HAEMATOLOGICAL REFERENCE INTERVALS FOR
ADOLESCENTS AND ADULTS IN NAKURU COUNTY, KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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I dedicate this project to my beloved husband Fredrick G Mbugua and my dear son George Mbugua whose moral support and understanding made my study a success. More dedication goes to my parent, my brothers, sisters and well wishers for their constant prayers and support. My dedication also goes to those fighting with various types of haematological disorders.
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TABLE OF CONTENTS

DEDICATION ........................................................................................................... iii
ACKNOWLEDGEMENT ........................................................................................... iv
LIST OF TABLES ..................................................................................................... vii
LIST OF FIGURES .................................................................................................. ix
LIST OF ABBREVIATIONS .................................................................................... x
DEFINITION OF TERMS ....................................................................................... xi
ABSTRACT ............................................................................................................. xii

CHAPTER ONE: INTRODUCTION ........................................................................... 1
  1.2 Problem statement ......................................................................................... 3
  1.3 Justification .................................................................................................... 4
  1.4 Objectives ....................................................................................................... 5
    1.4.1 Main objective ......................................................................................... 5
    1.4.2 Specific objectives ................................................................................... 5
  1.5 Limitations ..................................................................................................... 5
  1.5 Delimitations .................................................................................................. 6
  1.6 Conceptual framework ................................................................................. 6

CHAPTER TWO: LITERATURE REVIEW .............................................................. 8
  2.1 Reference values ............................................................................................ 8
  2.5 The erythrocytes ............................................................................................ 17
    2.5.1 Erythrocyte parameters ......................................................................... 18
    2.5.2 The erythrocyte count ............................................................................ 19
    2.5.5 Mean cell volume ................................................................................... 20
    2.5.7 Mean cell haemoglobin concentration ................................................ 21
    2.5.8 Red cell distribution width ..................................................................... 21
    2.6.1 Anaemia .................................................................................................. 23
  2.7 The leukocytes and its parameters ................................................................. 26
    2.7.2 Lymphocytes ........................................................................................... 27
2.7.3 Monocytes................................. 28
2.7.4 Neutrophils........................................... 29
2.7.5 Eosinophils............................................ 30
2.7.6 Basophils.............................................. 31

2.8 The leucocyte disorders.............................. 32
2.8.1 Disorders of Neutrophils.......................... 33
2.8.2 Disorders of lymphocytes......................... 33
2.8.3 The leukemoid reaction............................ 34

2.9 Thrombocytes and thrombocyte parameters....... 36
2.9.1 Thrombocytes....................................... 36
2.8.2 Disorders of thrombocytes......................... 37
2.8.3 The platelet count.................................. 38
2.3.4 Mean platelet volume............................ 39
2.5.5 Platelet distribution width......................... 39

CHAPTER THREE: METHODOLOGY........................ 40
3.1 Study area.............................................. 40
3.2 Study design.......................................... 41
3.3 Variables.............................................. 41
3.3.1 Independent variables............................ 41

3.4 Study population...................................... 41
3.5: Inclusion and exclusion criteria..................... 43
3.5.1: Inclusion criteria................................. 43
3.5.2: Exclusion criteria............................... 43

3.6 Sampling techniques.................................. 43
3.7 Sample size determination........................... 45
3.9 Procedure for urine and stool collection............ 47
3.10 Laboratory procedures............................... 47
3.11. Ethical Considerations............................. 56
3.12 Data analysis and presentation...................... 56

CHAPTER FOUR: RESULTS.................................. 58
4.1: Socio-demographic characteristics of the study participants.......................... 58
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.2 Erythrocytic reference intervals for adults and adolescents</td>
<td>61</td>
</tr>
<tr>
<td>4.3 Leukocytic reference intervals for adults and adolescents</td>
<td>64</td>
</tr>
<tr>
<td>4.4 Thrombocytic reference intervals</td>
<td>68</td>
</tr>
<tr>
<td>CHAPTER FIVE: DISCUSSION, CONCLUSIONS &amp; RECOMMENDATIONS</td>
<td>70</td>
</tr>
<tr>
<td>5.1 Discussion</td>
<td>70</td>
</tr>
<tr>
<td>5.2 Conclusions</td>
<td>77</td>
</tr>
<tr>
<td>5.3 Recommendations</td>
<td>77</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>78</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>93</td>
</tr>
</tbody>
</table>
LIST OF TABLES

Table 4.1: Social-demographic characteristics of the study participants……………………………81
Table 4.1: Erythrocytic reference intervals for adults and adolescents…………………………82
Table 4.1: Reference intervals for reticulocyte indices…………………………………………83
Table 4.1: Reference intervals for leukocytic absolute counts for adults and adolescents……86
Table 4.1: Reference intervals for leukocytic differential counts for adults and adolescents……87
Table 4.1: Thrombocytic reference intervals for adults and adolescents……………………….89
LIST OF FIGURES

Figure 2.1: Haemopoiesis.................................................................................. 20
Figure 2.1: Erythrocytes................................................................................ 22
Figure 2.1: The lymphocytes................................................................. 40
Figure 2.1: Monocyte................................................................................... 42
Figure 2.1: The neutrophils................................................................. 44
Figure 2.1: The eosinophil................................................................. 45
Figure 2.1: The basophil.......................................................................... 46
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical and Laboratory Standards Institute</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>ESR</td>
<td>Erythrocyte sedimentation rate</td>
</tr>
<tr>
<td>HbsAg</td>
<td>Hepatitis B surface antigen</td>
</tr>
<tr>
<td>HCT</td>
<td>Haematocrit</td>
</tr>
<tr>
<td>HCV</td>
<td>Hepatitis C virus</td>
</tr>
<tr>
<td>HGB</td>
<td>Haemoglobin</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>IFCC</td>
<td>International Federation of Clinical Chemistry</td>
</tr>
<tr>
<td>K2 – EDTA</td>
<td>Di-potassium ethylene diaminetetra acetic acid</td>
</tr>
<tr>
<td>MCH</td>
<td>Mean Corpuscular Haemoglobin</td>
</tr>
<tr>
<td>MCHC</td>
<td>Mean Corpuscular Haemoglobin Concentration</td>
</tr>
<tr>
<td>MCV</td>
<td>Mean Corpuscular Volume</td>
</tr>
<tr>
<td>MPV</td>
<td>Mean platelet volume</td>
</tr>
<tr>
<td>PDW</td>
<td>Platelet distribution width</td>
</tr>
<tr>
<td>PLT</td>
<td>Platelets</td>
</tr>
<tr>
<td>RBC</td>
<td>Red Blood Cells</td>
</tr>
<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>TMA</td>
<td>Thrombotic microangiopathies</td>
</tr>
<tr>
<td>TTP</td>
<td>Thrombotic thrombocytopenic purpura</td>
</tr>
<tr>
<td>VDRL</td>
<td>Venereal Disease Research Laboratory</td>
</tr>
<tr>
<td>WBC</td>
<td>White Blood Cell</td>
</tr>
<tr>
<td>WHO</td>
<td>World health organization</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>---------------------------------------------------------------------------</td>
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<tr>
<td>Reference individuals</td>
<td>Individuals selected for comparison using defined criteria</td>
</tr>
<tr>
<td>Reference value</td>
<td>Values of a measurement from a reference group</td>
</tr>
<tr>
<td>Reference Interval/Range</td>
<td>interval between and including two reference limits</td>
</tr>
<tr>
<td>Reference limit</td>
<td>derived from reference distribution</td>
</tr>
<tr>
<td>Reference population</td>
<td>consists of all possible reference individuals</td>
</tr>
<tr>
<td>Reference sample group</td>
<td>subset of the reference population</td>
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ABSTRACT

