1. Yes 2. No
29	History of TB for the last two years	1. Yes 2. No
30	History of Cancer	1. Yes 2. No
31	History of Cardiac illness	1. Yes 2. No
32	History of Bleeding disorders	1. Yes 2. No

33	History of allergy	1. Yes 2. No
34	History of Wheezing	1. Yes 2. No
35	History of kidney problem	1. Yes 2. No
36	History of anemia	1. Yes 2. No
37	History of liver disease	1. Yes 2. No
38	History of thyroid disease	1. Yes 2. No
39	History of chronic diseases in the family	1. Yes 2. No
Sign and symptom		
40	Do you feel any sign of pain?	1. Yes 2. No
41	If yes for Q 40, what type of pain you feel?	_____ ___
42	Are you in menstrual period? (**For non-pregnant women only)**	1. Yes 2. No
Filled by examination of clinicians'		
43	What type of finding you observe?	1. Fever 2. Sign of dehydration 3. Stress 4. Mental illness 5. Others specify_____

Part V. Nutritional habit and your life style

How often do you eat the following food? (put a "√ " mark)							
No.	**Food type**	**A Once/day**	**B More than Once/ day**	**C 2-3 times/week**	**D Occasionally (e.g holidays, special ceremonies)**	**E Never**	**Remarks**
44	Roots and Tuber (Potato, sweet						

	potato, Enset, Cassava)						
45	Legumes (Beans, peas, chicken pea, etc)						
46	Cereals (Corn, Teff, Wheat, sorghum, etc)						
47	Vegetables (Tomato, cabbage, etc)						
48	Fruits (Orange, banana, etc)						
49	Meat (including poultry, fish, etc)						
50	Milk and Milk products (Butter, yoghurt, cheese, etc)						
51	Egg						
52	Tea and/or coffee						

How frequent do you consume/use the following (put a √ mark)							
		Once/day (Regular)	More than once/day	2-3 times/week	Once a week	Occasionally (holiday, special ceremony)	Never
53	Alcohol						

54	*Khat*						
55	Cigarettes						

	Part V. Life style/Habit Continued…	
56	Do you have Fasting habit?	1. Yes 2. No
57	Do you have the habit of physical Exercise?	1. Never 2. Sometimes 3. Always
58	Do have a frequent contact with chemicals and Benzene	1. Yes 2. No
	Part VI. Anthropometric measurement	
59	Height (in cm)	_____
60	Weight (in kg)	_____
61	Blood pressure (mm Hg)	_____
62	Body temperature (^{0}c)	_____

- We thank you for your cooperation!

Interview Date:_____

Interviewer's Name_____ Signature_____

Annex-XIV: Questionnaire Amharic version (ቃለ-መጠይቅ)

በጤና ባለሞያዎች የሚሞላ ቃለመጠይቅ

መመሪያ፡

በቅድሚያ ይህንን ቃለመጠይቅ ለመሙላት ለሰጡን ጊዜና ትብብር አድናቆቴን እገልፃለሁ፡፡የዚህ ቃለመጠይቅ አላማ "በደቡብ ወሎ ዞን ስር ለሚገኙ ነፍሰጡር እናቶች እና ነፍሰጡር ያልሆኑ ሴቶች ሴቶች የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ላቦራቶሪ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል ለመስራት መረጃ ለመሰብሰብ ነው፡፡የዚህ ጥናት ሃሳቡን ያመጡት የጥናቱ ዋና ተመራማሪዎች መስፍን ፍስሃ እና ሚፈታህ ሙሃመድ ሲሆኑ ጥናቱም በአዲስ አበባ ዩኒቨርስቲ፣ጤና ሳይንስ ኮሌጅ፣ህክምና ላቦራቶሪ ሳይንስ ትምህርት ክፍል ስር በመሆን ድጋፍ ይደረግልታል፡፡የጥናቱን ወጪ የሸፈነው ትምህርት ሚኒስቴር ነው፡፡ስለሆነም የእርስዎ ቅን ትክክለኛ መልስ በሰዓቱ መስጠት የዚህን ጥናት ስኬት ይወስናል፡፡ ስለሆነም ይህንን ቃለመጠይቅሃቀኝነትና ሃላፊነት በተሞላው መንገድ እንዲሞሉ በትህትና እንጠይቃለን፡፡

አመሰግናለሁ!!!

ክፍል 1.አጠቃላይ መረጃ

ኮድ_____ ክልል _____ ዞን_____ወረዳ_____

ከተማ/ከፍለከተማ _____ቀበሌ _____

ክፍል 2.የግል መረጃ

1. እድሜ _____
2. ጾታ_____
3. የትውልድ ቦታ_____
4. አሁን ያሉበት ቦታ ለምን ያህል ጊዜ ኖረዋል? _____ዓመት

ክፍል 3.ማህበራዊና ኢኮኖሚያዊ መረጃ		
ቁጥር.	ጥያቄ	ምላሽ
5	የትምህርት ደረጃ	1. ያልተማሩ 2. ማንበብናመፃፍ 3. አንደኛደረጃ (1-8) 4. ሁለተኛደረጃ (9-12) 5. ኮሌጅዲፕሎማ/ዲግሪእናከዚያበላይ
6	ሥራ	1. ተማሪ 2. የቤት እመቤት 3. የመንግስት ሠራተኛ 4. የግል ተቀጣሪ 5. ገበሬ 6. ሌላ ካለ ይግለፁ _____
7	የጋብቻ ሁኔታ	1. ያላገቡ 2. ያገቡ 3. የተፋቱ 4. ባል/ሚስትየሞተባቸው 5. አይመለከታቸውም (ህፃናት)
8	ሃይማኖት	1. አርቶዶክስ ክርስቲያን 2. ሙስሊም 3. ፕሮቴስታንት 4. ካቶሊክ 5. ሌላ ካለ ይግለፁ _____
9	ብሔረሰብ	_____ ድብልቅ ከሆኑ ይግለፁ _____
10	መኖሪያ ቦታ	2. ገጠር 2. ከተማ
11	የቤተሰብ ወርሃዊ ገቢ (በብር ከደሞዝ፣ኪራይ፣ እናሌሎች ገቢዎች)	_____ብር
	ክፍል 4.የጤና መረጃ	
	ከ 12-17 ያሉት ጥያቄዎች ለነፍሰጡር ሴቶች ብቻ ነው	
12	ከፀነሱ ስንት ጊዜዎ ነው?	_____(ሳምንት)

