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COLLEGE OF HEALTH SCIENCES

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Establishment of community based Hematological Reference Intervals among apparently healthy adolescents aged 12-17 years in Mekelle city, Tigrai, Northern Ethiopia; a Cross Sectional Study Design From December, 2018 – May, 2019

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This is to certify that the thesis prepared by Hagos H/slassie, entitled: **Establishment of community based hematological reference intervals among apparently healthy adolescents (12-17 years) in Mekelle city, Tigrai, Northern Ethiopia; a cross sectional study design, 2019** and submitted in partial fulfillment of the requirements for Master of Science degree in 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

BMI	Body mass index
CLSI	Clinical Laboratory and Standards Institute
CSA	Central Statistical Agency
K_2EDTA	Di-potassium Ethylenediamine tetra-acetic acid
ESR	Erythrocyte sedimentation rate
ELISA	Enzyme linked Immunosorbent assay
FACS	Fluorescence-activated cell sorting
Hgb	Hemoglobin
ICSH	International Committee for Standardization in Hematology
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MPV	Mean platelet volume
PDW	Platelet distribution width
PPS	probability proportional to size
QC	Quality control
RBC	Red blood cells
RDW	Red blood cell distribution width
SPSS	Statistical Package for Social Sciences
THRI	Tigrai health research institution
WBC	White blood cells
WHO	World health organization

Abstract

Background: Hematological reference ranges are important in clinical and diagnostic management for the assessment of health and disease conditions. Hematological reference intervals are better to be established based on sex and age differences as these are among the main factors affecting them.

Objective: The aim of this study was to establish hematological reference intervals among adolescents aged, 12-17 years in Mekelle, Tigrai, and Northern Ethiopia, 2019.

Study area: conducted in Mekelle city

Study design and period: using a cross sectional study design conducted from December 2018 to May 2019 G.C.

Method: A community-based cross-sectional study was conducted in 249 adolescents aged 12-17 years from December 2018 to May 2019. About 4ml of blood sample was collected using vacutainer tube containing di-potassium Ethylenediamine tetra-acetic acid. Hematological parameters were measured by Sysmex KX-21N hematology analyzer (Sysmex Corporation Kobe, Japan). The data were entered and analyzed by SPSS version 23 statistical software. The 97.5^{th} percentile and 2.5^{th} percentile were the upper and lower reference limit for the study population.

Result: A total of 249 adolescents consisting of 122 (49%) males and 127 (51%) females with the median age of 15.31 (range 12 to 17) years were recruited. The median, mean and 95% percentile ranges of hematological values were determined. The 95% RI values were: Red blood cells (10^{12}/Liter), 4.6-5.9 (Males) and 4.28-5.75 (Females); White blood cells (10^{9}/Liter), 2.9-9.6 (Males) and 3.4-10.2 (Females); Hemoglobin (g/dl), 12.6-17.6 (Males) and 12-15.4 (Females); Platelets(10^{9}/Liter), 138-364 (Males) and 150.6-461.8 (Females). Almost all of the hematological parameters showed significant differences ($p<0.05$) across the gender.

Conclusion: This study concluded that there were statistically significant higher values of WBC count, MCV, platelet count, lymphocyte count, and neutrophil percent in females than males. However the value of RBC count, hemoglobin, hematocrit, MCHC, RDW CV (%), mixed count, and mixed percentage were statistically significant higher in males than females.

Key words: Adolescents, Apparently healthy, CBC, Ethiopia, Hematological parameters, Reference interval

1. Introduction

1.1. Background

Reference intervals are the most common decision support tool used for interpretation of numerical pathology reports. As laboratory results may be interpreted by comparison with these intervals, the quality of the reference intervals can play as large a role in result interpretation as the quality of the result itself [1]. Nearly 70% of physicians' medical decisions are based on information provided by laboratory reports [2].

Reference interval (RI) is a standard component of reporting laboratory result and is important to transform a numerical value into clinically meaningful information. RI is intended to inform the clinical care provider that laboratory values within the interval indicate a non-diseased condition. The most common approach is to base RI on the central 95% of laboratory test values observed for a reference population that is free of diseases influences the laboratory test result. Because many diseases are asymptomatic [3].

Hematological reference ranges are important in clinical practice for the assessment of health and disease, underscoring the importance of establishing population-appropriate values. Such parameters are also important for measuring disease progression, response to therapy, and in the assessment of adverse reactions to therapy [4]. Due to the prevailing epidemic diseases in sub-Saharan Africa, the normal immunological and hematological indices for these population becomes critical in supporting decisions with regard to treatment initiation and disease management [5].

The RI is defined simply the prediction value which includes the central 95% of reference values (RVs), or test results from well-defined apparently healthy individuals. Establishment of well-controlled, reliable RI is an important mission for all clinical laboratories. The clinical laboratory and standards institute (CLSI) guidelines recommend that laboratories establish their own reference intervals from the local population or validate them if derived from a different setting. Reference intervals need to be verified periodically every 5 years, to capture changes in the community over time [4].

Several factors including age, sex, race, and socio-economic conditions affect RIs. Nutritional status is related to an inflammatory process, and adolescents with excess weight or body fat presented higher amounts of circulating white blood cells [6].

Human beings are characterized by a dynamic period of growth and development in their life. The different periods of life possess a special hematological development. The chemical makeup of the circulating red blood cells and fetal hemoglobin content in the first days and months is not identical with in later life. Secondly, the hematopoietic system of the infant and the child undergoes development modification and growth [7]. Thirdly, hemopoietic (red) marrow occupies the entire capacity of the bones at birth through adolescence. In old age there is increased replacement with fatty marrow [8]. This may affect the hematological parameters and result in different reference ranges for the difference among age groups [9] and sex [10]. Thus, knowledge of the reference values during the dynamic period of growth and development in an individual is important for correct interpretation of a disease condition.

Even though, Ethiopia has a heterogeneous population and different geographical areas, there is no nationally established reference values for hematological parameters except some studies conducted in Addis Ababa [11], Bahir-dar town [12], Gilgel Gibe [13], Gondar university Hospital [14]for adult population and Southwest Ethiopia [15] for children. Therefore, this study is designed to assess the adolescents' reference intervals among the age of 12-17 years in Mekelle, Tigrai, and Northern Ethiopia.

1.2. Statement of the problem

Most reference values of hematologic tests currently used in Africa are derived from data collected for populations living in developed countries [16]. The reference intervals used for population living in industrialized countries is not similar to the populations living in the sub-Saharan countries which are endemic to different disease [17]. An adult Africans have been reported to have lower levels of hemoglobin, red blood cells, platelets, neutrophils counts compared with populations in the Western countries [18-21]. Similarly, hematology tests of Eastern and Southern African populations show differences from the European reference values. The values are against the standard ranges recommended by World Health Organization(WHO) [18, 22]. Most blood specimens of Africans were found to have lower Hemoglobin (Hgb), Hematocrit (Hct), Red blood cell count (RBC), mean corpuscular volume (MCV), Neutrophils and platelet counts [23]. Reference intervals for hematological parameters show significant differences by age variability [9].

The reference intervals and toxicity grading scales for hematological parameters which are used for clinical trials in sub-Saharan Africa follows the WHO reference standard guidelines which is different to our country. However, typical laboratory parameters in different communities may vary based on race, age, gender, diet, local disease patterns and environmental characteristics [24].

The reference intervals determined for hematology test parameters from apparently healthy individuals in southwest Ethiopia shows a difference in hematological RIs from the other Africa countries and the Caucasian populations. The RBC, Hct, Hgb, PLT, MPV, WBC, lymphocyte and neutrophil count reference values were also different in gender among adults but not among children's below the age of 12 years [15]. Locally, reference interval studies were performed in Ethiopia more than fifteen years ago and none of them addressed children's as a separate age groups except the currently published study done in southwest Ethiopia [15]. Therefore, this study aimed to assess the hematological reference range among apparently healthy adolescents in Mekelle, Tigrai, and Northern Ethiopia.

1.3. Significance of the study

As the main purpose of this study is to establish hematological reference intervals among adolescents, the findings will be an important input for physicians to compare the patient's hematological results with the apparently healthy adolescents easily using the local reference intervals.

Important for researchers to do clinical trials using locally appropriate RIs

Furthermore, it will also be used as a reference for further study in large and detail to prepare a reference interval in other areas.

2. Literature review

To establish hematological Reference values a study was conducted for African American children and adolescents among 2,161 participants between the ages of 2 and 18 years. The result showed that the African Americans hemoglobin was lower than whites. The respective mean values for males and females of African American 11-15 years old children were WBC 6.3, 6.6; Hgb 13.2, 12.7; HCT 39.3, 38; MCV 83.2, 83.6. For those in the age group 16-18 years WBC 5.9, 6.9; Hgb 14.4, 12.6; HCT 43.5, 37.4; MCV 86, 85.3 [25].

A blood result of 1200 normal children was collected from electronic data of 95,501 children and adults in Kuwaiti. The children divided into 8 groups according to age and gender. The range of the RBC, Hgb, Hct, MCV, MCH, MCHC, RDW, WBC, lymphocyte, PLT, MPV was evaluated to generate hematological reference range. There was no significance difference among sex below 13 years which is similar with the Ukraine study. But above the age of 13 years WBC and PLT counts are higher in Kuwaiti but lower in RBC, Hgb, MCV and MCH than Ukraine but not significant among sex difference. For the age 13-17 year old Kuwaiti, the study result showed the following: RBC 4.3-5.1 (Females) and 4.7-5.7 (Males); Hgb 11.9-14 (Females) and13.1-15.9 (Males); MCV 77-90 (Females) and 77-90 (Males); WBC 5.9-12 (Females) and 5.5-12 (Males) and Platelet 218-345 (Females) and 201-318 (Males) [26].

In the establishment of hematological reference intervals conducted on the healthy children from 1 month to 18 years from the Kilimanjaro Region of Tanzanian shows that median Hgb and Hct levels are higher than the east African countries but lower than the U.S/European intervals even for platelets and MCV. Platelet counts are decreased from 384 $x10^9$/L to 271$x10^9$/L in males and to 282$x10^9$/L for females. The study also showed a significant difference among gender in the mean Hgb, Hct, RBC and MCV values in the age of 13-18 years; increment of hematocrit in males during adolescence were related to evidence of progressive maturation by secondary sex changes. The study noted that, altitude-induced Erythropoiesis does not appear to explain higher values in related to the developed countries intervals. Rather the differences may be due to several factors, including iron-deficiency anemia, chronic blood loss because of parasitic infestation (especially hookworm), or undiagnosed hemoglobinopathies. Both geohelminth and schistosome infections are serious public health problems in Tanzania [27].

A study conducted in Port Harcourt Teaching Hospital, Nigeria involved a total of 1,021 apparently healthy children aged 0–17 years. It shows mean and 95th percentile reference intervals for the total and differential WBC counts have significant differences among the age groups and decline significantly until 14 years ($P < 0.001$). The normal differential leukocyte count did not vary with gender. The mean and 95th percentile reference ranges of the hematological parameters between 14 and 17 years according to gender also shows significant changes in the values of Hgb, PCV and Platelets ($P < 0.01$) but not in the WBC ($P > 0.05$) [28].

A study recruiting a total of 302 aged ≥ 12 to < 18years old Zimbabwean adolescents for the establishment of hematological reference intervals shows a significant difference between males and females in hematological parameters except platelets, and red cell distribution width. The study shows that females had significantly higher WBC, MCV, MCHC, platelet, lymphocyte, and monocyte absolute counts than males. RBC counts, Hct, and Hgb concentration were the only parameters significantly higher in males than females. The hematological parameter result with 95% RI shows as WBC (10^9/L) 3.31-9.84 (Females), and 3.25- 8.64 (Males); RBC(10^{12}/L) 4.16 - 5.83 (Females) and 4.47- 6.47 (Males); Hgb (g/dl) 11.1-15.7 (Females) and 12.1- 17.4 (Males); Hct(%) 34.6- 46.7 (Females), and 36.1-49.7 (Males); MCV (fl) 68.7- 96.9 (Females) and 70.1-93.2 (Males); and platelets (10^9/L) 214-476 (Females) and 186-415(Males) [29].