The haematologic reference intervals are important in the assessment of health and diseases conditions in a certain population and they vary with to age, sex, altitude and genetic. The current study intended to establish the haematological reference intervals in Nakuru County, Kenya. A cross sectional design was used where samples of blood were collected from consenting participants. The number of participants recruited for the study was 627 and they aged 13 to 18 years for the adolescents and 19 to 55 years for the adults. The erythrocyte median and reference indices (RBC count, $P < 0.0001$; Hb, $P < 0.0001$; HCT, $P < 0.0001$; MCH, $P = 0.034$; and MCHC, $P < 0.0001$) were significantly different across the study groups. Post-hoc analyses showed that the median and reference HB, RBC, MCH, HCT, and MCHC counts were lower in female adults compared to male adults ($P < 0.05$). Furthermore, median and reference values for MCHC were lower in the female adults compared to male adults, female adolescents and male adolescents ($P < 0.0001$, respectively). The median and reference for Hb, MCH, RBC, Hct, and MCHC values in the male adults were however, higher in comparison to female adolescents ($P < 0.0001$, respectively) while Hct was also lower in comparison to male adolescents ($P < 0.01$). Finally, the median and reference Hb, Hct, RBC and MCH values were lower in the female adolescents compared to the male adolescents ($P < 0.0001$ for all). The median and the reference levels for the absolute monocyte and eosinophil counts showed statistical significance (monocyte, $P < 0.0001$; eosinophil, $P < 0.001$). Consequent analysis indicated differences with females adolescents having higher values than male adolescents $p < 0.0001$ for monocytes counts. The females indicated higher median and reference differential count levels for [lymphocytes ($P < 0.012$), monocytes ($P < 0.031$) and basophils ($P < 0.0001$)] than male and the values showed statistical significant ($p < 0.05$). Post hoc indicated statistical differences within study groups with females having higher median and higher reference values for a lymphocytes and monocytes than the males adults ($P < 0.05$) and male adolescents ($P < 0.0001$). Age differences were also noted for lymphocytes, monocytes and basophils with female adults having lower values than the adults ($P < 0.0001$). Females had higher median levels for absolute platelet counts than males and there was statistical difference ($P < 0.0001$). Adult males also showed higher reference values than the male adolescents ($P < 0.0001$). MPV also illustrated higher medium levels in females than males for adolescent ($P < 0.05$). Further analysis indicated statistical significance with females having higher values ($P < 0.0001$). Age differences were noted with male adults versus male adolescents for MPV ($P < 0.05$). The results indicated low haematological reference values as compared to the currently utilized values by the clinicians in Kenya and also in Nakuru County. These data will therefore provide haematological reference values for this specific region which can also be interpolated in other regions for correct patients’ management and for clinical care.
CHAPTER ONE: INTRODUCTION

1.1 Background

Haematology reference values are a part of the clinical laboratory systems which have a worldwide a responsibility of providing health care to the populations of which they are derived and also elsewhere in the world (Kratz et al., 2004). A blood specimen is usually used to estimate these haematological reference intervals values and these measurements are also known as complete blood count (CBC) (Lewis et al., 2003). Reference hematological parameters are important for screening and diagnosis of diseases and also in detecting blood disorders (Haileamlak et al., 2012). The laboratory results become more practical when values obtained from research studies are then assigned to interval of values (Mohammad et al., 2011). The term reference intervals refer to the values within the upper and lower reference limit and it also includes the upper and the lower reference limits defined by a certain percentage (Zeh et al., 2011).

The hematological reference values commonly in use developing countries such as Asia and Africa were derived from western countries and may not apply to local populations (Prins et al., 1999). Studies from many parts of the world including some parts of Africa have indicated variations of the haematology reference intervals including sex, geographical location, race, altitude and diet (Luganda et al., 1999). It is therefore important to put into account these variations when dealing with particular group of people different from which they were obtained (Tsegaye et al., 1999). The reference values are commonly derived from samples of populations purported to be from healthy subjects. These may include people attending health facilities for routine medical investigations or from volunteer blood donors (Lipschitz et al., 1995; Fielding et al., 1981;
Haematological values have many applications in health care and at community level guiding public health decisions and interventions in health sectors (WHO and CDC, 2008). They are commonly utilized as surrogate markers in HIV/AIDS infections both for disease monitoring and initiation to therapy (O’Brien et al., 1997).

The clinician in the developing countries while interpreting the reported values compares them with those derived from the western countries which have been proved to be elevated (Horn et al., 2009). These leads to patient’s mismanagement as a result of failure to diagnose the health condition adequately which calls for the use of locally derived figures suited to a specific population (Chiu et al., 1998; Kibaya et al., 2008). Haematology reference interval values derived from Kenyan studies have also indicated differences from one locality to another citing the need for local values (Zeh et al., 2011).

The Clinical Laboratory and Standards Institute (CLSI) recommend that each laboratory to use its own reference intervals from local population or to validate reference intervals that have been obtained elsewhere (CLSI, 2008; Horn et al., 2009). Unfortunately, very few haematological reference ranges been established in Sub-Saharan Africa countries and there are non in Nakuru County which have often lead to the use of internationally derived values though proved different (Horn et al., 2009).

Clinically healthy participants in Africa and Asia may have been excluded from clinical trials or have been said to have had adverse drug events during clinical trials hence their discontinuation from such trials (Chou et al., 2007). It is therefore important to establish local reference intervals for a Nakuru County population.
1.2 Problem statement

Most laboratories including those in Kenyan have been using reagent test reference values from the manufacturers to aid in the clinical management of haematological disorders (Quinto et al., 2006). Most of the reagents used are derived from western countries therefore from a different population (Haddy et al., 1999). Studies have demonstrated that haematological reference intervals derived from African populations vary markedly from those of Caucasians (Choong et al., 1995; Romeo et al., 2009). As Clinical Laboratory and Standards Institute (CLSI) recommends that laboratories establish their own reference intervals from the local populations (Zeh et al., 2010) there is need to establish local values where there are none.