13	ለስንተኛ ጊዜ ነው የፀነሱት?	_____
14	ተጨማሪ የብረት ንጥረ ነገር ይወስዳሉ?	1. አዎን 2. የለም
15	ተጨማሪ ፎሌት ንጥረ ነገር ይወስዳሉ?	1. አዎን 2. የለም
16	ተጨማሪ የብረት ንጥረ ነገርና ፎሌት ይወስዳሉ?	1. አዎን 2. የለም
17	በመራቢያ አካልዎ የመድማት ችግር አለበዎት	1. አዎን 2. የለም
18	ከአንድ አመት በታች የሆነው ጡት የሚጠባ ልጅ አለዎት?	1. አዎን 2. የለም
	ከመድሃኒት ጋር የተያያዙጥያቄዎች	
19	ባለፉት ሶስት ወራት ለማንኛውም ዓይነት ህመም ማንኛውንም ዓይነት መድሃኒት ወስደኋል?	1. አዎን 2. የለም
20	ለተራ ቁጥር-19 መልስዎ ወስጃለሁ ከሆነ የትኛውን ዓይነት መድሃኒት ነው ወሰዱት? (ከአንድ በላይ መልስ ይቻላል)	1. ፀረ-ፕሮቶዞአ 2. ፀረ-ሄልሚንትስ 3. ፀረ-አለርጂ 4. የወሊድ መከላከያ ኪኒን 5. ፀረ-ባክቴሪያ 6. ፀረ-ቲቢ 7. የባህል መድሃኒት 8. ሌላ ካለ ይግለፁ _____
	የሚከተሉት የህመም ዓይነቶች አሞዎት ያውቃል?	
21	የስኳር ህመም አለብዎት?	1. አዎን 2. የለም
22	የደም ግፊት ህመም አለብዎት?	1. አዎን 2. የለም
23	ባለፈው 1 ዓመት ደም ተሰጥቶዎ ያውቃሉ?	1. አዎን 2. የለም
24	ባለፈው 3 ወር ውስጥ ደም ለግሰው ያውቃሉ?	1. አዎን 2. የለም
25	ባለፈው 1 ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1. አዎን 2. የለም
26	ባለፉት 3 ዓመታት የቀዶ ህክምና ተደርጎልዎ ያውቃሉ?	1. አዎን 2. የለም
27	የቆየ የጨጓራ ህመም አለብዎት?	1. አዎን 2. የለም
28	ባፉት 6 ወራት የወባ ህመም አጋጥሞዎት ያውቃሉ?	1. አዎን 2. የለም
29	ባለፉት 2 ዓመታት የቲቢ ህመም ታመውያውቃሉ?	1. አዎን 2. የለም
30	ካንሰር ህመም አለብዎት?	1. አዎን 2. የለም

31	የልብ ህመም አለብዎት?	1. አዎን 2. የለም
32	የመድማት ችግር/ህመም አለብዎት?	1. አዎን 2. የለም
33	አለርጂ (የሰውነት መቆጣት) አለብዎት?	1. አዎን 2. የለም
34	የመተንፈስ ችግር (ሲተነፍሱ ሲርሲር የሚል ድምፅ)አለብዎት?	1. አዎን 2. የለም
35	የኩላሊት ህመም አለብዎት?	1. አዎን 2. የለም
36	የደም ማነስ ችግር አለብዎት?	1. አዎን 2. የለም
37	የጉበት ህመም አለብዎት?	1. አዎን 2. የለም
38	የእንቅርት ህመም አለብዎት?	1. አዎን 2. የለም
39	በቤተሰብ ውስጥ ስር የሰደደ ህመም (ስኳር፣የደም ግፊት) የታመመ አለ ?	1. አዎን 2. የለም
የህመም ስሜትና ምልክት		
40	የህመም ስሜት አለዎት?	1. አዎን 2. የለም
41	በተራቁጥር 41 መልስዎ አዎ ከሆነ ምን አይነት ስሜት ይሰማዎታል?	_____
42	የወር አበባ ላይ ነዎት? (**ለሴት ተሳታፊዎች ብቻ**)	1. አዎን 2. የለም
በጤና ባለሙያዉ ምልከታ የሚሞላ		
43	ከተሳታፊዉ የትኛዉን የህመም ስሜት እና ምልክት (የጤና ችግር) ተመልክተዋል?	1. ትኩሳት(የሙቀት መጠኑ ከ 37^{0}cበላይ የሆነ) 2. የሰውነት ፈሳሽ ድርቀት(ዲሀይድሬሽን) 3. ጭንቀት 4. የአዕምሮ ውስንነት 5. ሌላ ካለ_____

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
44	ሥራ ሥር (ድንች፤ ስኳር ድንች፣እንሰት፣ ካሳቫ ወዘተ)						
45	አዝዕርት (ባቄል፣ አተር፣ሽንብራ ወዘተ)						
46	ጥራጥሬ (በቆሎ፣ ጤፍ፣ስንዴ፣ማሽላ)						
47	አትክልት (ቲማቲም፣ ጎመን፣ወዘተ)						
48	ፍራፍሬ (ብርትኳን፣ ሙዝ፣ወዘተ)						
49	ሥጋ(የዶሮ፣የአሳን ጨምሮ)						
50	ወተትና የወተት ተዋፅዎ (እርጎ፣ቅቤ፣ አይብ፣ወዘተ)						
51	እንቁላል						
52	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 (ለም ሳሌ፣ ለበዓ ል፣ ልዩ ዝግጅ ቶች ሲኖ ሩ)	ተጠቅሜ አላውቅም
53	አልኮል						
54	ጫት						
55	ሲጋራ						

	ክክፍል 5.የቀጠለየህይወት አመራርና ልምዶች	
56	የመዖም ልምድ አለዎት?	1. አዎን 2. የለም
57	የሰውነት እንቅስቃሴ የማድረግ ልምድ አለዎት?	1. የለም2. አልፎ አልፎ 3. ሁል ጊዜ
58	ዘወተር ከነዳጅ እና ኬሚካሎች ጋር ግንኙነት አለዎት?	1. አዎን 2. የለም
	ክፍል 6.ክብደት፣ቁመት፣የክንድ፣የሰውነት ሙቀትና የደም ግፊት ልኬት	
59	ቁመት	_____ሴንቲሜትር
60	ክብደት	_____ኪሎግራም
61	የደም ግፊት (በሚሊሜትር ሜርኩሪ)	_____(mm Hg)
62	የሰውነት ሙቀት መጠን	_____(^{0}c)

❖ ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን:_____

ቃለ መጠይቁን ያካሄደው ስም_____ ፊርማ_____

Annex-XV: Standard operating procedure for specimen collection, handling, transportation and storage

1. Purpose

To ensure consistent procedural approach for specimen collection, handling, transportation and storage from research participant.

2. Principle

The specimen must be appropriate, collected at the right time, collected in a way that minimizes contamination, collected in a way that reduces health and safety risk to all staff handling the specimen (including laboratory staff), collected using the correct equipment, and documented clearly using appropriate forms, stored/transported appropriately.

3. Type of specimen/Sample

- Venous blood(whole blood and plasma) , Random urine and Stool specimen

4. Type of container and additives

- Purple top EDTA test tube- for venous blood collection
- Nunc tube-for plasma
- Leak-proof screw cap container- for stool and urine

5. Required material, equipment and reagents

Type of specimen	Material and Equipment required	Reagents required
Venous blood (whole blood and plasma)	Cotton wool, tourniquet, vacutainer tube and needle, Purple top EDTA test tube, marker, safety box, vaccine carrier, refrigerator, Nunc tube, micropipette tip and micropipette	70% alcohol
Stool	Leak-proof screw cap container, vaccine carrier, marker	10% formalin
Urine	Leak-proof screw cap container, marker	

6. Storage conditions

Type of specimen	Storage condition
Whole blood	○ 8 hrs. at room temperature

Plasma	○ 2-8^{o}c for one weeks or store at -20^{o}c more than a week.
Stool	○ Store it in fridge but not more than 24 hours. ○ With 10% formalin for long period
Urine	○ Stored at 2-8^{0}c for up to 48 hours prior to testing. For prolonged storage, specimen may be frozen and stored below -20^{0}c.