Gender and age specific differences for selected hematological parameters were also demonstrated by a study which recruited wider age ranged population in Uganda. Among the 3,311 human immunodeficiency virus (HIV)-negative Ugandans aged 1 week to 92 years the RBC, Hgb, Hct and MCV all significantly increased with age ($P < 0.001$) but it is independent of gender until the age of 13 years, after 13 years the levels were higher in males than in females ($P < 0.001$). But White blood cell and their differentials significantly declined with age until the age of 13 years ($P < 0.001$), with no differences by gender, while platelet counts declined with age ($P <0.001$) and gender difference occurs among adults older than 24 years [18].

A study in Iganga, Uganda in the establishment of hematological reference intervals from 1 to 5 year children showed that there were no statistically significant differences in hematological values between male and female children ($p > 0.05$). It is notable that the lower limits of Hgb, Hct, MCV and platelet counts for the Ugandan children are all lower than conventional reference values. On the other hand, the white blood cell count (WBC 10^3/μl) reference values

(median: 8.9, interval: 5.9 - 14.3) for Iganga children are higher compared to the Caucasian normal reference value (interval 4.0 - 11.0) [30].

The 95% hematological reference ranges determined from a total of 1,070 young children in Kifili, Kenya whereas follows: RBC indices Hgb (g/dl) 7.2-7.7, Hct (%) 23.8-38.3, MCHC (g/dl) 29.4-34.4, MCV(fl) 52-97, platelet counts (10^3 cells/µl) 84-773, WBC (10^3 cells/µl) 5.7-16.7, Neutrophils (10^3 cells/µl) 0.7-4.39, Lymphocytes (10^3 cells/µl) 3.13-10.2. There were no statistically significant differences by gender for all assessed hematological parameters at the very young age [24].

Among the 499 clinically healthy males and 454 females' participants in the establishment of hematological reference intervals conducted in western Kenya, 22.0% (110) were adolescent males and 78.0% (389) were young adults. While adolescents and young adults constituted 29.1% (132) and 70.9% (322) of the female participants respectively. There were significant differences in the hematological indices among males by age; young adults having a higher median as compared to adolescents in Hgb (15.1 g/dL vs. 14.2 g/dL), Hct (45.4% vs. 42.6%), RBC ($5.4X10^6$/µL vs. 5.2 $X10^6$ /µL), and neutrophils ($2.6X10^3$ /µL vs. $2.2X10^3$ / µL). As compared to males, female adolescents and adults have not significant difference. However, females had significantly higher PLT, lymphocytes and WBC than males in both adolescent and young adult. There were significant differences in neutrophil counts between male and female adolescents [31].

Another research which is conducted in western Kenya among the age groups of 13-34 years also shows a significant difference in the Hgb, Hct, WBC and RBC by gender in 13-17 years [23].

A study which takes place in southwest Ethiopia from a total of 334 children who participated in the study revealed the following findings. The median and 95% RI of RBC count, Hgb, WBC count, and PLT were: 5.04×10^{12}/L (4.06–6.57), 141 g/L(120–196), 7.05×10^9/L (4.04–11.72), and 326.5×10^9/L (158.5–469.9), respectively for males and 4.96×10^{12}/L (4.32–5.63), 140 g/L(115.7–159.4), 7.02×10^9/L (3.74–11.42), and 321×10^9/L (197.7–460.4) respectively for females [15].

Although published national pediatric hematological RIs is lacking, data generated from apparently healthy adult Ethiopians also underscore the need for age and sex specific locally determined RIs. For example, a total of 405 adults consisting of 238 (58.7%) males and 167 (41.3%) females with the median age of 24 (range 18 to 60) years were included in the assessment of immunological and hematological reference values for apparently healthy HIV-negative adults in Bahir Dar Town, Ethiopia. The median, mean (± SD) and 95 percentile ranges of hematological values were determined. The mean (±SD) values were: RBC (10^{12}/L), 4.9±0.4 (female) and (5.4±0.5 male); Hgb (g/dl), 14.7±2 (females) and 16.5±1.8 (males); Hct (%), 44±4 (females) and 49±4.5 (males); platelets (10^9 /liter), 277 ± 20 (both sex); absolute leukocyte (WBC) counts 6.6±3.6 x10^9 /L (both sexes); lymphocyte, 2.15±.59 x10^9 /L (both sexes); granulocytes (neutrophils) 3.7±1.6 x10^9/L (both sexes) [12].

A study which takes place on the assessment of anemia among elementary and high school students living in Gorgora, Gondar, Ethiopia from 156 males (52.9%) and 139 females (47.1%) enrolled in the age distribution as follows; 79 (26.8%) 7-10 years, 157 (53.2%) 11-15 years, and 59 (20.0%) 16 years and above. The study shows the mean hematocrit value rises with age similarly for males and females up to 15 years, but the mean in women over 15 years being approximately 4% less than males [32].

3. Objectives

3.1. General objectives

- ✓ To establish community based hematological reference intervals among apparently healthy adolescents aged 12 to17 years in Mekelle city, Tigrai, Northern Ethiopia from December 2018 –May 2019.

3.2. Specific objectives

- ✓ To establish sex specific reference values among apparently healthy adolescents aged 12 to17 years.
- ✓ To compare the hematological reference intervals by gender difference among adolescents aged 12 to17 years

4. Materials and Methods

4.1. Study Area

The study was conducted from December 2018- May 2019 in Mekelle City. Mekelle was formerly the capital of Enderta awraja, but today the capital city of Tigrai regional state. It is located around 780 kilometers (480 mi) north of the capital city of Addis Ababa, Ethiopia with an elevation of 2,254 meters (7,395 ft) above sea level with Weyna-Dega climatic conditions. Administratively, Mekelle is considered a special zone, which is divided into seven sub-cities namely Hawelti, Adi-Haki, Kedamay-Weyane, Hadnet, Ayder, Semien and Quiha and also subdivided into 33 Kebelles. Mekelle is the economic, cultural and political hub of northern Ethiopia [33].

Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), Mekelle has a total population of 215,914 people (104,925 men and 110,989 women) [34].The sample was collected from apparently healthy individuals and also analyzed at Tigrai health research institute and Wukro general hospital. The institute is found in Hawelti sub-City and Wukro general hospital is 45 km far from Mekelle to the eastern zone. The hospital facility was utilized due to the availability of functional automated hematology analyzer during the study period and their willingness [33].

4.2. Study design and period

A cross-sectional study was conducted from December 2018 - May 2019.

4.3. Population

4.3.1. Source population

All adolescents who are living in Mekelle City

4.3.2. Study population

Apparently healthy volunteering adolescents aged 12-17 years who fulfilled the eligibility criteria and available in the selected sub-cities during the study period

4.4. Inclusion and Exclusion Criteria

4.4.1. Inclusion Criteria

Apparently healthy individuals aged 12-17 years and lived at least for 5 years in the study area

4.4.2. Exclusion Criteria

- ✓ Individuals with any chronic and acute illnesses
- ✓ Individuals taking antibiotic treatment
- ✓ Individuals who received blood transfusion
- ✓ Individuals who have any intestinal and hemoparasites
- ✓ Individuals who haven't full filled the questionnaire criteria

4.5. Study Variables

4.5.1. Dependent Variable

- ✓ Hematological parameters

4.5.2. Independent Variables

- ✓ Sex

4.6. Sample size determination and sampling technique

4.6.1. Sample Size determination

The CLSI guideline for the global application which was developed through the clinical and laboratory standards institute consensus process was employed. CLSI recommended that the best means to establish a reference interval was to collect samples from a sufficient number of reference individuals to yield a minimum of 120 samples for analysis, by non-parametric means for each partition (e.g. sex, age range) [4]. Growth is an extremely complex and non-linear biological process, driven by hormonal mechanisms, characterized by an intrinsic variability reflecting environmental, genetic influences and individual adaptive responses. Growth charts are very helpful as reference tools for pediatricians, in order to monitor individual growth and provide therapeutic interventions. Most female's puberty starts around 12 years but males from 14 years [35]. Therefore starting from the age of 12 years males and females must separate for the establishment of reference intervals (thus, 240 participants are needed for both sexes). According to previous studies in other African countries, in large scale studies about 30% of apparently healthy individuals [5] do not qualify for reference interval determination for various reasons when tested for the common infections. Considering a 30% exclusion from data analysis, to reach the CLSI recommended sample size of 240 for the reference interval determination, a total of 344 individuals were enrolled (i.e. assuming 30% of 344 around 104 of the participants may excluded during data analysis).

4.6.2. Sampling technique

A total of 3 sub-cities were selected from the total of seven sub-cities (Ayder, Hawelti and Semen) through random sampling method and then the total sample (344) was categorized based on the relative house hold size in each sub-city using Probability Proportional to Size (PPS). The 3 sub-cities have a total of 68477 households (18266, 33319 and 16892 in Semen, Hawelti and Ayder respectively).The total study participants were proportionally distributed to each sub-cities based on their number of households. Accordingly, from Semen 92, Hawelti 167 and Ayder 85 children were recruited. In each sub-city there are 5 Kebelles, and then the numbers were distributed to recruit participants from each Kebelles based on the number of house hold. Finally the study participants were selected using the systematic sampling techniques (k^{th}). If the K^{th} house hold is not fulfilled the eligible criteria or doesn't have children they were passed to the nearby household either to the next or former which fulfilled the criteria. Once volunteering participants fulfilling the eligibility criteria are identified by the health extension workers, they were invited to go to nearby health facilities or other selected collection areas for biological sample collection.

Table 1: Total number of households to be sampled from each sub cities and Kebelles of Mekelle, Tigrai, Northern Ethiopia 2019

sub-city	Kebelles	Total number of house hold	Total number of sample to be recruited	Age of 12-17 Females	Age of 12-17 Males
Semen	Mesfin	4615	24	12	12
	Dedebit	2870	14	7	7
	Yekatit	3216	16	8	8
	Endistry	2451	12	6	6
	Meles	5114	26	13	13
	Total	**18266**	**92**	**46**	**46**
Hawelti	Selam	3397	17	8	9
	Hayelom	4614	23	12	11
	Adi shimduhun	8793	44	22	22
	Momona	7731	39	19	20
	Hidase	8784	44	22	22
	Total	**33319**	**167**	83	84
Ayder	Sertse	3483	17	8	9
	Ginbot 20	3487	18	9	9
	Marta	3637	19	10	9
	Adi ha	5046	25	13	12
	Maryam Dihan	1239	6	3	3
	Total	**16892**	**85**	43	42

Note: Source for total population and number of households is from municipal of Mekelle city

4.7. Measurement and Data Collection

From the study participants who give assent based on their family consent, demographic information and a brief medical history was collected. The physical examination was performed by physicians. Blood specimen was collected for analyzing hematological parameters, blood grouping and hemoparasites. Stool specimen was collected for intestinal parasites examination and urine samples for urinalysis. The Laboratory results were shown to the participants upon their request through the health extension workers. But when their result shows above or below the normal value and presence of intestinal parasite in their stool examination, the health extension workers were linked the participant to nearby health institutes for proper management and treatment according to the facilities guideline.

4.7.1. Demographic and clinical data

Socio-demographic and clinical data were collected using a structured questionnaire by translating in to local language Amharic/Tigrigna by experienced professionalisms. Data was collected by trained data collectors, physical examination and anthropometric measurements were carried out by physicians.

4.7.2. Sample collection for laboratory analysis

About 4ml of venous blood sample was collected using K_2EDTA anticoagulant test tubes using multisampling needle from 8 am to 11 am. The sample collected in K_2EDTA anticoagulant was gently mixed by inverting the tube about 8 times immediately after drawing. Leak proof clean containers were used to collect urine and stool samples. All samples were labeled with unique identification number. The hematological samples were placed in cooled ice-box for transporting to Wukro general hospital within the allowable time. Left over plasma was collected and stored at -20^{o}c in the Tigrai health research institution (THRI) for further analysis as necessary.

4.7.3. Laboratory testing and analysis

Whole blood sample was used for analyzing hematological tests, blood morphology, blood-grouping and hemoparasites identification. Complete blood count and differential was analyzed using Sysmex KX-21N an automated 3-part differential hematology analyzer (Sysmex Corporation Kobe, Japan).The machine automatically dilutes whole-blood sample of 50µl in the CBC/differential mode, lyses and enumerates WBC, RBC, Hgb, HCT, platelets and their indices (PCT, MPV, PDW) absolute and relative lymphocytes, neutrophils and mixed populations, and the red cells indices.