Nakuru County serves a high population (1,187,037 as per the census of 2009) with different ethnic groups and therefore local reference values are important for this diverse population. This is particularly for the purpose of harmonizing the haematological reference values for the proper clinical management of the huge population in Nakuru County. The results from this study will also be utilized by the Ministry of health for diagnosis, initiating treatment and monitoring disease progress in health and immune-compromised patients and also for the screening of the community for haematological disorders within the County.
1.3 Justification

Haematological reference intervals are lacking for most African countries including Kenya and despite this, clinicians have been using the same reference values which have been established from Caucasian populations in patient care and in clinical trials which have proved different (Jaoko et al.; 2008). As many people from African continue to participate in many clinical trials it is important to establishment reference intervals to suit such trials. Clinical reference intervals which are not applicable to a particular population may lead to exclusion of people who would otherwise have participated making the process of clinical trials difficulty, very costly, and the results less representative which also applies for the toxicity tables (Eller et al., 2008; Karita et al., 2009).

Most reference values varies with age, sex, genetics, diet, and altitude and therefore reference intervals obtained in a certain locality may not adequately represent the population a particular patient originate from (Kibaya et al., 2008). Previous studies conducted in Kenya on haematological reference intervals both in Kisumu and in Kericho indicated differences in a number of haematological parameters, which suggests the need for the establishment of reference values for specific geographical regions (Zeh et al., 2011). These factors prompted this study in order to obtain reference interval values for Nakuru county population to ensure that the healthy participants who intend to participate in clinical trials are not left out of such trials and for proper diagnosis and management of haematological diseases within the community.
1.4 Objectives

1.4.1 Main objective

To establish haematological indices reference intervals for healthy adolescents and adults in Nakuru County.

1.4.2 Specific objectives

1. To establish erythrocytic parameters reference intervals for adolescents and adults in Nakuru County.

2. To determine leukocytic parameters reference intervals for adolescents and adults in Nakuru County.

3. To establish thrombocytic parameters reference ranges for adolescents and adults in Nakuru County.

1.4 Research questions

1) What are the erythrocytic parameters reference intervals for adolescents and adults in Nakuru County?

2) What are the leukocytic parameters reference intervals for adolescents and adults in Nakuru County in?

3) What are the thrombocytic parameters reference ranges for adolescents and adults in Nakuru County?

1.5 Limitations

Identifying suitable healthy reference individuals was a major challenge due to difficulties in diagnosing some diseases particularly the genetic diseases where the participant may appear healthy yet sick. Also some participants were unwilling to reveal some important information which might have influenced the results. Diurnal variation is
a factor that influences the haematological values and might have influenced the results due to some technical delays

1.5 Delimitations

Moderate number of study participants was used for this study due to difficulties in indentifying healthy individuals. The participants were assured of total confidentiality by the research team in order to obtain as much information as possible. The research team tried as much as possible to collect sample within s specific time (before noon) and analyze within four hours of sample collection to minimize the diurnal variations

1.6 Conceptual framework

Haematological reference values are essential for the interpretation of haematologic data in routine and clinical practice and also in research studies. The reference values of erythrocytes, leucocytes, and thrombocytes indices are known to vary with age and gender. Other factors influencing the haematological indices include pregnancy, genetic, ethnicity and environmental factors and most infectious diseases. It is of particular importance that these factors be considered by clinicians in different settings. Also disease conditions need to be evaluated and if found present, the participant to be excluded from the study before establishing the reference intervals. This is to try and establish a health population as much as possible. Therefore, in this study the participants who tested positive for pregnancy, HIV 1&2, hepatitis B surface antigen, hepatitis C, malaria and hookworms and also those who were clinically ill were excluded from the study
CHAPTER TWO: LITERATURE REVIEW

2.1 Reference values

Reference values are often obtained from quantitative analysis of a sample from a selected purportedly health population according to a certain criteria (Solberg, 1986). The use of reference values obtained elsewhere and utilized to manage a different group of people from which it was obtained has been questioned owing to the noted differences from various studies (Lugada et al. 2004; Quinto´ et al., 2006) and this suggests a need to provide values for a particular population in order to improve patients care (Koram et al., 2007). There are however few clinical laboratories that have established their own reference ranges particularly in Africa and clinician has continued to use the European and American established reference values (Ferre et al., 1999).

The Clinical Laboratory and Standards Institute and the International Federation of Clinical Chemistry both recommend that each laboratory establishes its own reference ranges for each parameter (CLSI, 2008, Jagalinec et al., 1998). In establishing reference values the data may come from healthy population such as those visiting hospital clinics for routine checkup or as volunteer blood donors or may be obtained from patients’ records with an assumption that the patients were healthy (Ferre et al., 1999). Laboratory reference values including Haematological reference intervals are essential for clinical practice but there is insufficiency data for African populations including Kenya (Alexander et al., 2011). Clinical trials have been taking part in African countries due to increase of the infectious diseases such as tuberculosis, HIV/AIDS and other endemic diseases such as soil helminthes to try to ease the disease burden (Jaoko et al., 2008; UNAIDS 2010). Accurate laboratory reference intervals are very important tools in
clinical care and also in clinical trials for drug therapy and adverse effects but are unavailable for most African populations especially when they are required to participate in clinical trials (Zeh et al., 2011).

The reference interval values commonly used for clinical diagnosis and research in sub-Saharan Africa and Asia are from the western countries despite the fact that differences have been noted for these values from one region to another due to age, gender, altitude, ethnicity, environmental exposure and infections (Tugume et al., 1995; Kibaya et al., 2008). It has been established that haematological reference intervals for most red blood cell parameters for Africa are lower compared to such values from western countries which has been attributed diet, genetic and geographical locations (Kibaya et al., 2008).

Some of the white blood cell parameters such as monocyte and eosinophil levels are higher in African population when compared to the European and the American white populations while neutrophils are lower. This is most probably due to chronic exposure to soil helminths, malaria and schistosomiasis which are endemic in African countries (Zeh et al., 2011, Gill et al., 1995).

Females have indicated higher haematological levels for RBC, Hb and Hct, MCV and MCH with increased values for the adult females as compared to adult males in African populations (Karita et al., 2009, Koram et al., 2007). Age differences were also noted with male adults having higher levels than male adolescents for the red blood cell parameters (Zeh et al., 2011). This concurs with western population for adolescents (Omosa-Manyonyi et al., 2011). Platelet counts have showed increased reference values for adults than the adolescents among the Africans. Gender differences also exist for the
platelet values with females having higher values than male which is influenced by hormonal differences and menstrual blood in females (Zeh et al., 2011).

WBC counts, neutrophil counts and lymphocytes have showed lower values in reference intervals than the western values. The WBC counts are increased in females compared to their male counterparts both African and Caucasian populations (Karita et al., 2009, Saathoff et al., 2006). Statistical differences in age have shown that adolescents have higher WBC counts than the adults (Lugada et al., 2004). Higher median levels for monocytes, basophil and eosinophil counts than the western reference values have been shown in East African studies done both in Kenya and Uganda (Lugada et al., 2004; Zeh et al., 2011). The reference intervals for RDW, MPV and PDW have not been estimated for the African populations but a study conducted in India indicated lower values for these parameters than those from the western nations (Subhashree et al., 2012).