7. Safety

Apply standard precautions during collection, handling, transporting and disposing of all biological specimens.

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

<u>Vacutainer venous blood collection</u>

1. Identify the participant's and avail the necessary material and supplies
2. The participant is seated comfortably in the phlebotomy chair, which has arm rests.
3. The sample collector look for a suitable vein in front of the elbow
4. A tourniquet is applied to the upper arm on the chosen side. The tourniquet should not be in place for longer long period of time.
5. If veins are not very visible, the sample collector taps the skin lightly over the vein, and instructs the participant to clench and unclench their fist
6. Apply 70% alcohol cotton swab and allow to dry for least 30 seconds, since alcohol may cause pain on venipuncture and/or contaminate the blood sample.
7. Inserts the needle through skin into the vein in which needle along the line of the vein at an approximately 30-45 degree angle to skin
8. Release the tourniquet when the blood starts to enter in to the test tube.

9. Once enough amount of blood draw, clean dry gauze or cotton wool will be placed on the venipuncture site and the needle will be removed in a swift backward motion
10. The tube must be properly mixed immediately after each drawn by inverting the tube.
11. The sample collector will press down on the gauze/cotton wool once the needle has been drawn out of the vein applying adequate pressure to avoid formation of a hematoma.
12. The research participant's arm will not be placed in a bent position at any time following venipuncture.
13. The research participants arm will be inspected to ensure bleeding has stopped.
14. All contaminated materials/supplies will be disposed of in the designated containers.
15. All blood collection tubes will be labelled immediately following collection the sample label with three digit identification number, and date and time of collection.
16. Finally, the blood sample will be placed in the vaccine carrier and transported to analysis within 3 hour of collection at room temperature.

Stool sample collection

- Identify the participant
- Avail the necessary materials and supplies
- The participants are given a screw capped collection container and instructed to collect a stool specimen in the container.
  - ✓ Use the scoop inside the lid of the container to transfer the sample in to the container and then screw the lid and wash his/her hands.
  - ✓ Tell them not to contaminate with urine.
- When the sample comes to the data collection site, the data collector labels the container with a three-digit unique identification number, and time and date of collection.
- Deliver the sample as soon as possible for direct stool examination

Random/routine Urine sample collection

- Identify the participant
- Avail the necessary materials and supplies
- The participants are given a non-sterile collection container and instructed to collect a midstream specimen in the container. This type of specimen is routinely used for urinalysis and may not be used for a culture and sensitivity.

- When the sample comes to the data collection site, the data collector labels the container with a three-digit unique identification number, and time and date of collection.
- Immediately perform the urine pregnancy test.

10. Quality control: Strictly follow this SOP

11. Calculation- Non-applicable (N/A)

12. Interference- Non-applicable (N/A)

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation- Non-applicable (N/A)

Annex-XVI: Standard operating procedure for HIV 1/2Ab STAT-PACK

1. Purpose

To ensure consistent procedural approach and for qualitative detection of antibodies to Human Immunodeficiency Viruses (HIV) type 1 and 2 in the human plasma specimens.

2. Principle

The chembio HIV 1/2 stat-pack employs a unique combination of a specific antibody binding protein, which is conjugated to colloidal gold dye particles, and HIV 1/2 antigens, which bound to the membrane solid phase. The sample is applied to the sample (S) well followed by the addition of running buffer. The buffer facilitates the lateral flow of the released products and promotes the binding of antibodies to the antigens. If present, the antibodies bind to the gold conjugated antibody binding protein. In a reactive sample, the dye conjugated-immune complex migrates on the nitrocellulose membrane and is captured by the antigens immobilized in the test (T) area producing a pink/purple line in. in the absence of HIV antibodies, there is no pink/purple line in the test (T) area. The sample continues to migrate along the membrane and produces a pink/purple line in the control (C) area containing immunoglobulin G antigens. This procedural control serves to demonstrate that specimen and reagents have been properly applied and have migrated through the device.

3. Type of specimen/Sample

- Plasma

4. Type of container and additives

- Nunc Tube and EDTA test tube

5. Required material, equipment and reagents

Material and equipment required	Reagents required
Disposable glove, Stat pack individually pouched test device, micropipette tip, micropipette, Timer and biohazard disposal container	HIV running buffer

6. Storage conditions

Storage and stability condition
○ **For the test device and running buffer** ✓ Should be stored in unopened pouches at 8 -30^{0}C and don't freeze. ✓ Stable until the expiration date marked on the pouch, when stored as indicated ○ **For sample** ✓ Store the specimens at 2-8^{0}c and can be used up to 3 days after collection. If testing within 3 days is not possible, specimens should be frozen at -20^{0}C.

7. Safety

Follow standard safety precautions while handling, analyzing and disposing the plasma specimens.

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

A. Collect test items and other necessary lab supplies

B. Remove device from package and label device with client identification number

C. Collect approximately 5 ul of specimen using a new disposable loop or pipette

D. Dispense the sample in the center of SAMPLE well

E. Add 3 drop of buffer, holding vial vertically over the SAMPLE well

F. Wait for 15 minutes before reading the results. Do not read the result after 20 minutes.

G. Read and record the results

10. Quality control: The inbuilt control also serves as quality control checker.

11. Calculation-Non-applicable (N/A)

12. Interference- Recent flu vaccination, syphilis infection and elevated albumin

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation

- **Reactive:** Two lines of any intensity appear in both the control and test areas.
- **Non-reactive:** One line appears in the control area and no line in the test area.
- **Invalid:** No line appears in the control area. Do not report the result rather repeats the test with a new test device even if a line appears in the test area.

Annex-XVII: Standard operating procedure for SD BIO LINE HIV-1/2

1. Purpose

To ensure consistent procedural approach and for qualitative detection of antibodies specific to HIV-1 including subtype-O and HIV-2 simultaneously in the human plasma specimens.

2. Principle

The SD BIOLINE HIV 1/2 contains a membrane strip, which is coated with recombinant HIV-1 capture antigen (gp41, p24) on the test band 1 region and with recombinant HIV-2 capture antigen (gp36) on test band 2 region, respectively. The recombinant HIV-1/2 antigen (gp41, p24 and gp36)-colloid gold conjugate and the specimen sample move along the membrane chromatographically to the test region (T) and form a visible line as the antigen-antibody-antigen gold particle complex forms with high degree of sensitivity and specificity. This test device has a letter of 1, 2 and C as test line 1(HIV-1), test line 2 (hiv-2) and control line on the surface of the device. Both the test lines and control line in result window are not visible before applying any sample. The control line is used for procedural control. Control line should always appear if the test procedure is performed properly and the test reagents of control line are working.