PRINCIPLE:

The Sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use for determining 17 hematological parameters. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemia's, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex KX-21N analyzer directly measures the WBC, RBC, Hgb, HCT, and PLT, LYM #, MIXED #and NEUT #. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT%.

The KX-21N counts and sizes RBC and PLT using electronic resistance detection, HCT is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hgb is converted to methemoglobin, and read photometrically at 555 nm. WBC are analyzed by direct current and discriminated into a three-part differential using Particle Distribution Analysis (PDA). The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mixed cell populations. The middle-size cell population contains Monocytes, basophiles and Eosinophils**.**

DC Detection Method

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

Non-Cyanide Hemoglobin Analysis Method

To analyze hemoglobin by automated methods, the Cyanmethemoglobin method or Oxyhemoglobin method have so far been the main stream. Cyanmethemoglobin method was recommended as the international standard method in 1966 by ICSH (International Committee for Standardization in hematology). This method, however, is so low in hemoglobin conversion rate that it cannot be said an appropriate method in the automated process in which multi-sample processing is the pre-condition. In addition, this method uses the reagent of cyanide compound

which is a poisonous substance and requires waste processing; thus, it can hardly be called an environmentally favorable method. At present, this method cannot be said suitable for a fully-automated instrument which is required to handle a large amount of waste. The Oxyhemoglobin method, on the other hand, is faster in hemoglobin conversion rate; in fact, blood hemoglobin is converted instantaneously into Oxyhemoglobin. Also, it does not contain poisonous substance as Cyanmethemoglobin method, making the method suitable for automation. This method, however, is unable to convert methemoglobin into Oxyhemoglobin. Consequently, when a great amount of methemoglobin is contained as in control blood, lower-than-real values result, although usual human blood poses no problems.

Non-cyanide hemoglobin analysis method utilizes the advantages of both of the above methods. Non-cyanide hemoglobin analysis method rapidly converts blood hemoglobin as the Oxyhemoglobin method and contains no poisonous substance, making it suitable for automated method. Being capable of analyzing methemoglobin, this method can accurately analyze control blood, etc. which contain methemoglobin.

Reagents

Cell Pack: - is a whole blood diluent for use in the determination of hemoglobin and electric counting and sizing of blood cells. Its ingredients are: sodium chloride, boric acid, sodium tetra borate, and K_2EDTA.

Stromatolyser WH: - is ready to use lysing reagent to analyze the leucocytes by lysing the RBC and left the WBC free and easy to count; whole blood sample by resistance measurement and photometric measurement and its ingredients are: nonionic surfactant, organic quaternary ammonium salt.

Cell Clean: - is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex analyzer.

Reagents preparation: Reagents are commercially prepared.

All laboratory assays were carried out following standard operating procedures by experienced medical laboratory technologists. ABO and RH typing was performed using the corresponding antiserum.

Stool samples were collected for parasitological analysis, urine for determining chemical and microscopic examination and pregnancy tests of young girls.

4.8. Data quality control

The questionnaire was pre-tested with 5% (18) of individuals who are living in Wukro city which is 45 km far away from Mekelle, rather than study subjects. This pre-testing of a research instrument was entailed a critical examination of each question as to its clarity, understanding, wording, and meaning as understood by potential respondents to remove possible problems with the question. Besides, adequate training was given to the data collectors before the collection period. Participants were also being adequately oriented on how to collect specimens. The quality of laboratory analysis was maintained by following standard operating procedures of the pre-analytical, analytical, and post-analytical stages, which involves the following steps.

Pre-analytical stage

- ✓ Regular supervision and orientation of participants on specimen collection
- ✓ Checking specimen containers for leakage, contamination, and label
- ✓ Proper labeling, processing, preservation, storage, and transporting of specimen to the working area
- ✓ Checking reagents for expiry date
- ✓ Make sure specimens reach laboratory as soon as possible
- ✓ Cross-checking of the sample with its questionnaire

Analytical stage

- ✓ Implementing the Internal Quality Controls (IQC) sample for the complete blood count and peripheral morphology through the whole processes of laboratory works
- ✓ Since, the Laboratory is on the process of accreditation they always done the three (**Low**, **Normal** and **High**) levels of QC tests daily. Without passing the QC tests they cannot do the patients result.
- ✓ Controlling stains by checking normal cells and reagents

Post-analytical stage

- ✓ Recording of results on appropriate reporting format
- ✓ Comparing of test results
- ✓ Interpreting test results correctly

4.9. Data Analysis and interpretation

Data were cleaned, double entered into a computer and statistical analysis was made using Statistical Package for Social Sciences (SPSS) version 23. Both parametric and non-parametric analyses were performed. Data that was observed to be lower than first quartile (Q1-1.5 × IQR), or higher than third quartile (Q3+1.5 × IQR) (Box and Whisker blot method) was considered as outliers and the outlier was excluded. The Mean, median and 95^{th} percentile reference intervals was determined by using 2.5^{th} and 97.5^{th} percentiles of each hematological parameter with simple descriptive statistics based on sex. Differences on median between males and females were evaluated using the Mann–Whitney U test to calculate the p values. "P-value < 0.05" was considered to be statistically significant at 95% confidence intervals.

4.10. Ethical Consideration

Ethical clearance was obtained from the research and an ethical review committee of Department of Medical Laboratory Sciences of Addis Ababa University, Ethiopia. Before starting the study, permission was obtained from Tigrai health bureau and the selected sub-cities. Also, after explaining the purpose and relevance of the study, written consent was obtained from each guardian of study participant and assent from the children before data collection. No name was mentioned during the entire data collection and identification was made based on the unique identification number given for each questionnaire and corresponding specimen. Confidentiality of information (results) was kept between the study participant, investigator and authorized body. All participants who were diagnosed positive for intestinal parasites were linked to nearby health institutions for standard treatment immediately. Cooperation letter was obtained from Wukro General Hospital to do the hematological tests.

4.11. Dissemination of result

Findings of this study will be disseminated to the study were local and zonal health administrations, Tigray regional health bureau, Medical laboratory science, College of Health Sciences, Addis Ababa University, and other concerned bodies. More ever the results would be presented to the scientific community in the AAU, national and international conferences and manuscript would be prepared and submitted for publication.

4.12. Operational Definition

Adolescents: is a period of development that occurs after childhood but before adulthood (12-17 years)

Apparently healthy: An individual who has no sign and symptoms and history for any disease in the previous and current time.

Hematological parameters: are tests which are done under the hematology analyzer including total WBC and its differentials (Neutrophils, lymphocytes and mixed valued cells), RBC and its parameters (Hgb, Hct, MCV, MCH, MCHC, and RDW), platelets and its parameters (MPV, PDW).

Reference individual: A person selected for testing based on well-defined criteria.

Reference population: A group consisting of all the reference individuals.

Reference interval: It is the 95% interval between 97.5^{th} and 2.5^{th} percentile which form upper and lower reference limit.

5. Work flow

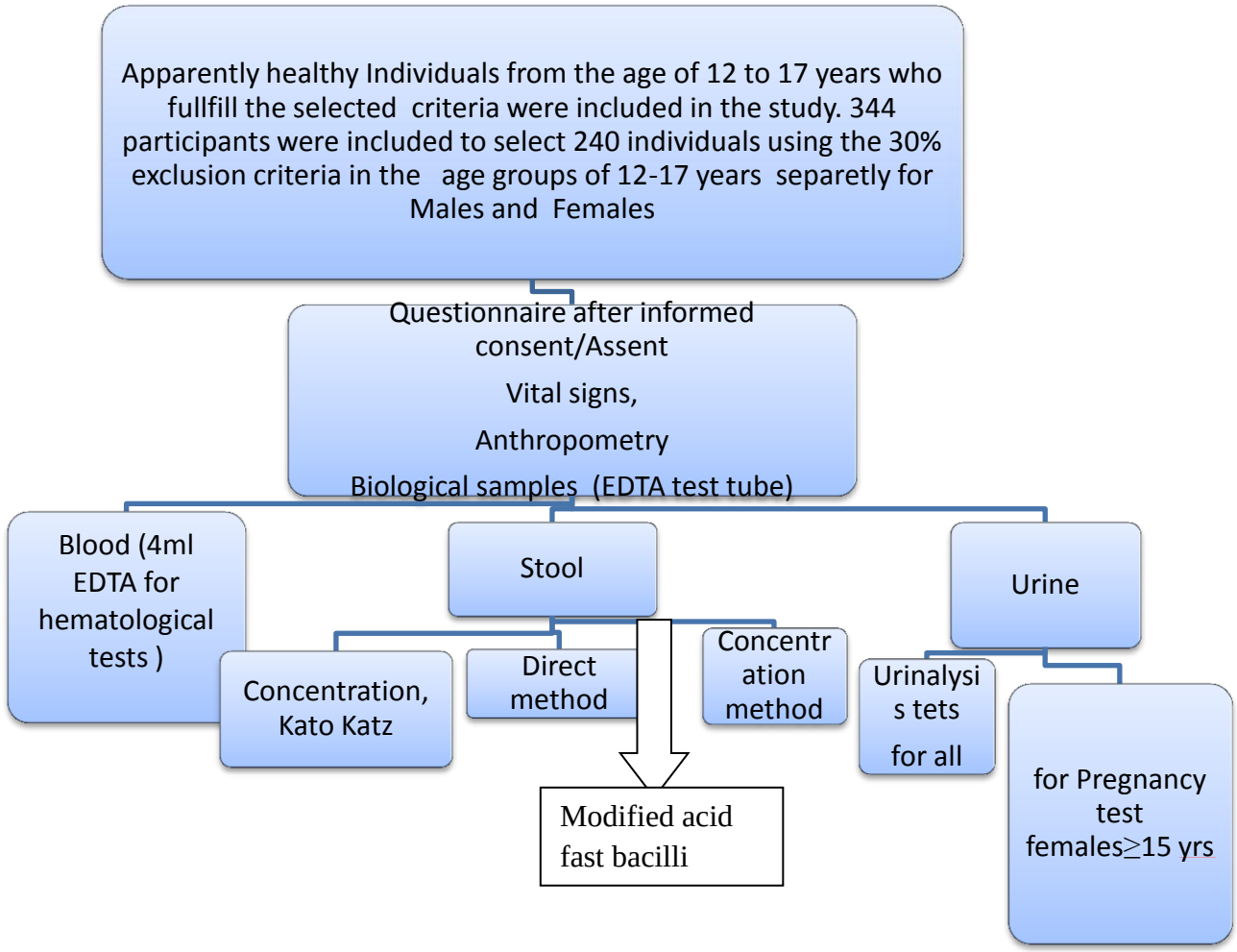

Figure 1 Work flow of the study

6. Results

6.1. Socio-Demographic characteristics

In this study a total of 344 adolescents were participated and 249 study participants were eligible for final analysis. Of the 249 study participants, 127 (51%) were females (Table 2). The mean age of study participants was 15.31 ±1.09 ranged 12-17 years old; figure 1 showed the age distribution of the study participants. The overall exclusion rate was 27.6%. Out of the total sample collected 50 (14.5%) of them were excluded due to the presence of intestinal parasites (ova of *S.mansoni* (33), ova of *H.nana* (12) and ova of *E.vermicularis* (5) and 45(13.1%) and also excluded due to incomplete information (15), outliers (20) and presence of hemolysis (10).

Table 2: Socio demographic characteristic of study participants in Mekelle city, Tigrai, Ethiopia, 2019 (n=249).