2.2 Haematological reference intervals

Haematological reference intervals are obtained from the evaluation of blood samples (Wintrobe 1999). This haematological parameters includes values such as red cell parameters (red blood cell count, haemoglobin concentration (HB), haematocrit (HCT), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), white cell counts and its indices (absolute total white blood cell count, neutrophils, eosinophils, basophils, monocytes and lymphocytes, and the WBC differential count) and platelet and its indices (platelet count, mean platelet volume (MPV), and platelet distribution width (PDW). These measurements are also referred to as complete blood count (CBC) (Lewis et al., 2006). The complete blood count (CBC) is
the most common test which is requested by the clinicians for the evaluation of blood conditions such as increased or decreased number of cells or cell abnormalities in which all cases indicates disease conditions (Kee et al., 2001). Other parameters include the erythrocyte sedimentation rate (ESR), the reticulocyte count and many others all of which have often been expressed in terms of reference intervals (Omosa-Manyonyi et al., 2011). The haematological values are useful in clinical and vaccine trials and also in epidemiology to make amicable decision in disease management within the community and appropriate values for the participating population is very important (WHO and CDC, 2008).

The last decade has witnessed a significant increase in the number of clinical trials taking place in sub-Saharan Africa in an effort to identify safe and effective prevention and treatment strategies to combat the heavy burden of infectious diseases in this region (Jaoko et al., 2008; UNAIDS 2010). This is because of the numerous endemic diseases in this region such as HIV/AIDS infections, tuberculosis infections, malaria cases and helminthes (UNISAIDS 2010) infections among others. Routine capacity for clinical laboratory testing is also increasing in Africa. Clinical trials and clinical care in sub-Saharan Africa require accurate laboratory reference intervals for appropriate assessment of patients/participants, monitoring disease progression, and reporting of possible toxicity and adverse events (Zeh et al., 2011). However, in African regions, there are insufficient published reference intervals, and these are therefore often taken from textbooks, whose values are based on those of Western populations (Alexander et al., 2011).
2.3 The blood

Blood is fluid tissue in the body which is made up of the cellular portion (the red cells, white cells and platelets and the fluid portion commonly known as the plasma (Lewis, 2005). Blood is transported in the body within blood vessels and is maintained in a liquid state by homeostasis (Wintrobe 1999; Lewis et al., 2006). The erythrocytes, the white blood cells and the platelets are collectively referred to as the formed elements of the blood with the red cells forming the majority of the formed elements (Richardson et al., 1996). They all perform their functions within the blood stream except for some white blood cells which can be found both in the blood stream and also in the lymphoid tissues (Richardson et al., 1996). Plasma is made of Water (90 – 92%) and Solid materials like proteins, anticomplement, sodium, calcium, potassium and magnesium (Lewis et al., 2006). Each of these cellular elements has its own unique function and differs morphologically from each other. They each have a finite life span and a balance between production and destruction which is maintained in health human (Wintrobe 1999; Lewis et al., 2006). Blood has many functions which include respiration, nutrition, excretion and maintenance of water content of the tissue, regulation of body temperature and protection with each blood component performing its own role (Richardson et al., 1996).

2.4 Haemopoiesis

Haemopoiesis is the formation, multiplication, composition and pathophysiology of human blood and is products. It is divided into two parts namely the the fetal haemopoiesis and after birth haemopoiesis (Wintrobe, 1999). Foetal haemopoiesis starts 1-2 weeks of fertilization/conception whereby during the first two months, the blood
formation occurs only in the yolk sac and the type of cells produced are called blood cells Myoblast. From the third and the fourth month of life, the liver and spleen take over the process of haemopoiesis which is also known as the hepatic phase and the cells formed in this phase are almost mature (Lewis et al., 2006). At the third to fourth month the bone marrow starts assisting in the manufacturing the blood cell and at 7th month, the bone marrow is the only haemopoetic site. At full term (36-40 weeks) the process of haemopoiesis is called the myoloid phase and in this phase the bone marrow produces granulocytes, monocytes and erythrocytes but the spleen, lymphoid tissue and lymph nodes will produce mainly lymphocytes. Immediately after birth other organs apart from bone marrow ceases to produce erythrocyte, granulocytes and platelets (Lewis et al., 2006).

Myeloid hyperplasia also known as abnormal haemopoiesis is the production of erythrocytes and platelets by other organs other than the bone marrow. In this case the following organs are included which are the spleen, liver, lymph node and lymphoid tissues (Bain et al., 1996). This happens when there is a disorder in the body and these tissues when they lost their function remained with their reticular cells in them which retained their potential haemopoetic activity. This happens under some conditions whereby the bone marrow is unable to cope with erythrocyte destruction such chronic or severe anaemia (Lewis et al., 2006).

The haemopoiesis process throughout the live of the mammals and its control is achieved through a coordination of cytokines. Haematopoietic cytokines stimulate hematopoietic cells to differentiate into many types of blood cells (Metcalf, 2008). These cytokines
includes granulocyte colony stimulating factor (G-CSF), granulocyte-monocyte colony stimulating factor (G-M CSF), monocyte colony stimulating factor (M-CSF), interleukin-3 (IL-3), interleukins, erythropoietin (EPO) and thrombopoietin (TPO). The colony stimulating factors (CSFs) promote the cellular viability and multiplication and also enhance the mature granulocytes and macrophage cells to function properly (Kovalovich et al., 2001). The erythropoietin and thrombopoietin stimulates the bone marrow to produce more erythrocytes and thrombocytes respectively as the body demands (Lewis, 2005).

Erythropoiesis is the formation of erythrocytes and the first primitive cells of erythropoiesis is the proerythroblast. It has a big nuclear size which gradually decreases in size with later condensation of their chromatin to develop into early normoblast, intermediate normoblast and late normoblasts which has a pyknotic nuclear. The nucleus is eventually lost or disappears and the cell then enters circulation as a young immature red cell known as the reticulocyte which later matures into erythrocytes (Lewis et al., 2006). The production and maturation of the erythrocytes is influenced by erythropoietin, a hormone produced in the kidney in the event of low tissue oxygen tension. The erythropoietin on reaching the bone marrow stimulates the stem cells to differentiate into pronormoblast, thus starting the process of erythropoiesis (Handin et al., 2003).