3. Type of specimen/Sample

- Plasma

4. Type of container and additives

- Nunc Tube and EDTA test tube

5. Required material, equipment and reagents

Material and equipment required	Reagents required
Disposable glove, SD BIOLINE HIV 1/2 individually pouched test device, micropipette tip, micropipette, Timer and	Assay diluent

biohazard disposal container	

6. Storage conditions

Storage and stability condition
○ **For the test device and running buffer** ✓ Should be stored in unopened pouches at 1-30^{0}C and don't freeze. ✓ Stable until the expiration date marked on the pouch, when stored as indicated ✓ The test device is sensitive for humidity ○ **For sample** ✓ Store the specimens at 2-8^{0}c and can be used up to 3 days after collection. If testing within 3 days is not possible, specimens should be frozen at -20^{0}C

7. Safety

Follow standard safety precautions while handling, analyzing and disposing the plasma specimens.

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

A. Collect test items and other necessary lab supplies
B. Remove device from package and label device with client identification number
C. Add 10 ul of plasma in to the sample well (S) using micropipette
D. Add 4 drops of assay diluents into the sample well (S) vertically
E. Read the result after 10 minutes but not more than 20 minutes.

10. Quality control: The inbuilt control also serves as quality control checker.

11. Calculation-Non-applicable (N/A)

12. Interference- Non-applicable (N/A)

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation

○ **Reactive:**
✓ The presence of two lines as control line (C) and test line 1 (1) within the result window indicate **reactive result for HIV-1**

- ✓ The presence of two lines as control line (C) and test line 2 (2) within the result window indicate **reactive result for HIV-2**
- ✓ The presence of two lines as control line (C) test line 1 (1) and test line 2 (2) within the result window indicate **reactive result for HIV-1 and HIV-2**
- ✓ If the color intensity of the test line 1 is darker than one of the test line 2 in the result window, you can interpret the result as a **reactive for HIV-1**
- ✓ If the color intensity of the test line 2 is darker than one of the test line 1 in the result window, you can interpret the result as a **reactive for HIV-2**

- **Non-reactive:** The presence of only control line (C) within the result window
- **Invalid:** No presence of control line in the result window. Do not report the result rather repeats the test with a new test device even if a line appears in the test area.

Annex-XVIII: Standard operating procedure for ABON HIV-1/2

1. Purpose

To ensure consistent procedural approach and for qualitative detection of antibodies to HIV-1, including subtype-O and HIV-2 in the human plasma specimens.

2. Principle

A rapid test device strip is pre-coated with HIV-1 and subtype O antigens on T1 test line and HIV-2 antigen on T2 test line. Firstly, specimen and then buffer is added to the specimen well, thus starting the migration of the specimen/buffer. The specimen/buffer passes the conjugate pad, which contains a mixture of HIV-1 envelope and core antigens and HIV-2 envelope antigen. These detection antigens are conjugated to latex particles. If present, the HIV-1 of HIV-2 antibodies reacts and binds to the detection antigen-conjugate. The antibody/antigen-conjugate mixture then migrates further and binds to antigens present on the test lines. If the specimen contains antibodies to HIV-1, the specimen will bind to the T1 test line and produce a line, if specimen contains antibodies to HIV-2, the specimen will bind to the T2 test line. As liquid continues to migrate down the test strip, the control line will appear. If the control line is present, in addition to either or both test lines, then the test lines region indicating a non-reactive result.

3. Type of specimen/Sample

- Plasma

4. Type of container and additives

- Nunc Tube and EDTA test tube

5. Required material, equipment and reagents

Material and equipment required	Reagents required
Disposable glove, ABON HIV 1/2/0 individually pouched test device, micropipette tip, micropipette, Timer and biohazard disposal container	Buffer

6. Storage conditions

Storage and stability condition
○ **For the test device and running buffer** ✓ Should be stored in unopened pouches at 2 -30^{0}C and don't store in the freezer. ✓ Stable until the expiration date marked on the pouch, when stored as indicated ○ **For sample** ✓ Store the specimens at 2-8^{0}c and can be used up to 3 days after collection. If testing within 3 days is not possible, specimens should be frozen at -20^{0}C

7. Safety

Follow standard safety precautions while handling, analyzing and disposing the plasma specimens.

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

A. Collect test items and other necessary lab supplies
B. Open the package and check the content and the expire date
C. Wear glove
D. Open the pouch, label with specimen ID use it as soon as possible(within one hour)
E. Draw the plasma specimen from the specimen tube with dropper
F. Transfer 1 drop of plasma (approximately 25 ul), then add 1 drop of buffer (approximately 40 ul)
G. Start the timer and read results within 10-20 minutes.

10. Quality control: The inbuilt control also serves as quality control checker.

11. Calculation-Non-applicable (N/A)

12. Interference- Non-applicable (N/A)

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation

- **Reactive:** Two or three distinct colored lines appear
- **Non-reactive:** The presence of only one colored line appear in the control region (C)
- **Invalid:** No line appears in the control line region

Annex-IXX: Standard operating procedure for HBsAg test

1. Purpose

To ensure consistent procedural approach and for qualitative detection of HBsAg in the human plasma specimens.

2. Principle

The HBsAg rapid test cassette is a lateral flow chromatographic immunoassay based on the principle of the double antibody-sandwich technique. The membrane is pre-coated with anti-HBsAg antibodies on the test line region of the test. During testing, Hepatitis B surface Antigen in the plasma specimen reacts with the particle coated with anti-HBsAg antibody. The mixture migrates upward on the membrane chromatographically by capillary action to react with anti-HBsAg antibodies on the membrane and generate a colored line. The presence of this colored line in the test region indicates a positive result, while its absence indicates a negative result.

3. Type of specimen/Sample

- Plasma

4. Type of container and additives

- Nunc Tube and EDTA test tube

5. Required material, equipment and reagents

Material and Equipment	Reagents
Disposable glove, test cassette, disposable specimen dropper, safety box, Nunc tube, micropipette tip and micropipette, Timer	Buffer

6. Storage and stability conditions

- The test kit can be stored at room temperature or refrigerated (2-30^{0}C) and stable through the expiration date printed on the sealed pouch.

- The plasma specimen may be stored at 2-8^{0}C for up to 3 days but for long period should be kept at -20^{0}C.

7. Safety

- Standard precautions should be followed when handling, analyzing and disposing the plasma specimens.

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

A. Remove the test device from the foil pouch and use it as soon as possible.

B. Place the test device on a clean and level surface, then hold the dropper vertically and transfer 3 drop of plasma (approximately 90 ul) to the specimen well (S) of the test device

C. Add 1 drop of buffer (approximately 40 ul) and start the timer

D. Read the result at 15 minutes but do not report result after 20 minutes

10. Quality control: The inbuilt control also serves as quality control checker.

11. Calculation-Non-applicable (N/A)

12. Interference- Heterophile antibodies, Rheumatoid factors

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation

- **Negative results:** One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).
- **Positive results:** Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T).
- **Invalid:** Color line fails to appear in control region.

Annex-XX: Standard operating procedure for HCV rapid test strip

1. Purpose

To ensure consistent procedural approach and for qualitative detection of antibodies to hepatitis C virus (HCV) in the human plasma specimens.