Variables	Frequency	Percent (%)
Sex		
Female	127	51
Male	122	49
Level of education		
Primary (1-8)	9	3.6
Secondary (9-12)	240	96.4
Religion		
Orthodox Christian	226	90.8
Muslim	23	9.2
Total	249	100

6.2. Hematological parameters of the study participants

The distributions of the parameters (median RBC p<0.001, Hgb p<0.001, Hct p<0.001, mixed p<0.001, MCHC p=0.027 were statistically different by gender; females had lower values than males (p<0.05). Statistically significant higher MCV p=0.019, platelets p<0.001, RDW-CV p=0.002, Abs Lymphocyte count p=0.004 and Neutrophils (%) p=0.036 were found in females (p<0.005). Males had higher RBC, Hgb, HCT, MCHC, RDW-CV, Absolute and percentile of mixed values of WBC differential than females. Median and 95% RIs shows as for males and females respectively were as follows: **RBC** (10^{12}/L) 5.2, 4.6-5.9; **Hgb** (g/dl) 14.8,12.6-17.1; **HCT** (%) 44.8, 40-55; **MCHC (**g/dl) 32.5, 30-35.8; **WBC** (10^{9}/L) 5.4, 2.9-10.49; **Plt** (10^{9}/L) 261, 138-364 for males and **RBC** (10^{12}/L) 4.9, 4.28-5.61**; Hgb** (g/dl) 14,12-15.4; **HCT** (%) 43.3, 38-47; **MCHC (**g/dl) 32.2, 30.4-34.2; **WBC** (10^{9}/L) 5.9, 3.4-10.96; **Plt** (10^{9}/L) 288,151-448 for Females. For detail information see **Table 3**.

Table 3: Mean, Median and 95% RI of hematological parameters among study participants in Mekelle, Tigrai, Northern Ethiopia, 2019 (n=249).

Parameter	Sex	N	Median	Mean	95%CI	95% RI		p-value
						2.5thcentile(90% CI	97.5thcentile(90%CI)	
WBC(10^9/L)	M	122	5.4	5.85	5.48-6.2	2.9 (2.6-3.3)	9.6(9-9.7)	
	F	127	5.9	6.28	5.9-6.6	3.4(2.7-3.6)	10.2(9.2-10.7)	0.027*
RBC(10^{12}/L)	M	122	5.2	5.3	5.2-5.36	4.59 (4.45-4.63)	5.91(5.88-5.98)	<0.001*
	F	127	4.9	4.92	4.8-4.98	4.28 (4.13-4.42)	5.61(5.4-5.76)	
Hgb(g/dl)	M	122	14.8	14.8	14.6-15	12.6 (12.1- 13)	17.1(17-18.6)	<0.001*
	F	127	14	13.9	13.76-14	12 (11.4-12.4)	15.4(15-16)	
HCT (%)	M	122	44.8	45.4	44.8-46	40 (38- 40.8)	55 (51-58)	<0.001*
	F	127	43.3	43.2	42.8-44	38 (36-39)	47(46- 50)	
MCV(fl)	M	122	86.7	86.7	86-87.4	76(74-80)	94(93-95)	0.019*
	F	127	88	88	87-88.6	80(73- 82)	98 (94-99)	
MCH(pg)	M	122	28.3	28.3	28-28.6	23.5(22- 25)	31.5(30.9-34.5)	0.085
	F	127	28.6	28.3	28-28.5	24.5 (21- 25.8)	30.8(30- 31.7)	
	C	249	28.3	28.5	28-28.5	24(23-24.8)	31(30.8-31.8)	
MCHC(g/dl)	M	122	32.5	32.6	32.4-33	30(29.7-30.8)	35.8(34.3-41.3)	0.027*
	F	127	32.2	32	32-32.4	30.4(29-30.9)	34.2(33.8-34.3)	
PLT(10^9/L)	M	122	261	261	251-271	138(133-188)	364(353-391)	<0.001*
	F	127	288	294	281-307	151(140-181)	448(413-467)	
RDW-CV (%)	M	122	14.1	14.3	14-14.4	13(12.8-13.2)	16(16-16.6)	0.002*
	F	127	13.7	13.8	13.7-13.9	12.5(12.4-12.8)	15.6(15-17.6)	
Neutrophil(10^9/L)	M	122	2.4	2.75	2.48-3	0.9(0.8-0.9)	6.7(5.7-7.7)	0.113
	F	127	3.0	3.23	2.9-3.55	1.0(0.8-1.2)	6.9(6.3-12.7)	
	C	249	3.0	2.7	2.8-3.2	0.9(0.8-1)	6.8(6.3-7.2)	
Mixed value(10^9/L)	M	122	0.8	0.97	0.85-1.1	0.3(0.2-0.4)	3.8(2.- 4.2)	<0.001*
	F	127	0.6	0.69	0.6-0.75	0.2(0.1-0.3)	1.6(1.2-2.4)	
Lymphocyte(10^9/L)	M	122	2.00	2.12	2.0-2.22	1.2(0.9-1.3)	3.3(3.1-3.7)	0.004*
	F	127	2.3	2.36	2.3-2.5	1.1(1-1.3)	3.7(3.5-3.8)	
Neutrophil (%)	M	122	45.5	44.8	42.6-47	23.7(21-24.9)	64.7(62-74.5)	0.036*
	F	127	50.6	49	46.8-51.	24.8(22-28.5)	71(66- 89.5)	
Mixed value (%)	M	122	14.5	16.3	15.-17.5	7.2(5.5-9.4)	36.8(29-43.5)	<0.001*
	F	127	10.5	11.4	10.5-12	4.8(1-5.1)	22.7(19- 37.7)	

Lymphocyte (%)	M	122	38.5	38.9	37-40.8	18.8(15-23)	63.4(59-68)	0.48
	F	127	37.5	39.6	37.8-41	20.6(8.8-26)	61.2(56.3-65.3)	
	C	249	39	38	38-40.6	19.8(17-23)	61.5(59-64)	
MPV(fl)	M	122	10.6	10.7	10.5-11	8.6(8.4-8.8)	13.3(12.6-14.5)	0.567
	F	127	10.7	10.7	10.5-11	8.9(8.6-9.1)	12.8(12.4-14)	
	C	249	10.6	10.6	10-10.8	8.8(8.6-8.9)	13.1(12.6-13.6)	

***WBC**: white blood cell; **RBC**: red blood cell; **Hgb**: hemoglobin; **Hct**: hematocrit; **MCV**: mean corpuscular volume; **MCH**: mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration; **PLT**: platelet; **MPV**: platelet mean volume **M**: male; **F**: female; **C**: Combined **N**: number of participants. *P < 0.05 by (Mann–Whitney U test) for comparison of medians between males and females*.

Comparing the upper and lower limits of this study established reference intervals to the company's reference intervals (**Table 4**). There were higher proportions of out of range values observed for RBCs 19(15%), MCHC 31(25.4%), Hgb 30(24%), Hct 33(24%), Mixed value 35(28%), MPV 67(54%) and percentile neutrophils 39(31%) in males. In females, a higher proportion with out of range values was observed for RBCs 12(9%), Hct 25 (19%), MCHC 49(38%), percentile neutrophils 28(21%) and MPV 28(22%). The combined out of company given ranges were WBC 56 (22%), Lymphocyte percentile 104 (42%) and absolute neutrophils 69(27%). The greatest proportion of the study participants with values outside the lower reference limits of reference intervals was observed for absolute neutrophil 68 (27%) while Lymphocyte percentile104 (42%) had the greatest proportion of participants with values above the upper reference limits of the old reference intervals. When comparing between this study and company's reference intervals methods for the WBC test parameters 51(20%) out of the 249 participants were considered leucopenia by the company's method while 5(2%) were considered as having leukocytosis.

Table 4: comparison of out of range values between new and old reference intervals for hematological parameters in Mekelle city, Tigrai, Northern Ethiopia.

Parameter	Sex	Current value	Company value	Lower range	Upper range	Total out of range
				Frequency (%)	Frequency (%)	Frequency (%)
WBC($x10^9$/L)	M F C	2.9-9.6 3.4-10.2 3.1-10.6	4.5-13	51(20)	5(2)	56(22)
RBC($x10^{12}$/L)	M F	4.6-5.9 4.3-5.6	4.2-5.6 4.1-5.3	2(1) 3(2)	17(14) 9(7)	19(15) 12(9)
Hgb(g/dl)	M F	12.6-17.1 12-15.4	12.5-16.1 12 -15	2(1) 0	28(23) 4(3)	30(24) 4(3)
HCT (%)	M F	40-55 38-47	36 - 47 35 - 45	5(4) 3(2)	28(23) 22(17)	33(27) 25(19)
MCV(fl)	M F	76-94 80-98	78 - 95 78 -95	1(0.8) 2(1)	1(0.8) 0	2(1) 2(1)
MCH(pg)	C	24-31	26 - 32	13(5)	2(0.8)	15(6)
MCHC(g/dl)	M F	30-36 30.4-34	32 - 36	31(25) 46(36)	0 3(2)	31(25.4) 49(38)
PLT($x10^9$/L)	M F	138-364 151-462	140 - 385	0 2(1.6)	1(0.8) 11(8)	1(0.8) 13(2.4)
Neutrophil($x10^9$/L)	C	0.9-6.8	2-7	68(27)	1(0.4)	69(27)
Mid value($x10^9$/L)	M F	0.3-3.8 0.2-1.6	0.24-1.6	0 0	7(2.8) 0	7(2.8) 0(0)
Lymphocyte ($x10^9$/L)	M F	1.2-3.3 1.1-3.7	1-3	0 0	3(2.4) 16(12.6)	3(2.4) 16(12.6)
Neutrophil (%)	M F	24-65 24.78-71	40 - 80	37(30) 26(20)	2(1.6) 2(1.6)	39(31) 28(21)
Mid-size value (%)	M F	7-37 5-23	4-18	2(1.6) 2(1.6)	33(27) 6(4.7)	35(28) 8(6)
Lymphocyte (%)	C	20-62	20 - 40	0	104(42)	104(42)
MPV(fl)	M F C	8.6-13.3 8.9-12.8 8.8-13.1	7.2-10.4 7.5-11.5	2(1.6) 2(1.6)	65(53) 26(20)	67(54) 28(22)

***WBC**: white blood cell; **RBC**: red blood cell; **Hgb**: hemoglobin; **Hct**: hematocrit; **MCV**: mean corpuscular volume; **MCH**: mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration; **PLT**: platelet; **MPV**: platelet mean volume **M**: male; **F**: female; **C**: Combined **N**: number of participants.*

Finally, the current study established RI was compared with others studies. As shown in **Table 5**, variations among the difference parameters were noted. For example, the white cell count of these participants as well as the values from other Africans was lower compared to the instrument or American/European values. RBC parameters were higher in the lower range than southwest Ethiopia. Some inconsistencies were also noted in Platelet counts.

Table 5: WBC, Hgb, Hct, MCV, Plt and WBC subsets for apparently healthy Mekelle city Children Compared to Previously Published Tanzanian, Ugandan, Zimbabwe and Industrialized Country reference Intervals

Parameter	Sex	Current Study (95% RI)	Instruments Values [36]	South west Ethiopia [15]	Tanzanian (95% RI) [27]	Uganda (95% RI) [18]	United States/Europe (95% RI)	Zimbabwe (95% RI) [29]
WBC(10^9/L)	M F C	2.9-9.6 3.4-10.2 3.1-9.6	4.5-13	4.0-11.7 3.7-11.4	3.2-10.3	4.1-10.7	4.5-13	3.25-8.64 3.3-9.8
RBC(10^{12}/L)	M F	4.6-5.9 4.3-5.6	4.2-5.6 4.1-4.5	4.06- 6.57 4.32-5.63	-	-	-	4.47-6.47 4.8-5.83
Hgb(g/dl)	M F	12.6-17.1 12-15.4	12.5 –16.1 12 – 15.4	12- 19.6 11.6- 15.9	10.8-17 10-14.9	11.2-15.9 9.9-14.5	13-16 12-16	12.1-17.4 11.1-15.7
HCT (%)	M F	40-55 38-47	36 – 47 35 – 45	35.6-55.2 36-47	33-48.1 30.8-44.7	32.3-45.5 28.1-42.4	37-49 36-46	36.1-49.7 34.6-46.7
MCV(fl)	M F	76-94 80-98	78 – 95 78 – 95	75-93 74.5-91	63.2-91 62.2-94.5	65-89.5 67.4-89.9	78-98 80-100	70.1-93.2 68.7-96.9
MCH(pg)	M F C	23.5-31.5 24.5-30.8 24-31	26 – 32 26 – 32	25-31 25- 30.8	-	-	-	22.5-30.9 22.1-32
MCHC(g/dl)	M F	30.1-35.8 30.4-34.2	32 – 36 32 – 36	32-36 32-35	-	-	-	30.3-36.1 29.8-35.8
PLT(10^9/L)	M F	138-364 151-462	140 – 385	158.5-470 198-460.4	119-458 107-482	110-327 124-353	150-400 150-400	186-415 214-476
Neutrophil (10^9/L)	M F C	0.9-6.7 1.02-6.9 0.9-6.8	2-7	1.3 -7.4 1-7	0.9-4.6	0.9-3.5	1.5-6	1.03-3.9 1.13-5.7
Mid value(x10^9/L)	M F	0.3-3.8 0.2-1.6	0.24-1.6	-	0.2-2.5	0.5-3.6	0.6-1.5	0.41.5 0.3-1.5
Lymphocyte(10^9/L)	M F	1.2-3.3 1.1-3.7	1-3	-	1.4-4.2	1.7-4.7	1.5-4.5	1.4-3.9 1.4-3.9
Neutrophil (%)	M F	23.7-64.7 24.78-71	40 – 80	-	-	-	-	23.3-58.5 24-61.2