Leukopoiesis is the process by which the leukocytes are synthesized in the body. The leukocytes which are also known as the white blood have their origin in a very primitive cell called the hemocytoblasts which develops into myeloid stem cells and lymphoid stem cells. The myeloid stem cells produce the cells of the myeloid series which includes the
eosinophils, neutrophils, and basophils and the Monocytes. myeloblasts or monoblasts
and the lymphoid stem cells becomes lymphoblasts which later develop into lymphocytes
(Bain et al., 1996; NCCLS, 2003). Lymphocytosis, the process of the formation of
lymphocytes takes place in the lymph nodes and lymphoid tissues. They develop from the
pluripotent stem cell (precursor cell) into lymphoid stem cell. The lymphoid stem cell
later proliferates into lymphoblast, prolymphocyte, large lymphocyte and finally to small
lymphocyte.Monocytosis is the formation of Monocytes which originates from the
committed stem cell and later develops into monoblast, promonoblast and lastly to a
mature monocyte. The Monocytes may then migrate to different parts of the body
including the spleen, liver and lymph nodes. Granulocytosis is the process by which the
granulocytes are synthesized. The first stage is the myeloblast which proliferates to form
the promyelocytes which then matures into myelocytes and has characteristic granules in
the cytoplasm undergoing changes in nuclear morphology. The myelocytes later
transforms into metamyelocytes which have a C-shaped nucleus and is capable of
differentiating into the cells of the granulocytic series to include the neutrophils
Eosinophils and the basophils (Handin et al., 2003; Bain et al., 1996).

Thrombopoiesis is the formation of platelets also known as thrombocytes. The platelets
are formed as a result of fragmentation of the cytoplasm of megakaryocytes. Megakaryocytes are multilobulated large cells measuring approximately 160 μm in
diameter which later different into basophilic megakaryoblasts (Handin et al., 2003; Bain
et al., 1996). The dominant hormone controlling megakaryocyte development is
thrombopoietin. The platelets appear as biconvex discoid structures with a size of 2–3 μm
in diameter (Machlus et al., 2014).
Figure 2.1: The haemopoiesis process

2.5 The erythrocytes

Red blood cells are circular, discs shaped cells possessing no nuclear with a size mean diameter of 7.2 µm or 0.007mm and a thickness of 2.2µm. The central position of the red cells is much thinner than the circumference thus giving the cell the biconcave shape (Lewis et al., 2006). The cytoplasm is composed of 90-97% of haemoglobin and possesses no cytoplasmic structures. The cell wall is made up of elastic membrane which enables the cell to regain their unique biconcave shape once they reenter the blood vessels. The elastic membrane is made up of protein (lecithin) and fat material (cholesterol) which contribute to the elastic nature. The cell membrane and cytoskeleton of the RBC are collectively called stoma. Due to abundant supply of haemoglobin in the RBC they give blood a characteristic red colour (Handin et al., 2003). Haemoglobin also carries the waste product such as carbon dioxide from the expiring tissues and is transported back to the lungs for expiration. Myoglobin which is a form of haemoglobin is used as a storage site for oxygen in the muscle cells (Maton et al., 1993). The old erythrocyte undergoes changes in its plasma membrane where they are recognized as defective by the splenic macrophages and they are removed by phagocytosis (Bain et al., 1984). The process occurs as erythropoiesis takes place such that the amount of the erythrocytes is balanced within the circulation (Lang et al., 2012). Reference values for adults male is 4.5-6.5 million. An increase in Rbcs’ causes polycythaemia and decrease in Rbcs’ causes anaemia (Lewis et al., 2006).
2.5.1 Erythrocyte parameters

There are several parameters that are estimated in the clinical laboratory and the result of these parameters reflects the structure and function of red blood cells (Lewis et al., 2003). These measurements may determine the ability of the red blood cells to deliver oxygen adequately to the tissues for respiration. They are also useful in the diagnosis of haematological disorders including the types of anaemias where they may detect the red cell and shape abnormalities (Handin et al., 2003). These parameters include erythrocyte count, haemoglobin estimation, haematocrit, MCV, MCH and MCHC. Other erythrocyte parameters include reticulocyte indices and erythrocyte sedimentation rate and the
increase or decrease of this parameter values may indicate a disease condition (Blann, 2014).

2.5.2 The erythrocyte count

Red cell count estimates of the number of red blood cells per liter of blood. Low red blood cell count in most cases indicates a condition known as anaemia which may maybe as a result of acute or chronic blood loss, defective bone marrow, destruction of red cells and malnutrition (Lewis et al., 2009; Wintrobe, 2009). Red blood cells when increased may indicate a condition such as polycythaemia and reduced values indicates anaemia (Eric 1995); Estridge et al., 2000). The red blood cell count varies with gender and age with males having higher values and aged male having lower values than younger males (Kratz et al., 2004).

2.5.3 Haemoglobin

Haemoglobin is a red pigment which is made up of globin and iron and is responsible for the characteristic red colour of the blood (Maton et al., 1993). Haemoglobin has a high affinity for oxygen and transports oxygen and carbon dioxide around the body for respiration and expiration respectively (Lewis et al., 2003). The haem part is synthesized in the mitochondria and the globin protein parts are synthesized by ribosome and its production occurs throughout the life of the mammal. Haemoglobin is composed of a protein molecule and haem group with iron (ferous or in the ferric state) (Linberg et al., 1998). Measurement of haemoglobin concentration in the blood usually helps in the diagnosis of anemia (Bain et al., 1996; Ema, 2012). Haemoglobin is usually measured as total haemoglobin and the result is expressed as the amount of haemoglobin in grams per
deciliter of whole blood. The normal ranges for haemoglobin are 14-18 gm% for adult males and 12-16 gm% for adult females (Kratz et al., 2004).

2.5.4 Haematocrit

The haematocrit which is also referred to as the packed cell volume (PCV) is the value of erythrocyte expressed as percentage (%) of the volume of the whole blood in a sample (Pendse et al., 2005). The significance of determining the haematocrit value is that it can evaluate the severity of anaemia, level of blood loss, bone marrow failure and also leukaemia. A high haematocrit may indicate dehydration or polycythaemia vera (Purves et al., 2004). Haematocrit values may vary from place to place and from person to person (Wintrobe, 2009). High haematocrit levels indicate that the blood can transport enough oxygen throughout the body for energy production (Birchard et al., 1997; Wintrobe, 2009).

2.5.5 Mean cell volume

Mean cell volume is the average volume of a red blood cell in femitoleriter or cubic microns (Wintrobe, 2009). Mean cell volume is used to classify the red blood cells into either normal cell, smaller cells or larger cells and the classification by size is later used in the classification of the anaemias as normocytic, microcytic and macrocytic anaemias respectively (Snow, 1999). Increased mean cell volume may be caused by alcoholism, lack of Vitamin B₁₂ and/or Folic Acid and also the liver diseases causing the macrocytic anaemias ((Vinay et al., 2010); Wallach & Jacques, 2000). Microcytic cells are associated iron deficiency anaemia, thalassemia, or chronic disease (Zittoun, 1998; Lewis, 2003).
2.5.6 Mean cell haemoglobin
The mean cell haemoglobin is the average amount of haemoglobin by weight in a red blood cell and is expressed in picogram (Teffe ri 2003). Low MCH values usually indicated low haemoglobin and the cells are referred to as hypochromic cells whereas the cells with high MCH values and are called hyperchromaic cells. The MCH is found increased macrocytic cells as a result of deficiency of vitamin B₁₂ and folate (Pagana et al., 1998).