2. Principle

The HCV rapid test strip is a qualitative, membrane and double antigen based immunoassay for detection of antibodies to HCV in plasma. The membrane is coated with recombinant HCV antigen on the test line region of the strip. The conjugated pad is treated with gold particles which are conjugated with antigen. During testing, the plasma specimen reacts with the recombinant HCV antigen coated particles. The mixture migrates upward on the membrane chromatographically by capillary action to react with recombinant HCV antigen on the membrane and generate a colored line. Presence of this colored line indicates a positive result, while its absence indicates a negative result.

3. Type of specimen/Sample

- Plasma

4. Type of container and additives

- Nunc Tube and EDTA test tube

5. Required material, equipment and reagents

Material and Equipment	Reagents
Disposable glove, test cassette, disposable specimen dropper, safety box, Nunc tube, micropipette tip and micropipette, Timer	Buffer

6. Storage and stability conditions

- The test kit can be stored at room temperature or refrigerated (2-30^{0}C) and stable through the expiration date printed on the sealed pouch.
- The plasma specimen may be stored at 2-8^{0}C for up to 3 days but for long period should be kept at -20^{0}C.

7. Safety

- Standard precautions should be followed when handling, analyzing and disposing the plasma specimens.

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

A. Remove the test device from the foil pouch and use it as soon as possible.

B. Place the test device on a clean and level surface, then hold the dropper vertically and transfer 2 drop of plasma (approximately 80 ul) to the specimen well (S) of the test device and start the timer

C. Read the result at 10 minutes but do not report result after 20 minutes

10. Quality control: The inbuilt control also serves as quality control checker.

11. Calculation-Non-applicable (N/A)

12. Interference- Non-applicable (N/A)

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation

- **Negative results:** One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).
- **Positive results:** Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T).
- **Invalid:** Color line fails to appear in control region.

Annex-XXI: Standard operating procedure for Syphilis rapid test cassette

1. Purpose

To ensure consistent procedural approach and for qualitative detection of antibodies to Treponema *Pallidum* in the human plasma specimens to aid in the diagnosis of syphilis.

2. Principle

The syphilis rapid test cassette is a qualitative membrane based immunoassay for the detection of *Treponema palladium* (TP) antibodies in plasma. In this test procedure, recombinant syphilis antigen is immobilized in the test line region of the test. After a specimen is added to the specimen pad it reacts with syphilis antigen coated particles that have been to the specimen pad. This mixture migrates chromatographically along the length of the test and interacts with the immobilized syphilis antigen. The double antigen test format can detect both IgG and IgM in specimens. If the specimen contains TP antibodies, a colored line will appear in the test line region, indicating a positive result.

3. Type of specimen/Sample

- Plasma

4. Type of container and additives

- Nunc Tube and EDTA test tube

5. Required material, equipment and reagents

Material and Equipment	Reagents
Disposable glove, test cassette, disposable specimen dropper, safety box, Nunc tube, micropipette tip and micropipette, Timer	Buffer

6. Storage and stability conditions

- The test kit can be stored at room temperature or refrigerated (2-30^{0}C) and stable through the expiration date printed on the sealed pouch.
- The plasma specimen may be stored at 2-8^{0}C for up to 3 days but for long period should be kept at -20^{0}C.

7. Safety

- Standard precautions should be followed when handling, analyzing and disposing the plasma specimens.

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

A. Remove the test device from the foil pouch and use it as soon as possible.

B. Place the test device on a clean and level surface, then hold the dropper vertically and transfer 2 drop of plasma (approximately 50 ul) to the specimen well (S) of the test device.

C. Add 1 drop of buffer (approximately 40 ul) and start the timer

D. Read the result at 10 minutes but do not report result after 20 minutes

10. Quality control: The inbuilt control also serves as quality control checker.

11. Calculation-Non-applicable (N/A)

12. Interference- Non-applicable (N/A)

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation

- **Negative results:** One red line appears in the control region (C). No apparent red or pink line appears in the test region (T).

- **Positive results:** Two distinct red lines appear. One line should be in the control region (C) and another line should be in the test region (T).
- **Invalid:** Color line fails to appear in control region.

Annex-XXII: Standard operating procedure for urine pregnancy test

1. Purpose

To ensure consistent procedural approach and for the qualitative detection of HCG in urine.

2. Principle

It is a rapid chromatographic immunoassay for the qualitative detection of human chorionic gonadotropin (HCG) in urine to aid in the detection of pregnancy. The pregnancy test kit consists of a sample window including a sample cavity on which the urine sample is placed. A permeable membrane holds the pad in the cavity. The membrane consists of three antibody areas includes monoclonal antibodies and color sensitive colloidial gold particles. The stable area that forms the test line includes anti-HCG antibodies on the membrane. The 3rd area that forms the control line includes an ant-mouse immunoglobulin. If the sample includes enough HCG in the sensitivity range of the device, anti-HCG forms a compound with colloidal gold conjugate and moves towards to the test area that is indicated by the letter ‘‘T’’. Once the compound is bound, a line is formed in the area ‘‘T’’. The absence or presence of this line shows a negative or a positive test result. The colloidal gold particles that are not bound in the test area move towards to the control line to area to form control line regardless to the presence of HCG. The line that forms in this area acts as a control device by confirming that there is enough sample in the device and that the sample followed the correct flow course.

3. Type of specimen/Sample

- Random urine specimen

4. Type of container and additives

- Leak-proof screw cap container

5. Required material, equipment and reagents

- Leak-proof screw cap container, marker, timer, test strip and package insert

6. Storage and stability conditions

- **For the test strip**
  - ✓ Store as packaged in the sealed pouch at 4-30^{0}c and stable through the expiration date printed on the sealed
- **For sample**
  - ✓ Stored room temperature and used within 24 hours.

7. Safety

- Follow standard precautions for processing and examination of urine pregnancy test.

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

- Collect the urine in a clean dry and contaminant free plastic or glass container
- Remove the test from the package just before starting the test.
- Dip the strip until Maximum line region
- Place the test strips on a non-absorbent flat surface, start the timer and wait for the red line(s) to appear. The result should be read at 3 minutes.

10. Quality control

Internal procedural controls are included. If the control region red line does not appear, don't report the result and review the procedure and repeat the test with a new test strip because it may be due to insufficient sample volume and incorrect procedural techniques.

11. Calculation- Non-applicable (N/A)

12. Interference- Non-applicable (N/A)

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation

Positive- Two distinct red lines appear

Negative- One red line appear in the control region

Invalid – control line fail to appear (insufficient sample volume and incorrect procedural techniques are most likely reasons for failure. Therefore, review the procedure and repeat the test with a new test strip)

Annex-XXIII: Standard operating procedure for direct saline wet mount stool examination

1. Purpose

To ensure consistent procedural approach for direct saline wet mount stool examination to detect intestinal parasites.

2. Principle

It is made by mixing a small quantity (about 2 mg) of faeces in a drop of saline placed on a clean glass slide. The smear is then examined under microscope. Saline wet mount is used for the detection of trophozoites and cysts of protozoa, and eggs and larvae of helminthes.