Mid-size value (%)	M F	7.2-36.8 4.8-22.7	4-18	-	-	-	-	5.4-67.3 5-73.4
Lymphocyte (%)	M F C	18.8-63.4 20.6-61.2 19.8-61.5	20 – 40	-	-	-	-	27.7-62.6 28.4-65
MPV(fl)	M F C	8.6-13.3 8.9-12.8 8.8-13.1	7.2-10.4 7.5-11.5	-	-	-	-	8.6-12.3 8.4-11.2

***WBC**: white blood cell; **RBC**: red blood cell; **Hgb**: hemoglobin; **Hct**: hematocrit; **MCV**: mean corpuscular volume; **MCH**: mean corpuscular hemoglobin; **MCHC**: mean corpuscular hemoglobin concentration; **PLT**: platelet; **MPV**: platelet mean volume **M**: male; **F**: female; **C**: Combined **N**: number of participants.*

7. Discussion

Reference Intervals are essential for decision making in clinical diagnosis, to initiate and monitor therapeutic actions, or to provide accurate data for epidemiological purposes. Several factors including age, sex, race, environment, socio-economic conditions, dietary pattern etc. influence laboratory parameters. RIs also depend on the type of instrument, reagents and methods used. Hematological parameters tested in this study were WBC and its differential, RBC with indices and also platelets were analyzed using the automated Sysmex KX 21-N 3 diff hematology analyzer. Most of the parameters showed a significant difference among gender which is similar to studies done in Western Kenya [23], Tanzania [27], Southwest Ethiopia [15], African American [25], Kuwaiti [26] and Port Harcourt Nigeria [28]. The RBC, Hgb and HCT were significantly higher in males than females ($p< 0.001$). The reason for the difference b/n male and female may be due to hormonal variations among sex may affects erythropoietin release in response to the hormonal production [27] and also progressive maturation increase in muscle mass resulting in increased RBCs production in males but females having lower level may be due to menstrual blood loss [29].

In contrast in our study the mean value of RBC and Hgb results were significantly different from mean values of studies reported in Kuwaiti [26] and African American [25] among children. The differences may be due to ethnicity [25], altitude variation since Kuwaiti have an average mean altitude of 108m above sea level and nutritional differences [26].

There is also a significant difference among sex for WBC RI in our study which is higher in females than males similar with studies done in Kuwaiti [26], African American [25], Zimbabwe [29], and western Kenya [31].This sex difference in the total WBC counts is almost in all ethnic groups. This difference may be due to a genuine biological difference [37], and also due to body composition difference between men and women because women have proportionally more fat mass than men [10, 38] so, fatty mass is the critical component that influences leukocyte counts [39]. However, the Kenyan study did not find significant differences between males and females [23].

When comparing WBC results with similar studies which are done in African American, Kuwaiti, Zimbabwe [25, 26, 29] results were higher than our study population. This difference

may be due to the dietary or other life style differences [37], and also we Sub-Saharan countries are known exposed to chronic viral and bacterial infections [5].

The current finding also showed that the mean, median and 95th percentile reference intervals for the platelet counts varies significantly between females and males ($p<0.05$). This study indicated that the platelet value is higher in females than males similar to other studies done in Kuwaiti [26], Zimbabwe [29], Port Harcourt Nigeria [28] and Southwest Ethiopia [15]. The sex difference in the platelets counts is almost in all ethnic groups and it may be due to biological difference [37]; proteomic variability males have higher cytokine and growth factor levels in platelet rich plasma when compared with females for inflammatory cytokines such as interleukin–1 beta and tumor necrosis factor–alpha [40]; and also due to the differences on serum estrogen level in females play a role in the increased platelet counts, many studies have revealed that estrogen favorably benefits platelet production [41, 42]. Thrombopoietin release increases in response to regular menstruation cross-stimulating thrombopoiesis [43, 44], the total body iron storage is generally lower in women because of menstruation, so iron depletion is a well-known factor to stimulate platelet production [45, 46]. However, the Kenyan study didn't find significant differences between males and females [23].

The platelet counts are also lower in our study population than similar studies done in Southwest Ethiopia [15], and Zimbabwean adolescents [29]. The cause of platelet count differences among different study areas is unknown [28].

Though limited studies are available on children and adolescents, the neutrophil range in the current study which is lower than the currently in use company derived ranges, is consistent with the findings from Zimbabwean adolescents [29]. In addition, the upper limit of the newly established lymphocyte RI is higher than the company derived value but again consistent with values generated from Zimbabwean adolescents [29], as well as finding from adult [11]. Comparing to the reference interval generated in southwest Ethiopia on a total of 334 children, the WBC count of both lower and upper limit was higher than the current study especially in males. On the other hand, both the lower and upper limit of RBC count was slightly higher in the current study whereas slightly higher upper limit for Hgb was recorded in male children of the Southeast Ethiopian study. Similarly higher lower & upper limit for PLT count in males and higher lower limit only in Females was recorded as compared to the current study [15].

Taken together, when comparing the newly developed RI with the old method almost all tests have a variation. The newly developed RI for WBC results is lower than the old method may be, as we know Africans are more exposed to chronic infections [5]. But the RBC and RBC indices are higher than the currently in use method, the reason for the increment may be the current study was undertaken in the Weyna-dega climatic conditions with an altitude of 2,254 meters above sea level.

Finally the study checked how much misclassification could have happened as a result of utilizing company derived RIs, which is widely being practiced in our country as in most resource limited settings. The reported result showed such misclassification is noted in most of the hematological parameters by the RI established by manufacturer of the instrument Sysmex KX-21N as compared to the RI established by this study. This may be due to nutritional difference, climate, parasitic and viral infections, ethnic background differences between the current study population and the population used for the company derived values, mostly Caucasians. So, it is better to use this established RI for the local populations.

8. Strength and limitation of the study

8.1. Strength of the study

The sample was collected at morning to minimize the diurnal variation. Wet mount, concentration (formol-ether), Kato Katz and modified acid-fast technique were performed to exclude participants who are infected with intestinal parasites. Besides this, blood films were done and well-prepared questionnaire to check individual's current or previous apparently healthy status.

Since this study is community based study it is more representative than the other studies

8.2. Limitations of the study

- ✓ The findings in this study was limited to be used in the 3 Diff analyzer until validated

9. Conclusion and Recommendation

9.1. Conclusion

This study concluded that there were statistically significant higher values of WBC count, MCV, platelet count, lymphocyte count, and neutrophil percent in females than males. However the value of RBC count, hemoglobin, hematocrit, MCHC, RDW CV (%), mixed count, and mixed percentage were statistically significant higher in males than females. Hematological reference values established in this study was different from text book reference. Our data confirms that it is important and mandatory to prepare local reference intervals separately for males and females.

9.2. Recommendations

- ✓ It is better every regions and also every health facility to set their own reference intervals because they may have difference in geographical, altitudinal and climatic condition difference
- ✓ It is better clinicians to use locally established reference intervals than the reference interval from text books and other company values.

10. References

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Annexes

Aannex I: Informed assent / English version

(The questionnaire is translated into the study participant's local language)

This informed assent form is for children apparently healthy individuals, who are invited to participate in this study on the reference interval of hematological parameters.

Information Sheet

Introduction: I am Hagos Haileslasie W/haweriat (BSc) studying medical hematology and Immunohematology at Addis Ababa University College of health science department of medical laboratory sciences. I am researching the reference interval. I am going to give you the information and invite you to be part of this research. There may be words that you do not understand. Please ask me to stop as we go through the information and I will take time to explain.

Title of the project: Establishment of community based hematological reference intervals among the apparently healthy adolescent's aged 12-17 years in Mekelle city, Tigrai, North Ethiopia; A cross sectional study design from December 2018- May 2019

Introduction: You are kindly invited to participate on the establishment of hematological reference intervals among the apparently healthy individuals in Mekelle City, Tigrai, and Northern Ethiopia.

Purpose of the study: The purpose of the study is to establish community based hematological reference intervals which is important to the local population to screen for physiological or pathological conditions in routine health assessment

Duration: the duration of this study depend on the availability of study subjects and it can take about 3-5 months. However, specimen from you is collected only once.

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

Risks and Discomfort: There will be minor discomfort or feel pain during collection of samples. During collection of samples from your hand appropriate precaution will be taken and all samples will be collected by trained health professionals. Appropriate medical care will be provided to you if needed.

Expected Benefit: the result of the study will be have direct benefit to you since you will be communicate with your results but there is no any financial benefit to you.

Confidentiality: Your name will not be written in the questioner and I assure that all the information you give and the laboratory results will be kept strictly confidential and could only be accessed by the researcher.

Termination of the study: The participation is based on your voluntary. You can resign participating in the study at any time. This decision will not affect in any way your current or future medical care in any health facility.

Agreement

After communicated in detail with guardians/parents about the study procedures and other related issues, the participant will be kindly requested to put your signature of the agreement. Your signature indicates that the participant is voluntary to participate in the study.

If you have any question or problems please contact the following address:

Principal Investigator: Mr. Hagos H/slassie (Bsc)

Mobile Phone: +251914404630

Email: hagoshaileslasie78@gmail.com

Advisors:

Dr. Aster Tsegaye (Msc, PhD)

Mobile Phone: +251911696085

Email: tsegayeaster@yahoo.com

Department of Medical Laboratory Science Research and Ethics Committee office

Telephone: +251 11275 5170 37

Certificate of assent

I am voluntary as I have communicated with my guardian/parent about the previous information, or as I have read it. I have had the opportunity to ask questions about it and any questions that I have asked have been answered to my satisfaction. My child is assented voluntarily to participate in your research.

Signature of Participant _____

Date _____Day/month/year

Statement by the researcher

I confirm that the participant was allowed to ask questions about the study, and all the questions asked by the participant have been answered correctly and to the best of my ability. I confirm

that the parents/guardians haven't been coerced into giving assent, and the assent has been given freely and voluntarily.

Name of guardian/parent taking the assent_____

Signature of guardian /parent taking the assent_____

Date _____

Day/month/year

Annex II: Questionnaire (English version)

Questionnaires to be filled by health professionals

Part I. General information

Code Number_____ Region _____ Zone _____

Sub city _____ Kebele _____

Part II. Personal information

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

No.	Questions	Responses
	Part III. SOCIO-DEMOGRAPHIC INFORMATION	
6.	Educational status	1. Illiterate 2. Read and write 3. Primary (1-8) 4. Secondary (9-12) 5. College diploma/degree and above
7.	Occupation	1. Student 2. House wife 3. Government employee 4. Private employee 5. Farmer 6. Others (specify) _____
	History of common diseases	
8.	History of diabetes	1. Yes 2. No
9.	History of Hypertension	1. Yes 2. No
10.	History of Blood transfusion for the last 1 year	1. Yes 2. No
11.	History of Hospital Admission for the last 1 year	1. Yes 2. No

12.	History of Surgical procedure for the last three years?	1. Yes 2. No
13.	History of chronic gastritis	1. Yes 2. No
14.	History of Malaria for the last 6 months	1. Yes 2. No
15.	History of TB for the last two years	1. Yes 2. No
16.	History of Cancer	1. Yes 2. No
17.	History of Cardiac illness	1. Yes 2. No
18.	History of Bleeding disorders	1. Yes 2. No
19.	History of allergy	1. Yes 2. No
20.	History of Wheezing	1. Yes 2. No
	Part IV. Anthropometric measurement	
21.	Height (in cm)	_____
22.	Weight (in kg)	_____
23.	MUAC	_____in cm (will be interpreted later)
24.	Blood pressure (mm Hg)	_____

NB: If a participant answers **yes for one of the** questionnaire from No. 8 to No. 20 and the blood pressure is out of **90-120 systolic** and **60-90 diastolic** except with some preconditions they cannot include in the analysis.