2.5.7 Mean cell haemoglobin concentration
Mean cell haemoglobin measures the average concentration of haemoglobin in grams percent (%) (Tefferi, 2003). Mean cell haemoglobin concentration is used to classify the red cells hence the anaemias into either normochromic or hypochromic depending with their haemoglobin concentration within the cell (Vinay et al., 2010). There are however no hyperchromic cells since there is a limitation of haemoglobin a cell can contain (Pagana et al., 1998; Gale Encyclopedia of Medicine, 2008).

2.5.8 Red cell distribution width
Red cell distribution width refers to how much the size of the red blood cell can vary also termed as anisocytosis (Cetin, 2012). This parameter is used to differentiate anaemias with similar characteristics such as the iron deficiency anaemia which has an abnormally wide RDW and thalassemia minor both of which are microcytic hypochromic anaemias (National Institutes of Health, 2003). Due to pronounced poikilocytosis it may also be used in the detection of ineffective haemopoiesis and also as a biomarker in cardiovascular diseases (Oustamanolakis et al., 2009; Hammarsten et al., 2010).
2.5.9 Reticulocyte count and reticulocyte index

Reticulocytes are juvenile or immature RBCs which still contain ribonucleic acid (RNA) in form of fibrin strands (Hoffbrand, 2011; Vajpayee et al., 2011). Reticulocytes which are released into circulation due to increased erythroid activity in the bone marrow or due to haemorrhages may occasionally appear in the blood stream (Ryan et al., 2012). It takes approximately 24-48 hrs for the reticulocytes in the peripheral blood to mature into red blood cells (Prchal et al., 2010). The absolute reticulocyte count is a measurement of reticulocytes in a blood sample and may serve as a biomarker in red cells synthesis. It is obtained as number of reticulocytes counted divided by the number of erythrocytes counted and then multiplied by 100 (Marks et al., 2009; Prchalet al., 2010).

2.5.10 Erythrocyte sedimentation rate

The erythrocyte sedimentation rate (ESR) is defined as the rate at which red blood cells falls in plasma when whole blood is stood in a standard vertical position for one hour and is expressed in millimeters per hour (Lewis et al., 2006). There are several stages during the sedimentation of the erythrocytes which includes aggregation, precipitation and packing. These stages are affected by number, size and the viscosity of the red blood cells (Bull et al., 1993; Gabay et al., 1999). ESR is commonly measured by Westergren method using citrate as the anticoagulant (Lewis 2003; Bain, 1995). Other methods of ESR estimation include the Landau Adams which is less common and Wintrobe method (Lewis et al., 2006). Wintrobe method uses a narrow glass closed on the lower end and opened at the top which is known as the Wintrobe’s tube. It has a length of 11cm and has the marking 0 at the top and 10 at the bottom for ESR. This tube can also be used for PCV and the marking is 10 at the top and 0 at the bottom for PCV. Modifications on
The Westergren method has been done to accommodate other anticoagulants such as EDTA (Koepke et al., 2000b).

The ESR can diagnose many chronic diseases including multiple myeloma, autoimmune diseases and rheumatoid arthritis (Bharathi et al., 2011; Liu et al., 2013). ESR can also detect the acute phase inflammatory response and act as a prognostic marker in staging of Hodgkin's (Zlonis, 1993; Briggs, 2009).

2.6 The erythrocytic disorders

Red blood cells have uniformity in size, shape and in haemoglobin concentration and any variation from these features results to abnormal functioning of the cells (Lewis et al., 2005). Knowledge of any deviations in size (anisocytosis) and in shape (poikilocytosis) will assist in the diagnosis of the associated haematologic disorders (Pagana et al., 1998). The disorders of the erythrocytes may also be due to either increase (polycythemia) or reduction in the number of the red cells (anaemia) (Prchal et al., 2010).

2.6.1 Anaemia

Anaemia can be defined low amount of haemoglobin in the circulating red blood cells. It is caused by the deficient in the materials required for haemopoiesis and the most important are vitamin B$_{12}$, foliate and iron. Genetic or hereditary factors also play a major role in defining anaemia as in thalassaemia syndromes or sickle cell diseases. Anaemia results in reduction of oxygen tension in blood leading to tissue hypoxia (Tefferi 2003). Anaemia is usually a sign of an underlying cause of a disease and should be accompanied by a thorough clinical evaluation for proper diagnosis (Carmel 1999; Bain 2001).
are many underlying causes of anaemia which can be used in the aetiological classification of anaemias and they include chronic or acute blood loss such as hemorrhages and accidents, reduced red cell production in the bone marrow like in the cases of low iron intake in the diet and also in vit B\textsubscript{12} and folic acic deficient foods. Increased blood cell destruction which may occur within or outside the bone marrow as in sickle cell diseases, malaria infections and also in some autoimmune diseases can also cause anemia(Janz et al., 2013; Blann, 2014).

Anaemia can be classified morphologically based on the size of the cell as microcytic, normocytic, or macrocytic and also based on the absence or presence of anisocytosis (Aslan et al., 2002; Tefferi 2003). Anaemia caused by nutritional deficiencies such as iron, folate, or vitamin B12 have greater variation in size of the red blood cell than anaemia caused by genetic defects or primary bone marrow disorders (Oh et al., 2003). Anaemia can also be classified aetiologically depending on decreased red blood cell survival as intrinsic or inherited defects and those that are acquired or caused by extrinsic factors and this classification may help in understanding the underlying disease process and hence assist in the disease diagnosis (Pierre 2002; Cotelingam, 2003).

2.6.2 Polycythaemia
This describes an increase in red blood cell count per unit volume based on age, sex and attitude of the patient. Two terms are used to describe the increase in the number of the RBCs which are the erythrocytes referring to increase in numbers of red blood cell count and is usually secondary (known cause). Erythaema is defined as an increase in the number of cells which may be idiopathic or unknown (Lewis et al., 2003). Polycythaemia
as a condition is divided into absolute and relative polycythaemia. Absolute polycythemia is when the total number of RBC is actually increased and this may be secondary or acquired while relative polycythaemia is caused by a decrease in total plasma in the body thereby falsely raising the concentration of red blood cells (Wintrobe 1999). The causes of absolute polycythaemia are high altitude, congenital heart failure, chronic pulmonary disease, defective pigment metabolisis, Benign or familiar polycythemia and the causes of relative polycythaemia are nutritional, burns, infections and pseudo polycythaemia due to stress or shock (Vinay et al., 2010).