3. Type of specimen/Sample

- Stool

4. **Type of container and additives**

- Leak-proof screw cap container with no additives

5. **Required material, equipment and reagents**

Material and Equipment	Reagent
Leak-proof screw cap container, microscope slides, microscope cover slips, Pipettes, pencil Gloves, Microscopes and applicator stick	Normal Saline (0.85% NaCl)

6. Storage conditions

- Immediately perform the examination but can be store until 30 min at room temperature

7. Safety

- Apply universal precautions as well as standard microbiological laboratory practices

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

- Apply the participant sample (about 2mg) to a small area on a clean microscope slide.
- Immediately before the specimen dries, add 1 or 2 drops of saline with a pipette. Mix with applicator stick.
- Cover the specimen with a cover slip. (**Note:** Avoid air bubbles by drawing one edge of the cover slip slightly into the suspension and lowering it almost to the slide before letting it fall. The mount should be just thick enough that newspaper print can be read through the slide.)

- Examine the slide with low and high power objectives under microscope.

10. Quality control: Daily Check solutions with each use to be sure they are clear and free of any bacterial contamination.

11. Calculation- Non-applicable (N/A)

12. Interference- Artifacts, debris etc.

13. Biological reference range-Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation- Non-applicable (N/A)

Annex-XXIV: Standard operating procedure for peripheral blood film preparation and examination

1. Purpose

To ensure consistent procedural approach and for detection of hemoparasite, abnormalities in blood cells and manual differential WBC count.

2. Principle

Peripheral blood film or blood smear is a thin layer of blood smeared (thin and thick) on a glass microscope slide which is prepared by using the wedge techniques and then stained with Giemsa staining solution in such a way as to allow the various blood cells to be examined microscopically. Blood films are examined in the investigation of hematological (blood) disorders and are routinely employed to look for blood parasites. It also helps for manual differential WBC count.

3. Type of specimen/Sample

- Whole blood

4. Type of container and additives

- Vacutainer test tube with EDTA Anti-coagulate

5. **Required material, equipment and reagents**

Material and Equipment	Reagents
Pencil, micropipette, micropipette tip, Single-use non-sterile gloves, gauze, timer, microscope, microscopic glass slides, Bottle of 1000 ml, Clean water or tap water, Dropper	Stock Giemsa solution and Absolute methanol

bottle, Pasteur pipettes, 10 ml graduated cylinder, staining rack, drying Rack for slides, Timer , Microscope (objective 100 x), Immersion oil, touch counters, Storage box for slides , Tissue paper and filter paper	

6. Storage and stability conditions

- **For sample**
  - ✓ Maximum one hour at room temperature (to avoid morphological deformations of Plasmodium parasites)
- **For reagent**
  - ✓ Working Giemsa staining solution store at room temperature and stable for 24 hour.

7. Safety

- Apply universal precautions as well as standard microbiological laboratory practices

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

A. Label the slide

B. Prepare thick (placing 6ul of blood on a clean slide and make round by the other slide) and thin (by placing 2ul of blood on one end of a slide, and using a spreader slide to disperse the blood over the slide's length to found "feathered edge") blood smear.

C. The slide is left to air dry, after which only the thin blood smear is fixed by methanol.

D. After fixation, the slide is stained by Giemsa working solution (Prepared by mixing one part Giemsa stock and nine part distilled or clean tap water) to distinguish the cells from each other.

E. After staining, allow to air dry

F. Finally the stained smear is viewed under a microscope using magnification up to 1000x.

Note: During examining stained smear start with the field on the top left part of the film, and then move the slide to the right, field by field. When the other end of the film is reached, move the slide downwards, then to the left, field by field, and so forth.

G. Individual cells are examined and their morphology is characterized and recorded and differential count would be counted.

Note: Review the area where the red cells are evenly distributed and begin to overlap. Scan for abnormalities such as platelet clumping, rouleaux, agglutination or abnormally large leukocytes. Review all abnormally large leukocytes under 100X for appropriate classification and follow up testing. Select the best area for detailed morphological evaluation and differential count. The erythrocytes will be evenly distributed and not distorted. The field will also be devoid of broken areas caused by improper smear preparation.

10. Quality control

- Daily quality control should be performed by known positive and negative slides before the start of the actual sample analysis.

11. Calculation- Non-applicable (N/A)

12. Interference- Non-applicable (N/A)

13. Biological reference range- Non-applicable (N/A)

14. Critical value- Non-applicable (N/A)

15. Interpretation- Non-applicable (N/A)

Annex-XXV: Standard operating procedure for Mindary BC-3000 plus

1. Purpose

To ensure consistent procedural approach and gives a brief overview of how to run blood samples on the Mindary BC-3000 plus.

2. Principle

The machine utilized two basic principles for measurement of the parameter: the impedance method for determining the WBC, RBC, and PLT data and the colorimetric method for determining the Hgb. WBC, RBC and PLT were counted and sorted by the electrical impedance method, which were based on the measurement of changes in electrical impedance produced by a particle passing through an aperture. The Hgb is determined by the colorimetric method in which the lyse reagent releases Hgb when RBC is broken down and react with Hgb to generate a mixture for Hgb measurement. The WBC/Hgb dilution was delivered to the WBC bath where it is bubble mixed with a certain amount of lyse, which converts hemoglobin to a hemoglobin complex that is measurable at 525 nm. The value of Hgb expressed in g/dl. The machine

automatically dilutes a whole-blood sample, lyses and counts the cells, and then gives a printout result.

3. Type of specimen/Sample

- Whole blood specimen collected in K2EDTA anticoagulant tube

4. Type of container and additives

- Vacutainer test tube and EDTA additive

5. **Required material, equipment and reagents**

Material and Equipment	Reagents
Disposable glove, Mindary BC-300 plus machine, Electrical blood mixer and Electrical power stabilizer	M-30D Diluents, M-30CFL Lyse, M-30R Rinse, M-30E E-Z Cleanser, M-30P Probe Cleanser, Three level Controls

6. Storage and stability conditions

Storage and stability condition
○ **For the reagent** ✓ The reagents must be stored between 180c and 300c and used before the expire date indicated on the label. ○ **For sample** ✓ For the whole blood samples to be used for WBC differential or PLT count, you shall store them at the room temperature and run them within 8 hours after collection ✓ If you do not need the PLT, MCV and WBC differential results, you can store the samples in a refrigerator (2-8^{0}c) for 24 hours. You need to warm the refrigerated samples at room temperature for at least 30 minutes before running them.

7. Safety

- Apply universal precautions as well as standard microbiological laboratory practices

8. **Calibration**

The purpose of calibration is to maintain system accuracy. Run calibration program if

- It is the first time the analyzer has been used;
- Certain major components (s) of the analyzer has been changed;
- The quality control results indicate there may be a problem.

9**. Procedure**

A. Check and make sure: the waste container is empty; there are enough reagents; the diluent rinse and waste tubes properly connected; power cord of the analyzer is properly plugged.