❖ We thank you for your cooperation!

Interview Date:_____

Interviewer's Name____ _____

Signature_____

Annex III: Questionnaire Amharic version (ቃለመጠይቅ12—17 ዓመት ለሆኑ ህፃናት መረጃ)

የፕሮጀክቱ ርዕስ፡ “እድሜአቸው ከ 12-17 ዓመት ለሆኑ የመቀለ ነዋሪዎች የጤናማ ሰው ደም ውስጥ የሚገኙ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እና በላቦራቶሪ ውስጥ የጥራት መመርመሪያ ንጥረ ነገር መስራት ነው፡፡

የጥናቱ ተመራማሪ: ሓጎስ ሃይለስላሰ ወልደሃወርያት

መግቢያ:

ጤና ይስጥልኝ! ስሜ ሓጎስ ሃይለስላሰ እባላለሁ ፡፡ የኣዲስ ኣበባ ዩኒቨሪስቲ የድህረ ምረቃ ተማሪ ሲሆን የመመረቂያ ፅሑፌን በሄማቶሎጂ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው ከ 12-17 ዓመት ለሆኑ የ መቀለ ነዋሪዎች እየሰራሁ 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 እና በአገር ውስጥ የሚመረት የጥራት መመርመሪያ የለም፡፡ ስለሆነም የዚህ ጥናት ዓላማ በአገር ውስጥ በላቦራቶሪ ውስጥ የሚመረት የጥራት መመርመሪያ እና የጤናማ ሰው የሄማቶሎጂ ውጤት ማወዳደሪያ ሪፈረንስ ኢንተርቫል መሥራት ነው፡፡

አንተም/አንቺም በዚህ ጥናት እንድትሳተፍ/ፊ እየጋበዝን ወላጆችሽ/ወላጆችህ ፈቃዳቸውን ገልፀዋል፡፡፡ ስለዚህ በዚህ ጥናት በመሳተፍ በአገራችን በላቦራቶሪ ውስጥ የሚመረት የጥራት መመርመሪያ እና የጤናማ ሰው የክሊኒካል ላቦራቶሪ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለመስራት አስተዋፅዎ እንድታደርግ/ጊ ተጋብዘሃል/ሻል፡፡ ሁለቱም ጥራት ያለው የላቦራቶሪ አገልግሎት ለመስጠት አስፈላጊ ናቸው፡፡

የጥናቱ አካሄድ:

በጥናቱ ለመሳተፍ ከተስማማህ/ሽ የጥናቱ አባል/አባላት 10 ደቂቃ የሚወስድ ጥያቄ ይጠይቁሃል/ሻል፡፡ ክብደት፣ ቁመት፣ የክንድ እና የደም ግፊት ልኬት ይወሰዳል፡፡ ሽንትና አይነምድር በምንሰጠው እቃ እንድትሰጠን/ጭን እንጠይቃለን፡፡፣ በተጨማሪም 4 ሚሊ ሊትር (ግማሽ የሾርባ ማንኪያ የሚሆን) በንፁህ ቫኩቴይነር ብልቃጥ እና መርፌ ፡፡ ፓራሲቶሎጂ እና በሄማቶሎጂ 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፡፡ 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 መጎዳትና 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፡፡ በአገር ውስጥ በላቦራቶሪ ውስጥ የሚመረት የጥራት መመርመሪያ እና የጤናማ ሰው የሄማቶሎጂ ውጤት ማመዳደሪያ ሪፈረንስ ኢንተርቫል ለማሻሻል ይረዳል፡፡

በጥናቱ ለመሳተፍ ማትጊያ:

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

ያለመሳተፍ መብት:

በዚህ ጥናት ከተሳተፍክ/ሽ 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 የሰጠኽውን/የሰጠሽውን ሰው መጠየቅ ይቻላል፡፡

በጤና ባለሞያዎች የሚሞላ ቃለ መጠይቅ

መመሪያ፡

በቅድሚያ ይህንን ቃለ መጠይቅ ለመሙላት ለሰጡን ጊዜና ትብብር አድናቆቴን እገልፃለሁ፡፡ የዚህ ቃለ መጠይቅ አላማ "በላቦራቶሪ ውስጥ የጥራት መመርመሪያ ንጥረ ነገር እና የጤናማ ሰው ደም ውስጥ የሚገኙ የሄማቶሎጂ ምርመራዎች መጠን ሪፈረንስ ኢንተርቫል እድሜአቸው ከ 12-17 ዓመት ለሆኑ የመቀለ ነዋሪዎች ለመስራት መረጃ ለመሰብሰብ ነው፡፡ የዚህ ጥናት ሃሳቡን ያመጡት የጥናቱ ተመራማሪ **ሓጎስ ሃይለስላሰ** በአዲስ አበባ ዩኒቨርስቲ የህክምና ላቦራቶሪ ትምህርት ክፍል የድህረ ምረቃ ተማሪ ሲሆኑ የመመረቂያ ፅሁፋቸው ሊሰሩበት ነው፡፡ ስለሆነም የእርስዎ ቅን ትክክለኛ መልስ በሰዓቱ መስጠት የዚህን ጥናት ስኬት ይወስናል፡፡

አመሰግናለሁ!!!

ክፍል 1. አጠቃላይ መረጃ

ኮድ_____ ክልል _____ ዞን_____

ከተማ/ክፍለከተማ _____ቀበሌ _____

ክፍል 2. የግል መረጃ

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

	ክፍል 3. የጤና መረጃ	
6.	ባፉት ሶስት ወራ ለማንኛውም ዓይነት ህመም ማንኛውንም ዓይነት መድሃኒት ወስደኃል?	1. አዎን 2. የለም
7.	ለተራ ቁጥር 6 መልስዎ ወስጃለሁ ከሆነ የትኛውን ዓይነት መድሃኒት ነው ወሰዱት? (ከአንድ በላይ መልስ ይቻላል)	1. ፀረ-ፕሮቶዞአ 2. ፀረ-ሄልሚንትስ 3. ፀረ-አለርጂ 4. የወሊድ መከላከያ ኪኒን 5. ፀረ-ባክቴሪያ 6. ፀረ-ቲቢ 7. ሌላ ካለ ይግለፁ _____

	የሚከተሉት የህመም ዓይነቶች አሞዎት ያውቃል?	
8.	የስኳር ህመም?	2. አዎን 2. የለም
9.	የደም ግፊት ከፍ ማለት?	1. አዎን 2. የለም
10.	ባለፈው 1 ዓመት ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም
11.	ማንኛውም ጊዜ ደም ተሰጥቶዎ ያውቃል?	1. አዎን 2. የለም
12.	ባለፈው 1 ዓመት ሆስፒታል ተኝተው ያውቃሉ?	1. አዎን 2. የለም
13.	ባለፉት 3 ዓመታት የቀዶ ህክምና ተደርጎልዎ ያውቃል?	1. አዎን 2. የለም
14.	የቆየ የጨጓራ ህመም አለብዎት?	1. አዎን 2. የለም
15.	ባፉት 6 ወራት የወባ ህመም ኢጋጥሞዎት ያውቃል?	1. አዎን 2. የለም
16.	ባለፉት 2 ዓመታት የቲቢ ህመም ኖሮዎት ያውቃል?	1. አዎን 2. የለም
17.	ካንሰር ህመም	1. አዎን 2. የለም
18.	የልብ ህመም	1. አዎን 2. የለም
19.	የመድማት ችግር/ህመም	1. አዎን 2. የለም
20.	አለርጂ (የሰውነት መቆጣት)	1. አዎን 2. የለም
21.	የመተንፈስ ችግር (ሲተነፍሱ ሲር ሲር የሚል ድምፅ)	1. አዎን 2. የለም

	ክፍል 4. ክብደት፣ ቁመት፣ የክንድና የደም ግፊት ልኬት	
22.	ቁመት	_____ሴንቲ ሜትር
23.	ክብደት	_____ኪሎ ግራም
24.	የክንድ መሃለኛው ክፍል ዙሪያው (MUAC)	_____ሴንቲ ሜትር
25.	የደም ግፊት (በሚሊሜትር ሜርኩሪ)	_____(mm Hg)

ማሳሰብያ፡ ከተራ ቁጥር 6-21 መልስዎ አዎን ከሆነ ከጥናቱ ዉጪ ይሆናሉ

❖ ስለትብብርዎ እናመሰግናለን!

ቃለ መጠይቅ የተደረገበት ቀን:_____

ቃለ መጠይቁን ያካሄደው ስም_____ ፊርማ_____

Annex IV: ቃለ-ማሕላ

ብመጀመርታ እዚ ቃለ-መሕተት ዝምላእ ንዝሃብና /ንዝሃባና ንዝሃብከሙና ግዜን ትሕብብርን ኣድናቕተይ ይገልፅ ::ናይዚ ቃለ-መሕተት ዓላማ ኣብ ላቦራቶሪ ዉሽጢ መመርመሪ ንጥረ ነገር ኣብ ጥዑይ ሰብ ደም ዉሽጢ ዝርከብ ናይ ሄማቶሎጂ ምርመራታት መጠን ሪፈረንስ ኢንተርቫል ዕድሚኣም 12-17 ዓመት ዝኾኑ ናይ መቀለ ነበርቲ ንምስራሕ መረዳእታ ንምእካብ እዩ::ናይዚ ፅንዓት ሓሳብ መቐረቢናይዚ ፅንዓት ዋና ተመራማሪ **ሓጎስ ሃይለስላሰ** ኣብ ኣዲስ ኣበባ ዩኒቨርስቲ ናይ ሕክምና ላቦራቶሪ ትምህርት ክፍሊ ናይ ካልኣይ ዲግሪ ተመሃራይ :: ስለዚ ናቶም ቁኑዕ መልሲ ብግዚኡ ምሃብ ነዚ ፅንዓት ዕዉትነት ይዉስኖ ::

ምስቲ መፅናዕቲ ተታሒዙ ዝመፅእ ሳዕቤን፡- ንምርመራ ዝኽውን ደም ኣብ ዝህብሉ እዋን ምንም ዓይነት ዝኽፍኦ ፀገም ኣየጋጥሞምን። ነገር ግን ደም ኣብ ዝውሰደሉ እዋን ዝተወሰነ ናይ ምሕማም ስምዒት ክህሉ ይኽእል እዩ። ይኹን ዳኣምበር ደም ንምእካብ ልምዲ ብዘለዎም በዓል ሞያታት ስለ ዝምደቡን ኣድላይ ዝኾነ ጥንቃቐን ስለዝውሰድ ናይ ምሕማም ስምዐዒት ኣይህሉን።

ካብዚ መፅናዕቲ ዝረክብዎ ጥቕሚ፡ እዚ መፅናዕቲ ናይ ካልኣይ ድግሪ መመረቂ ፅሑፍ ከም ምኻኑ መጠን ኣብዚ መፅናዕቲ ብምስታፎም ዝረኽብዎ ናይ ገንዘብ ጥቕሚ ዋላኳ እንተዘይሃለዎ ካብቲ መፅናዕቲ ብዝርከብ ውፅኢት ግን ተጠቃሚ እዮም። ነቲ መፅናዕቲ ካብ ዝተወሰደ ደም ዝርከብ ውፅኢት ብነፃ ይረኽቡ እዮም። ብተወሳኺ ኣብቲ ናይ ደም ውፅኢት ለውጢ እንተሃልይዎ ምስ ሓኻይም ንክራኽቡን ንኽምርመሩን ይግበር እዩ።

ናይ ሕክምና መረዳእታ ብምሸጥር ምሕላዉ ዝምልከት ፡ ኣብዚ ጽንዓት ስለ ናቶም ወይ ናተን ንእክቦ ዝኮነ ዓይነት መረዳእታ ብሚስጥር ክም ንሕዘለኩም ነፍልጥ:: ነዚ መጽናዕቲ ኢልና ዘሎ ናቶም/ተን መንነት ዝገልጽ ኩሉ መረዳእታ ናብ ሚሽጥር ክንቐይሮ ኢና::ብተወሳኺ እቲ ትህቡና ደም ኮነ መረዳእታ ካብቲ ጽንዓት ወጻኢ ኣይንጥቀመሉን::

ካብቲ መፅናዕቲ ስለምቁራፅ: - ኣብቲ መፅናዕቲ ምስታፍ ብናቶም/ተን ፍቓደኝነት ዝተመስረተ ኮይኑ ኣብ ማእከል ምቐራፅን ዘይደለይዎ ሕቶ ዘይምምላስ ይኽእሉ/ላ እዮም/የን:: ኣብዚ መፅናዕቲ ዘለዎም/ወን ሕቶን/ ርኢቶን ኣብ ዝኾነ ይኹን ግዜ ክሓቱ/ታ ይኽእሉ/ላ:: ስለ ዝኾነ እዚ ቃለ-መሕተት ሓቅነትን ሓላፍነትን ብዝተመልአ መልክዕ ንኽመልኡ ብትሕትና ንሓትት::

ነምስግን!!!