2.6.3 Polycythaemia rubra vera

It is a chronic progressive and ultimate fatal condition and the basic abnormality is the excess production of formed blood cells by hyperplastic bone marrow and the increase in production is not due to any underlying disorder or due to any bone marrow stimuli (Vinay et al., 2010). Excess RBCS production result to rise in count in RBCS/unit volume and also increased PCV leading to absolute increase in blood volume. Such increase in blood volume does not reflect an increase in plasma volume because it is only the RBCs which has been increased (Lewis et al., 2003). The increased blood volume causes engorgements in bloody organs producing many symptoms such as cerebral symptoms where the patients present with cerebral symptoms such as headache, cardial vascular symptoms, bloody shot eyes red face, gastro intestinal syndromes visual disturbances and thrombotic complications. Macroscopic examination of blood will be dark in color, viscous tendency to clot easily and therefore it will be very difficult to make a blood film unless diluted (Vinay et al., 2010). Treatment of anaemia is usually aimed at correcting the underlying abnormality whether from blood loss, nutritional or
genetic (cetin, 2012; Tefferi, 2003) where some anaemias may require blood transfusion especially those causing hypoxia (Lewis, 2006).

2.7 The leukocytes and its parameters

Leukocytes also referred to as the white blood cells (WBCs) are immune cells which protect the body against foreign substances with the aim to destroy and remove them from the body (Pruthi et al., 1994; Wintrobe 1999). They have a half life of twenty days after which they are destroyed by macrophages (Orkin & Zon, 2008). The classification of the white blood cells is based on the presence or absence of granules within their cytoplasm hence the terms granulocytes and non granulocytes. The granulocytic cells include neutrophils, basophils and Eosinophils (Sahr et al., 1995; Wintrobe 1999) while the non granulocytes include lymphocytes and Monocytes. These different white blood cell types function independently whenever there is an inflammation in the body(Maton et al., 1997; Orkin & Zon, 2008).

2.7.1 The leukocyte count

Leukocyte count is one of the complete blood count tests which are used to diagnose the health status of an individual or a population (Lewis et al., 2006). Two important parameters are required to measure the white blood cells which are the absolute individual cell counts and the percentages of each of the cell also known as the differential count (Leguit & van den Tweel, 2010). These measurements are very important since an increased or decreased total white blood cell count could be due to abnormal bone marrow pathology which is a fatal haematological disorder (Estridge et al., 2000; Berliner et al., 2011).
2.7.2 Lymphocytes

Lymphocytes are variable in size ranging between the size of mature neutrophil and that of an erythrocyte. The nucleus is round staining deeply with the basic stain. There are 2 types of lymphocytes which includelarge lymphocytes which are the immature lymphocytes and Small lymphocyte which are mature lymphocytes (Denise et al., 2004). An increase in lymphocyte is called lymphocytosis associated with lymphocyte leukaemia, syphilis, measles, chronic infections, infectious mononucleosis and bacillary infection etc. A decrease is called lymphopaenia caused by severe infection especially due to cocci, and malignant leukaemia (Lewis et al., 2006).

Figure 2.3: The lymphocytes

Fernández & Alarcón, 2013
2.7.3 Monocytes

Monocytes are largest circulating leukocytes in body with a size of 1 – 20 µm and a less staining nucleus than that of lymphocytes in romaknowsky stains (Lewis et al., 2006). It has horse shoe shaped or are a round nucleus that closely resembling that of the lymphocyte making it difficult to differentiate them. The cytoplasm is usually round but assumes an irregular shape after phagocytosis. Monocytosis is an increase in monocytes in the periphery blood and is associated with typhoid fever, parasitic diseases, and leukemia (Wintrobe 1999).

Figure 2.4 The monocytes

Fernández & Alarcón, 2013
2.7.4 Neutrophils

Neutrophils have a lobulated or segmented nucleus hence referred to as polymorph nuclear leucocytes with a Size of 12 µm and the segments vary from 1-7 segments (Bain 1996). The cytoplasm contains numerous fines granules which do not stain with either the basic dyes or acid dyes hence referred to us neutral. Neutrophilia refers to raised number of neutrophils which may be due to many factors including severe infections acute loss of blood, infarct (cardiac) or myeloid leukaemia (Wheater et al., 2002). The reduction in the number of neutrophils is called neutropaenia caused by acute or chronic infection, leukaemia and anaemia (Wintrobe, 1999).

Figure 2.4: The neutrophils

http://www.anatomyatlases.org/MicroscopicAnatomy/Section04/Plate0452.shtml (1995)
2.7.5 Eosinophils

Eosinphils has a lobulated nucleus as that of neutrophils but are larger in size than neutrophils with large and oval granules in the cytoplasm and are such very prominent (Lewis et al., 2006). These granules are destructive and toxic and they granules stain deeply pink to bright red in colour and when demonstrated using wrights stain they take an orange colour (Saladin et al., 2012). The Eosinophils are formed in the bone marrow and later migrate from the peripheral blood system after a few hours of production. An increase in eosinophils is called eosinopaenia which is associated with parasitic infection, bronchial asthma, leukemia and fever. A decrease is termed as eosinophilia which may be due to acute infection. The normal adult count for Eosinophils is 2-6 x10^9/1 (Denise et al., 2004).

Figure 2.5: The eosinophil

http://www.anatomyatlases.org/MicroscopicAnatomy/Section04/Plate0452.shtml (1995)
2.7.6 Basophils

Basophils also called mast cells are few in number and contain large purple staining nucleus with wrights stain. They resemble the neutrophils with a lobulated nucli which is indistinctive. The cytoplasmic granules are water soluble which are not visible in a film. An increase in basophils is called basophilia and it is associated with provoked immunity (Falcone et al., 2000). Basophils excrete chemical substances such as histamine and heparin which boosts the body immune system. Histamine causes the blood flow to increase and move towards the injured site while heparin is an anticoagulant that inhibits the coagulation cascade and enhances the white blood cells to move to the injured site.

The eosinophils and neutrophils moves towards the infection site in response to chemical signals produced by the basophils (Saladin et al., 2012). An increase in the number of basophils is known as basophilia and it is associated with provoked immunity and a decrease in basophils in the peripheral blood film is insignificant. The normal count in the peripheral blood is $<0.1 \times 10^9/\text{l}$ (Lewis et al., 2006).
2.8 The leukocyte disorders

There white blood cell disorders include proliferative disorders and leukopaenias (Vinay et al., 2010). The proliferative disorders may either be due to an increased white blood cells count probably as a result of infection or it may be due to malignancy. Leukopaenia is caused by a reduction in the amount of circulating leukocytes. These proliferative disorders and leukopaenias belong to the quantitative disorders of white blood cells. The disorder in which the number of white blood cells is normal but the cells do not function normally is known as a qualitative disorder (Lewis et al., 2006).
2.8.1 Disorders of Neutrophils

Neutrophilia is when there is an increased amount of neutrophils in the circulation which may have a primary as in hereditary neutropaenia and also Downs syndrome or a secondary cause such as inflammation (Bain, 1996). The appearance in the peripheral blood of more immature neutrophils and other abnormal features such as the dohle bodies, cytoplasmic vacuolation, and toxic granulation are as a result of changes in the neutrophil morphology (Vinay et al., 2010). Decreased production of neutrophils also known as neutropaenia may be caused by radiation, toxins and some medication which if severe may increase the risk of infections (Richardson, 1996)