B. Check the printer, keyboard connection, and then place the power switch at the back of the analyzer in the ON position

B. Prior to running your samples, you must execute a quality control.

C. The Sysmex is now ready to sample. To run your samples:

- Ensure your samples are rapidly and thoroughly mix the blood with the anticoagulant.
- Press [Menu] and select ‘‘Sample Mode’’ screen, then select the whole blood from the sample mode pull-down list.
- Press [Mean] and select ‘‘Count’’ to enter Count screen
- Enter sample information at the count screen, press [ID] and an edit window will pop up
- Present the mixed sample to the sample probe and press the aspirate key. The system status area will display ‘‘Running’’ and the analyzer will start aspirating sample.
- When you hear the beep and the sample probe is out of the tube, remove the sample tube. The sample probe will retract into the analyzer and the analysis progress will be displayed on the screen
- When the analysis is finished, the result will be displayed on the screen and the sample ID will automatically increase by 1 and the sample probe will be repositioned. And if the auto print function is enabled, the analysis result will be automatically printed out.

D. When you are finished with your sampling, you must complete the daily shut down procedure. To do this:

- Press [Menu] to enter the system menu and select ‘‘ Shutdown’’
- A message box will pop up to ask you to confirm the shutdown and click enter
- Present the E-Z cleanser to the sample probe and press the aspirate key
- When the cleaning is finished, place the switch at the back of the analyzer to OFF to turn off the analyzer.

NOTE: Perform daily, weekly and monthly preventive maintenance using maintenance program.

Every day- E-Z cleanser cleaning and Probe cleanser cleaning every three days (if the analyzer work 24 hours)

Weekly- You need to perform probe cleanser cleaning every week

Monthly- Perform probe cleanser cleaning.

10. Quality control

Daily quality control should be performed by using three level quality control materials to monitor an instrument's performance over time and also run after component replacement or after a service call.

11. Calculation- Non- applicable

12. Interference: Clot and fibrin strands, colder agglutinin, severely hemolyzed sample, extremely elevated WBC (> 100,000/UL), Giant and clumped platelet, lipemic and icteric sample etc.

13. Biological reference range

Sex	WBC (10^3/ul)	RBC (10^6/ul)	Hgb (g/dl)	HCT (%)	MVC (fl)	MCH (pg)	MCHC (g/dl)	RDW-CW (%)	PLAT (10^3/ul)
Female	4.0-10.0	3.5-5.0	11.0-15.0	37.0-47.0	80.0-100.0	27.0-34.0	32.0-36.0	11.0-16.0	100-300

14. Critical value- Non-applicable (N/A)

15. Interpretation- Non-applicable (N/A)

Annex-XXVI: Standard operating procedure for one step C-reactive protein

1. Purpose

To ensure consistent procedural approach and it is a rapid latex agglutination test kit for the detection of C-reactive Protein in human serum or plasma.

2. Principle

The AVITEX CRP latex particles are coated with antibodies to human CRP. When the latex suspension is mixed with the sample containing CRP on a slide, clear agglutination is seen within 2 minutes.

3. Type of specimen/Sample

- Serum/ plasma

4. Type of container and additives

- EDTA test tube and Nunc tube

5. **Required material, equipment and reagents**

Material and equipment required	reagents required
Disposable glove, micropipette tip and micropipette, Timer, stirrers, plastic slide	Suspension of polystyrene latex particles coated with Anti-CRP antibodies and isotonic saline

6. Storage conditions

- **For the reagent**
  - ✓ Reagents must be stored at temperature between 2^0C to 8^0C. Do not use the reagent after expiry date
- **For sample**
  - ✓ Sample may be stored at 2^0C to 8^0C for 48 hours prior to testing. If longer storage is required, store at -20^0C.

7. Safety

- Apply universal precautions as well as standard microbiological laboratory practices

8. **Calibration**- Non-applicable (N/A)

9**. Procedure**

A. Allow kit reagents and patient sample to come to room temperature

B. Transfer one drop (50ul) of patient's plasma to the test circle on the slide.

C. Shake the latex reagent, then using the dropper provided, add one drop of suspension to the test circle.

D. Mix the drops using the disposable stirrer ensuring coverage of the test circle with the mixture.

E. Gently and evenly, rock and rotate the test slide for 2 minutes whilst examining the test slide for agglutination.

10. Quality control: Run daily quality control using positive and negative control provided by manufacturer.

11. Calculation-Non-applicable (N/A)

12. Interference- Non-applicable (N/A)

13. Biological reference range- Non-applicable (N/A)

14. Critical value-Non-applicable (N/A)

15. Interpretation:

Positive result: it is indicated by the obvious agglutination pattern of the latex, in a clear solution.

Negative result: it is indicated by no change in the latex suspension on the test slide.

Annex-XXVII: Laboratory result form

Participant code _____Region _____

Woreda_____/ city /_sub city _____Kebel _____

- Stool examination
  - Consistency_____
  - Direct_____
- Urine HCG test (for female) _____
- Peripheral Blood film examination
  - Hemoparasites_____
- Serological Tests
  - HIV test_____
  - HBsAg test_____
  - HCV Test (anti-HCV Ab)_____
  - Syphilis screening (anti-TP Ab test)_____
  - CRP test_____
- Hematology (CBC attach print out) Annex-XXVIII: Comparison of Hematological parameters RIs for pregnant women with trimester from different countries

Annex-XXVIII: Comparison of hematological parameters RIs for pregnant women with gestational age (trimester) form different countries

Parameters	Trimester	Current study	Gondar, Ethiopia(42)	Addis Ababa, Ethiopia (55)	Sudan (51)	Central Uganda (53)	Northwest morocco (37)	China (46)	Text book (predominantly from Europeans) (62)
WBC ($x10^3/\mu l$)	1st	3.64-13.24	8.62–10.30	6.5-8	4.36-11.20	4.57-8.75	4.5-11.6	4.3-12.2	5.7–13.6
	2nd	4.56-13.59	8.67–9.90	8-8.7	5.48-12.13	4.71-8.85	4.6-12.6	5.1-13.8	6.2–14.8
	3rd	4.56-13.62	8.60–9.61	8-8.8	5.00-11.96	4.8-9.2	5.3-14.3	4.9-13.4	5.9–16.9
	Combined	4.00-13.21	8.90–9.60	8.1-8.6		4.51-8.79	4.6-13.0		
Lymph# ($x10^3/\mu l$)	1st	1.1-2.8	2.18–2.53		1.20-2.98	1.30-2.58	1.2-3.14		1.1–3.5
	2nd	1.03-2.6	1.96–2.22		1.28-2.63	1.13-2.45	1.2-3.6		0.9–3.9
	3rd	1.13-2.77	2.11–2.33		1.10-2.60	0.84-2.02	1.1-3.8		1–3.6
	Combined	1.1-2.71	2.13–2.28			1.08-2.38	1.2-3.6		
MID# ($x10^3/\mu l$)	1st	0.2-0.9			0.30-0.99	0.29-0.73			
	2nd	0.2-1.08			0.30-0.90	0.28-0.76			
	3rd	0.2-1.08			0.30-1.00	0.31-0.77			