Annex V. Consent form for parents/guardians

ኣብ ላዕሊ ከም ዝተገለፀ መረዳእታ ኣንቢቤ/ተነቢቡለይ፡፡ሕቶ ንምሕታት ዕድል ተዋሂብኒ ጠይቐ ብዘርክዕ መለክዑ ተመሊስለይ፡፡ቆልዓይ ክሳተፍ/ክትሳተፍ ተስማዕሚዐ ኣለኹ፡፡ከ 12-17 ዓመት ንታሕቲ ዝኾነ ቆልዓይ እንተተስማዕሚየምኮ/ን ኣብዚ ፅንዓት ንኽሳተፈ/ፍ ዝፈቐድኩም ምኻነይ ብክታመይ ኣብ ታሕቲ ይገልፅ፡፡

ናይ ዓይነምድር ናሙና ንምሃብ ☐

ናይ ሽንቲ ናሙና ንምሃብ ☐

ደም ናሙና ንምሃብ ☐ ኣብዚ ፅንዓት ተሳታፊ ንምኻን፣ኣብ ዝኮነ ሰዓት ካብ ፅንዓት ንምዉፃእ መብት ከምዘለኒ

*ናይ ተሳታፊ ስም፣መዓልትን ፈርማ(ወይም ክታም)*_____ ____ /____ /____ (መ/ወር/ዓመተ ምህረት)

ዘይተመሃሩ እንተኾይኖም;

ዝተመሃሩ ገለልተኛ እማኝ ሰብ ስም ፣ መዓልትን ፈርማን (እንተተኻኢሉ እዚ ሰብ ብተሳተፎ እንተምረፅ ካብ ተመራመርቲ ኣባላት ርክብ ዘይብሉ እንተዝኸውን) _____

____/____/_____ (መ/ወር/ዓመተ ምህረት) ናይ ተመራመሪ ስምን፣መዓልትን ፈርማን

Annex VI. Assent form for Adolescents

ኣብ ላዕሊ ከም ዝተገለፀ መረዳእታ ኣንቢቤ/ተነቢቡለይ፡፡ሕቶ ንምሕታት ዕድል ተዋሂብኒ ጠይቐ ብዘርክዕ መለክዑ ተመሊስለይ፡፡ክሳተፍ ተስማዕሚዐ ኣለኹ፡፡ ንኽሳተፈ/ፍ ዝፈቐድኩ ምኻነይ ብክታመይ ኣብ ታሕቲ ይገልፅ፡፡

ናይ ዓይነምድር ናሙና ንምሃብ ☐

ናይ ሽንቲ ናሙና ንምሃብ ☐

ደም ናሙና ንምሃብ ☐ ኣብዚ ፅንዓት ተሳታፊ ንምኻን፣ኣብ ዝኮነ ሰዓት ካብ ፅንዓት ንምዉፃእ መብት ከምዘለኒ

*ናይ ተሳታፊ ስም፣መዓልትን ፈርማ(ወይም ክታም)*_____ ____ /____ /____ (መ/ወር/ዓመተ ምህረት)

ዘይተመሃሩ እንተኾይኖም;

ዝተመሃሩ ገለልተኛ እማኝ ሰብ ስም ፣ መዓልትን ፈርማን (እንተተኻኢሉ እዚ ሰብ ብተሳተፎ እንተምረፅ ካብ ተመራመርቲ ኣባላት ርክብ ዘይብሉ እንተዝኸውን) _____

____/____/_____ (መ/ወር/ዓመተ ምህረት) ስምን፣መዓልትን ፈርማን

Annex VII: Questionnaire Tigrigna version (ቃለ መሕተት)

ብመጀመርታ እዚ ቃለ-መሕተት ዝምላእ ንዝሃብና /ንዝሃባና ንዝሃብከሙና ግዜን ትሕብብርን ኣድናቐተይ ይገልፅ ::ናይዚ ቃለ-መሕተት ዓላማ ኣብ ላቦራቶሪ ዉሽጢ መመርመሪ ንጥረ ነገር ኣብ ጥዑይ ሰብ ደም ዉሽጢ ዝርከብ ናይ ሄማቶሎጂ ምርመራታት መጠን ሪፈረንስ ኢንተርቫል ዕድሚኡም ካብ 12-17 ዓመት ዝኾኑ ናይ መቀለ ነበርቲ ንምስራሕ መረዳእታ ንምእካብ እዩ::ናይዚ ፅንዓት ዋና ተመራማራይ **ሓጎስ ሃይለስላሰ** (ኣብ ኣዲስ ኣበባ ዩኒቨርስቲ ናይ ሕክምና ላቦራቶሪ ትምህርቲ ክፍሊ ናይ ካልኣይ ድግሪ ተምሃራይ) :: ስለዚ ናቶም ቁኑዕ መልሲ ብግዚኡ ምሃብ ነዚ ፅንዓት ዕዉትነት ይዉስኖ ::

ክፍሊ 1. ኣጠቓላሊ መረዳእታ

ኮድ_____ ክልል ትግራይ ዞባ መቐለ

ክፍለ ከተማ _____ጣብያ _____

ክፍሊ 2. ናይ ዉልቀ መረዳእታ

1. ዕድመ (ብዓመት) _____
2. ፆታ_____
3. ናይ ትዉልዲ ቦታ_____
4. ኣብ ትዉልዲ ቦኦኡም ንኽንደይ ጊዜ ነቢሮም_____
5. ኣብ ሕዚ ዘለዉዎ ቦታ ንኽንደይ ጊዜ ነቢሮም? (ካብ ትዉልዲ ቦታ ዝተፈለየ እንተኾይኑ) _____ዓመት

ክፍሊ 3. ናይ ጥዕና መረዳእታ

6. ኣብ ዝሓለፉ ሰለስተ ወርሒ ዝኾነ ዓይነት መድሓኒት ንዝኾነ ዓይነት ሕማም ወሲዶም ዶ ይፈልጡ ?

   1. እወ 2. ኣይፋሉን

7. ንታራ ቁፅሪ 6 መልሶም እወ እንተኾይኑ ኣየናይ ዓይነት መድሓኒት እዮም ወሲዶም ? (ካብ ሓደ ንላዕሊ መልሲ ምምላስ ይካኣል እዩ)

   1. ፀረ-ፕሮቶዝዋ 2. ፀረ- ሓሰኻ 3. ፀረ-ኣለርጂ 4. ናይ ወሊድ መከላኸሊ ክኒና
   5 ፀረ-ባክቴሪያ 6. ፀረ-ቲቢ 7. ካለእ ተሃልዩ ይገለፅ _____

ናይ ዝስዕቡ ናይ ሕማም ዓይነት ሓሚሞም ይፈልጡ ዶ?

8. ናይ ሽኮር ሕማም? 1. እወ 2. ኣይፋሉን
9. ናይ ደም ድፍኢት ልዕል ምባል? 1.እወ 2. ኣይፋሉን
10. ኣብ ዝሓለፈ 1 ዓመት ደም ሂቦም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን
11. ኣብ ዝኾነ ጊዜ ደም ተዋሂብዎም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን
12. ኣብ ዝሓለፈ 1 ዓመት ሆስፒታል ሃሪሶም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን
13. ኣብ ዝሓለፈ 3 ዓመታት ናይ መጥባሕቲ ህክምና ተገይርሎም ይፈልጡ ዶ? 1.እወ 2. ኣይፋሉን
14. ዝፀንሐ ናይ ጨነራ ሕማም ኣለዎም ዶ? 1.እወ 2. ኣይፋሉን
15. ኣብ ዝሓለፈ 6 ኣዋርሕ ናይ ሕማም ዓሶ ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን
16. ኣብ ዝሓለፈ 2 ዓመት ናይ ቲቢ ሕማም ሒዝዎም ይፈልጥ ዶ ? 1.እወ 2. ኣይፋሉን
17. ናይ ካንሰር ሕማም 1. እወ 2. ኣይፋሉን
18. ናይ ልቢ ሕማም 1.እወ 2. ኣይፋሉን
19. ናይ ምድማይ ፀገም /ሕማም ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

20. አለርጂ (ናይ ሰውነት ቁጠዐ) ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

21. ናይ ምትንፋስ ችግር ኣጋጢምዎም ነይሩ ዶ? 1.እወ 2. ኣይፋሉን

	ክፍሊ 4. ክብደት፣ ቁመት፣ ጭዋዳን ናይ ደም ድፍኢትን	
22.	ቁመት (ሴንቲ ሜትር)	_____
23.	ክብደት (ኪሎ ግራም)	_____ኪሎ ግራም
24.	ናይ ጭዋዳ ማእኸላይ ክፍሊ ዙሪያ (MUAC)	_____ሴንቲ ሜትር
25.	ናይ ደም ድፍኢት (በሚሊሜትር ሜርኩሪ)	_____

መተሓሳሰቢ፡ ካብ ተራ ቁጽሪ 6-21 ምልሶም እወ እንተኾይኑ ካብቲ ጽንዓት ውጻኢ ይኾኑ

❖ ስለ ምትሕብባሮም ነምስግን!!!

ቃለ መሕተት ዝተገበረሉ መዓልቲ: _____

ቃለ መሕተት ዘካየደ ሽም_____ ፊርማ_____

Annex-VIII. Standard Operating Procedure (SOP)

SOP for Blood Collection

Equipment

- ✓ 21 gauge syringe
- ✓ Blood collection tubes (K_2EDTA test tube)
- ✓ Tourniquet
- ✓ Box of nitrile/vinyl gloves
- ✓ 70% alcohol
- ✓ Cotton

Laboratory blood sample collection procedure and processing

1. Assemble all the necessary materials for blood collection
2. Identify and prepare the person for collection
3. Label tubes with the specific identification number
4. Wear the rubber gloves and make the person at comfortable position
5. Tie the tourniquet around the arm of the person just above the bend in the elbow. The tourniquet should be positioned 7.5 cm to 10 cm above the puncture site.
6. using the tip of the index finger examine the phlebotomy site, feel the vein, and decide exactly where to place the puncture
7. Disinfect the phlebotomy site by swabbing the skin in small outward circles with alcohol swab.
8. Insert the needle directly into the vein and withdraw peripheral blood of approximately 4ml in K_2EDTA test tube
9. Withdraw the needle from the vein and cover the puncture site cotton swab and hold pressure at the puncture site for 3 minutes.
10. Properly discard the used materials in a safe sharp container.
11. Gently mix the blood with the anticoagulant

Urine Reagent strip procedure

- ✓ Dip the test – strip in the urine specimen. Remove the test-strip immediately and let the excess urine drain off on a paper towel, or tap the edge of the strip
- ✓ Read the color change
- ✓ Report the result according to the color chart provided by manufacturer

- ✓ Always read the test strip in good white light and ignore color developing on the test area after the period specified as the reading time of the test
- ✓ Be careful not to wet the reagent strip excessively. So that the acid buffer from the protein area runs into the pH area, causing an orange discoloration.

Urine Microscopy procedure

1. Mix the urine specimen
2. Transfer about 10 ml of urine into a labeled centrifuge tube
3. Centrifuge the specimen at a medium speed (from 1500 – 2000 rpm) for 3-5 minutes
4. Discard the supernatant by quick inversion of the tube
5. Re suspend the sediment that is at the bottom of the tube, by tapping the tube by your fingers
6. Take the sediment by Pasteur pipette from the tube and transfer a drop into the clean and dry slide
7. Apply cover slide on the urine sediment that is on the slide.
8. Put on the microscope and look under 10x objective of the microscope
9. Then after looking through the low power objective, change the objective in to 40x objective
10. Then report what you get under low power and high power objective on the laboratory request form of the patient

Procedure for Formal Ether concentration Technique

1. Wear gloves when handling stool specimens
2. In a suitable container, thoroughly mix a portion of stool specimen about the size of a walnut into 10mL of saline solution. Mix thoroughly
3. Filter the emulsion through fine mesh gauze into a conical centrifuge tube
4. Centrifuge the suspension at relative centrifugal force (RCF) of 600 g (about 2000 rpm) for no less than 10 minutes. The suspension should yield about 0.75mL of sediment for fresh specimens and 0.5 mL for formalized feces
5. Decant the supernatant and wash the sediment with 10 mL of saline solution. Centrifuge again and repeat washing until supernatant is clear

6. After the last wash, decant the supernatant and add 10 mL of 10% formalin to the sediment. Mix and let stand for 5 minutes to effect fixation
7. Add 1 to 2 mL of ethyl acetate, Stopper the tube and shake vigorously

SOP for Sysmex KX-21N hematology analyzer

PRINCIPLE:

The Sysmex KX-21N is a quantitative automated hematology analyzer for in vitro diagnostic use for determining 17 hematological parameters. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemia's, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex KX-21N analyzer directly measures the WBC, RBC, Hgb, HCT, and PLT, LYM #, MIXED #and NEUT #. The remaining parameters are calculated or derived, MCV, MCH, MCHC, MPV, RDW-CV and RDW-SD, and differential percentages LYM%, MIXED%, NEUT%.

The KX-21N counts and sizes RBC and PLT using electronic resistance detection, HCT is measured as the ratio of the total RBC volume to whole blood using cumulative pulse height detection. Hgb is converted to methemoglobin, and read photometrically at 555 nm. WBC are analyzed by direct current and discriminated into a three-part differential using Particle Distribution Analysis (PDA). The resulting WBC histogram is discriminated into lymphocyte, neutrophil and mixed cell populations. The middle-size cell population contains Monocytes, basophiles and Eosinophils**.**

DC Detection Method

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

.

Non-Cyanide Hemoglobin Analysis Method

To analyze hemoglobin by automated methods, the Cyanmethemoglobin method or Oxyhemoglobin method have so far been the main stream. Cyanmethemoglobin method was recommended as the international standard method in 1966 by ICSH (International Committee for Standardization in hematology). This method, however, is so low in hemoglobin conversion rate that it cannot be said an appropriate method in the automated process in which multi-sample processing is the pre-condition. In addition, this method uses the reagent of cyanide compound which is a poisonous substance and requires waste processing; thus, it can hardly be called an environmentally favorable method. At present, this method cannot be said suitable for a fully-automated instrument which is required to handle a large amount of waste. The Oxyhemoglobin method, on the other hand, is faster in hemoglobin conversion rate; in fact, blood hemoglobin is converted instantaneously into Oxyhemoglobin. Also, it does not contain poisonous substance as Cyanmethemoglobin method, making the method suitable for automation. This method, however, is unable to convert methemoglobin into Oxyhemoglobin. Consequently, when a great amount of methemoglobin is contained as in control blood, lower-than-real values result, although usual human blood poses no problems.

Non-cyanide hemoglobin analysis method utilizes the advantages of both of the above methods. Non-cyanide hemoglobin analysis method rapidly converts blood hemoglobin as the Oxyhemoglobin method and contains no poisonous substance, making it suitable for automated method. Being capable of analyzing methemoglobin, this method can accurately analyze control blood, etc. which contain methemoglobin.

Reagents

Cell Pack: - is a whole blood diluent for use in the determination of hemoglobin and electric counting and sizing of blood cells. Its ingredients are: sodium chloride, boric acid, sodium tetra borate, and K_2EDTA.

Stromatolyser WH: - is ready to use lysing reagent to analyze the leucocytes by lysing the RBC and left the WBC free and easy to count; whole blood sample by resistance measurement and photometric measurement and its ingredients are: nonionic surfactant, organic quaternary ammonium salt.

Cell Clean: - is a strong alkaline detergent to remove lysing reagents, cellular residuals and blood proteins remaining in the hydraulics of Sysmex analyzer.

Reagents preparation: Reagents are commercially prepared.

Reagents stability and storage: All reagents are stable at room temperature up to their expiry date.

Supplies

- Disposable glove
- BD Vacutainer tube (K_2EDTA anticoagulant tube)
- Dry gauze
- Cotton Swab
- Vacutainer
- Needle with holder
- 70% Ethanol alcohol
- Tourniquet

Equipment's:

- Sysmex KX-21N
- Electrical blood mixer
- Electrical power stabilizer (500 or 1000 w)

Sample

- Whole blood specimen collected in K_2EDTA anticoagulant tube.

Amount required

¾ of the collection test tube (4ml)

Transport and Storage: 2-8 ^{o}c

Aspiration

- Whole blood mode- Approximately 50µl
- Pre-diluted mode-Approximately 20µl

Dilution

In pre-dilution mode a sample is diluted in to 1:26 before analysis.

Instrument dilution

In whole blood mode

- The dilution for Hgb and WBC 1:500
- The dilution for RBC 1:25000

In pre-dilution mode

- The dilution for Hgb and WBC is 1:1000
- The dilution for RBC is 1:25000

CBC Analysis using Sysmex KX-21N

WBC/HGB Analysis procedure

In WBC and Hgb analysis, the volume of WBC and hemoglobin in the blood are measured. The flow of WBC/Hgb analysis is described below:

Whole Blood Mode

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

Pre-diluted Mode

1. Blood sample that was diluted beforehand to 1:26 dilution using CELLPACK. This sample is aspirated from the sample probe into the sample rotor valve.
2. 78 µl of diluted blood measured by the sample rotor valve is transferred to the WBC transducer chamber along with 1.922 mL of diluents. At this time, 1.0 mL of WBC/HGB lyse is added to prepare 1:1000 dilution sample. When the solution is made to react in

this status for approximately 10 seconds, RBC is hemolyzed and platelets shrink, with WBC membrane held as they are. At the same time, hemoglobin is converted into red colored methemoglobin.

3. Of the diluted/hemolyzed sample in the WBC transducer chamber, approximately 1 mL is transferred to the Hgb flow cell.
4. 500 µl of sample in the WBC transducer chamber is aspirated through the aperture. The pulses of the blood cells when passing through the aperture are counted by the DC detection method.
5. In the Hgb flow cell, 555 nm wavelength beam irradiated from the light emitting diode (LED) is applied to the sample in the HGB flow cell. Concentration of this sample is measured as absorbance. This absorbance is compared with that of the diluents alone that was measured before addition of the sample, thereby calculating HGB (hemoglobin value).

RBC/PLT analysis procedure

In RBC/PLT analysis, RBC and platelet count in the blood are measured. The flow of RBC/PLT analysis is described below:

Whole Blood Mode

1. Blood is aspirated from the sample probe into the sample rotor valve.
2. 4.0 µl of blood measured by the sample rotor valve is diluted into 1:500 with 1.996 mL of diluents and brought to the mixing chamber as diluted sample. (1st step dilution)
3. Out of the 1:500 dilution samples, 40 µL is measured by the sample rotor valve, diluted into 1:25000 with 1.960 mL of diluents, and then transferred to the RBC/PLT transducer chamber. (2nd step dilution)
4. 250 µl of the sample in the RBC/PLT transducer chamber is aspirated through the aperture. At this time, RBC and PLT are counted by the DC detection method. At the same time, HCT (hematocrit value) is calculated by RBC pulse height detection method.

Pre-diluted Mode

1. Blood sample that was diluted beforehand to 1:26 dilution using CELLPACK. This sample is aspirated from the sample probe into the sample rotor valve.

2. 2.08 µl of diluted blood measured by the sample rotor valve is transferred in 1.99792 mL of diluents to the RBC/PLT transducer chamber, and is made into 1:25000 dilution samples.
3. Of the sample in the RBC/PLT transducer chamber, 250 µL is aspirated through the aperture. At this time, RBC and PLT are calculated by the DC detection method. At the same time, HCT (hematocrit value) is calculated by RBC pulse height detection method.

Calculation of RBC Constant

RBC constant (mean RBC volume, mean RBC hemoglobin, mean RBC hemoglobin concentration) is calculated from RBC, HGB, and HCT.

1. Mean RBC Volume (MCV)

   Calculation is made from RBC and HCT by the formula below:

   $$MCV\ (fL) = \frac{HCT\ (\%)}{RBC\ (x10^6/\mu l)} \times 10$$

2. Mean RBC Hemoglobin (MCH)

   Calculation is made from RBC and HGB by the formula below:

   $$MCH\ (pg) = \frac{HGB\ (g/dL)}{RBC\ (x10^6/\mu l)} \times 10$$

4. Mean RBC Hemoglobin Concentration (MCHC)

   Calculation is made from HCT and HGB by the formula below:

   $$MCHC\ (g/dL) = \frac{HGB\ (g/dL)}{HCT\ (\%)} \times 100$$

General analytical procedure

1. Mix the sample sufficiently
2. Remove the plug while taking care not to allow blood scatter
3. Set the tube to the sample probe and in that condition, press the start switch
4. The buzzer sounds two times - "beep, beep" - and when the LCD screen displays "Analyzing, “remove the tube. After that, the unit executes automatic analysis and displays the result on the LCD screen. Then the unit turns to the Ready status, becoming ready for analysis of the next samples.

5. When the LCD screen displays "Ready," prepare the next samples and repeat the above procedures. To analyze the sample in Pre diluted Mode, first switch the analyzer to Pre diluted mode and follow the procedure as Whole Blood analysis.

Pediatric Reference Range

Parameter	**Reference range**
Red Blood Cell Count Men Female	4.2 – 5.6 x 10^{12}/l 4.1 – 5.3 x 10^{12}/l
Haemoglobin Men Female	 12.5 –16.1 g/dl 12 – 15 g/dl
Haematocrit Men Female	 36 – 47 % 35 – 45 %
Mean Cell Volume Male Female	 78 – 95 fl 78 – 95 fl
Mean Cell Haemoglobin Male Female	 26 – 32 pg 26 – 32 pg
Mean Cell Haemoglobin Concentration Male Female	 32 – 36 g/dl 32 – 36 g/dl
Red Cell Distribution Width Male Female	 11.8 – 15.6 % 11.8-15.6%
White Blood Cell Count (WBC) Male Female	 4.5-13 x 10^{9}/l 4.5-13 x 10^{9}/l
Differential White Cell Count Neutrophils Lymphocytes Monocytes Eosinophils Basophils	 40 – 80 % (2 - 7 x 10^{9}/l) 20 – 40 % (1 – 3 x 10^{9}/l) 2- 10 % (0.2 – 1.0 x 10^{9}/l) 1 – 6 % (0.02 – 0.5 x 10^{9}/l) < 1- 2 % (0.02 – 0.1 10^{9}/l)
Platelet Count MPV Female Male PDW	140 – 385 x 10^{9}/l 7.2-10.4 fl 7.5-11.5 fl 9-14 fl

Quality Control

The reliability of this instrument and reagents is monitored by quality control. By use of control blood or control materials the stability of the measured value is monitored over a certain period of time, and problems can be detected early or prevented.

Control material

The control materials, EIGHTCHECK-3WP-N (Normal), EIGHTCHECK-3WP-L (Low level) and EIGHTCHECK-3WPH (High level) are used. These are equivalent to Low, Normal and High level.

Declaration

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

MSc candidate: Hagos H/slassie (B.Sc.)

Signature:_____

Date of submission:_____

This thesis has been submitted with our approval as advisors.

Advisor: Aster Tsegaye (MSc, PhD)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.