	Combined	0.2-1.0				0.29-0.73			
GRAN# ($x10^3/\mu l$)	1st	2.23-8.62			2.56-8.68	3.55-5.83	2.1-8.2		3.6–10.1
	2nd	2.42-9.78			3.62-9.80	2.59-6.13	2.2-9.2		3.8–12.3
	3rd	2.61-10.23			3.22-9.30	2.7-5.68	3-11		3.9–13.1
	Combined	2.2-9.83				2.48-5.86	2.2-9.7		
Lymph (%)	1st	12.78-45.60		23.4-30		20.91-39.83			
	2nd	10.96-32.96		21.6-23.4		19.96-36.8			
	3rd	13.53-45.68		23-25		15.93-32.89			
	Combined	12.9-38.13		23-24.3		19.53-36.91			
MID (%)	1st	3.94-12.00		7-12		5.72-9.68			
	2nd	3.74-9.60		8-9		5.11-10.45			
	3rd	3.83-12.33		8-10		6.64-10.84			
	Combined	3.9-10.9		8.3-9.3		5.78-10.3			

GRAN (%)	1st	45.86-80.42		60-68					
	2nd	58.62-81.50		68-70					
	3rd	60.53-82.55		65.6-68					
	Combined	50.45-81.54		67-68.7					
HGB (g/dl)	1st	10.37-13.53	12.43–13.46	13.7-15	8.92-12.74	11.6-13.32	10-13.9	10.4-14.0	11.0–14.3
	2nd	9.99-12.90	12.82–13.33	12.6-16	9.00-12.10	10.71-12.29	9.6-13.6	9.5-13.0	10.0–13.7
	3rd	10.68-13.71	13.11–13.67	12.6-14	8.82-12.60	10.85-12.65	9.1-13.4	9.6-13..5	9.8–13.7
	Combined	10.1-13.67	12.99–13.36	13.3-14.7		10.79-12.79	9.4-13.7		
RBC ($x10^6/\mu l$)	1st	3.58-4.90	4.08–4.46	4.6-5	3.69-4.93	4.04-5.02	3.49-4.91	3.35-4.75	3.52–4.52
	2nd	3.35-4.01	4.21–4.43	4.4-4.5	3.69-4.93	3.77-4.67	3.26-4.82	3.01-4.31	3.2–4.41
	3rd	3.76-4.99	4.37–4.55	4.4-4.6	3.44-4.78	3.92- 4.7	3.19-4.78	3.08-4.50	3.1–4.44
	Combined	3.45-4.67	4.30–4.44	4.4-4.5		3.86-4.84	3.29-4.85		

HCT (%)	1st	34.86-47.80	37.17–41.19	40-43	30.12-40.30	36.94-45.3	29.8-40.9	31-41	31–41
	2nd	33.93-46.19	39.63–41.44	38-40	30.58-38.23	34.67-41.35	28.6-39.9	30-39	30-38
	3rd	32.33-45.98	41.17–42.75	39-40	29.66-40.04	32.47-44.79	27.34-39.3	30-41	28-39
	Combined	33.49-46.52	40.19–41.49	39-39.9		34.84-43.66	28.6-40.5		
MCV (fl)	1st	86.67-103.03	92.02–94.34	85-88	65.50-93.02	83.06-98.89	74.4-94.9	82.3-98.2	81–96
	2nd	86.10-103.58	93.18–95.20	86.7-88.4	71.35-94.70	83.71-97.09	74.7-97.7	84.2-102.7	82–97
	3rd	87.62-105.77	93.09–95.37	88-89	73.40-95.68	81.69-97.43	72.8-96.1	82.4-103.9	81–99
	Combined	84.76-103.52	93.33–94.63	87.4-88.5		82.52-97.16	74-96		
MCH (pg)	1st	26.40-32.94	29.82–31.14	29.4-30.5	19.40-28.74	24.96-30	24.2-32.9	27.8-33.4	
	2nd	26.89-33.20	29.84–31.11	30-30.6	20.83-30.15	24.86-29.94	24.0-33.3	27.6-34.7	
	3rd	27.51-33.99	26.73–40.79	30.4-31	21.34-30.18	23.95-28.81	23-33.4	27.0-35.0	

	Combined	27.5-33.00	28.88–34.81	30-30.7		24.72-29.78	23.7-33.2		
MCHC (g/dl)	1st	30.30-33.66	37.02–33.38	34-35	28.54-32.30	29.46-30.98	31.3-36.6	32.2-35.4	
	2nd	30.13-33.2	31.24–35.35	34.4-34.8	28.78-32.40	29.49-31.13	31.2-36.6	31.3-35.3	
	3rd	30.31-33.86	31.75–32.41	34.5-35	28.82-32.50	29.37-30.93	30.8-36.2	30.9-35.1	
	Combined	30.30-33.73	31.91–33.37	34.5-34.8		29.47-31.07	31.2-36.5		
RDW-CV	1st	12.44-15.99			12.00-17.94				
	2nd	12.52-17.00			12.40-18.81				
	3rd	12.62-16.20			12.11-17.50				
	Combined	12.5-16.145							
RDW-SD	1st	40.60-55.50							
	2nd	42.28-57.99							
	3rd	41.30-59.84							

	Combined	42.1-58.2							
Platelet ($x10^3/\mu l$)	1st	167.05-390.00	224.53–253.21	212-267	182.6-418.0	152.38-267.24	145-374	64-263	174–391
	2nd	149.58-373.32	213.70–247.86	220-239	163.8-381.8	145.41224.59	140-364	63-247	171–409
	3rd	124.60-356.90	209.50–237.38	216-235	150.4-346.2	128.48220.94	139-398	61-238	155–429
	Combined	131.7-373.15	221.25–240.14	221.6-235		148.88249.12	141-377		
MPV (fl)	1st	6.73-9.80			6.90-9.78	6.28-7.8	8.9-13.7		
	2nd	7.05-10.25			6.96-9.62	6.4-7.66	8.9-13.5		
	3rd	7.40-10.30			7.00-9.80	6.29-7.89	8.9-13.2		
	Combined	7.09-10.12				6.33-7.75	8.9-13.5		
PDW	1st	15.10-16.36			15.10-16.29				
	2nd	15.22-16.48			15.20-16.30				
	3rd	15.16-16.57			15.40-16.60				

	Combined	15.2-16.4							
PCT	1st	0.152-0.316			0.16-0.32				
	2nd	0.110-0.321			0.14-0.29				
	3rd	0.118-0.321			0.14-0.27				
	Combined	0.121-0.316							

Gondar, author : Genetu M *et al*. year:2017; Addis Ababa, author: Yeshanew AG *et al*. Year: 2017;Sudan, author: Rayis DA *et al*. Year:20
Uganda, author: Philip K *et al*. Year: 2018;Northwest Morocco, author:Bakrim S*et al*. Year: 2018; China, author: Shen C *et al*. Year: 2010

WBC-white blood cell count, Lymph#- Absolute lymphocyte count, MID#- Absolute mixed cell count, Gran#-Absolute granulocyte count, Lymph%- lymphocyte percentage, MID%- mixed cell percentage, Gran%- granulocyte percentage, Hgb- hemoglobin, RBC- red blood cell count, HCT- hematocrit, MCV- mean cell volume, MCH- mean cell hemoglobin, MCHC- mean cell hemoglobin concentration, RDW-CV- red cell distribution width coefficient of variation, RDW-SD- red cell distribution width standard deviation, MPV- mean platelet volume, PDW- platelet distribution width, PCT- Plateletcrit

Declaration

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

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