8.2 Disorders of lymphocytes

Lymphocytes commonly found in the lymphatic system than in blood stream. They have eccentric nucleus and a small amount of cytoplasm usually found at the periphery (Phillips et al., 2001). The main function of lymphocytes is their involvement in the body's immune system where they destroy foreign substances in the body (Lewis et al., 2006). Lymphocypaenia is defined as reduction in the number of lymphocytes in the circulation and the cells most commonly affected are CD4+ T cells (Bain, 1996). Like neutropaenia, lymphocytopenia may caused by either primary or secondary factors including the inherited immune deficiency, blood cell dysfunction, infectious diseases, medications and radiation among others. The symptoms and treatment of lymphocytopenia are aimed at looking for the cause of the change in cell counts (Vinay et al., 2010).
Lymphocytosis is an increase in lymphocytes. Lymphocytes are cells of the immune system that help fight off diseases and therefore they increase in number whenever there is an infection (Coates, 2016). Lymphocytes of about 3000 million are considered as lymphocytosis in adults and 9000 in children. Causes of lymphocytosis include chronic infection, leukaemia and other ongoing (chronic) inflammation (Coates, 2016).

Eosinophilia which is an increase in the number of eosinophils occurs more commonly in children and is affected by the time of the day being lower in the morning and higher at night, exercise, environment, exposure to allergens and also in parasitic infections (Bain, 1996; Wintrobe 1999). A decrease is termed as eosinopaenia due to acute infection especially by cocci, normal adult count 2–6 $\times 10^9$/l (Lewis et al, 2006). The disorders of a normal eosinophil count are considered to be less than 0.65$\times 10^9$/L (Wintrobe 1999). An increase in number of Monocytes is called monocytosis associated with typhoid fever, protozoic diseases, monocytic leukemia and hodgking disease. An increase in the number of basophils is called basophilia and it is associated with provoked immunity (immune response). Normal count of basophils is $<0.1x\ 10^9$/l. A decrease in Basophils in the peripheral blood film is insignificant (Bain 1996).

2.8.3 The leukemoid reaction
Leukemoid reaction is condition where there is abnormally high leukocyte counts seen in a non leukaemic state which may be lymphoid or granulocytic in nature (Bain, 1996). It is commonly seen in severe infections, extensive burns, malignancies with bone marrow infiltration and severe hemorrhage. Lymphoid reactions are usually in children in response to viral infections (Crowther et al., 1996)
Leukaemia is an abnormal progressive proliferation of the haemopoetic stem cells in the bone marrow and result in high numbers of the abnormal white blood cells called blasts or leukemia cells (National Cancer Institute, 2013). The exact cause of leukemia is unknown but genetic and environmental factors to play a major role. Risk factors include smoking, ionizing radiation, some chemicals (such as benzene), prior chemotherapy, and Down syndrome. People with a family history of leukemia are also at higher risk (Lewis et al., 1003).

Leukaemia can be divided in acute and chronic leukaemia (Hutter, 2010). Acute leukemia is characterized by a rapid increase in the number of immature blood cells causing such cell to infiltrate the bone marrow which renders it incapable of producing healthy blood cells. The immature cells finally spills into the blood stream and spread into other body organs (Hutter, 2010). Acute leukemias are more common in children while the chronic form is more common in older people (National Cancer Institute, 2014).

Leukemia can further be subclassified into acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), chronic lymphocytic leukemia (CLL) and chronic myeloid leukemia (CML) (Vardiman et al., 2009). Acute lymphoblastic leukemia (ALL) is the commonest in young children while chronic lymphocytic leukemia (CLL) is often seen in adults over the age of 55 (Vardiman et al., 2009; American Cancer Society 2014). Acute
myelogenous leukemia (AML) occurs more commonly in adults than in children, and more commonly in men than women (Colvin & Elfenbein, 2003).

2.9 Thrombocytes and thrombocyte parameters

2.9.1 Thrombocytes

Thrombocytes, also called platelets are a component of blood whose function (along with the coagulation factors) is to stop bleeding by formation of fibrin clots (Giles, 1981). Platelets are made as a result of the fragmentation of cells and they contain many structures that help to arrest bleeding in cases of vessel injury (George et al., 1998). They contain proteins on their surface that allow them to stick together, form plagues and to change their shape to seal blood vessel break (Crowther et al., 1996). The main function of platelets is to stop bleeding at the site of interrupted endothelium (Crowther et al., 1996; Dacie et al., 1991). All these processes result to the formation of the fibrin clot hence preventing more blood loss (Yip et al., 1984; Dacie et al., 1991)
Disorders of thrombocytes are as result of either increase in the number of platelets also known as thrombocytosis or decrease in numbers of platelets known as thrombocytopaenia. The disorders can also be as a result of platelet dysfunction (Geddis, 2013). Thrombocytopaenia can be caused by the reduction in the production of the platelets by the bone marrow resulting to low amount in the circulation or by increased destruction of platelets once they are produced and released into the circulating blood by the immune or non immune mechanisms. Immune platelet destruction occurs when platelets are coated with immune antibodies which are therefore recognized as foreign and are then destroyed by macrophages (Moake, 2002). The most common disorders of immune mechanisms include the idiopathic thrombocytopenic purpura (ITP) and the
heparin-induced thrombocytopenia (HIT) (Cines & Blanchette, 2002). Platelet aggregation or endothelial cell injury can also cause platelet destruction. Non immune mechanisms include thrombotic thrombocytopenic purpura (TTP), haemolytic uremic syndrome (HUS) and disseminated intravascular coagulation (DIC) (Kurata, 2001).

These problems are caused by autoantibodies that destroy the platelet and also due to chronic infections such as parasitic infections notably leishmaniasis. The autoantibodies destroy the platelets rapidly after they are produced and they also inhibit their production in the bone marrow (Cines, 2002). Thrombotic microangiopathy are disorders due to abnormal blood clotting in the smallest blood vessels in the body. Drug-induced thrombocytopenia, the most common disorder of platelet function and is caused by aspirin. Aspirin blocks the ability of the platelets to stick together whereby the drug sticks to the platelet after which it is recognized as foreign and is destroyed by the immune system (Giles et al., 1981; Mammen et al., 1998). This reaction makes aspirin an effective treatment for patients with thrombosis (Osselaer et al., 1997; Tefferi et al., 2001).

2.8.3 The platelet count
Platelet count is an estimation of the number of platelets per litre of blood in a sample of blood. Low numbers of platelets is known as thrombocytopenia, while high level of platelets than normal is called thrombocytosis (Dacie et al., 1991). Platelet counts can be used in the monitoring of toxic medications to the bone marrow, or conditions such as thrombocytopenia. They may also be used to help diagnose problems associated with abnormal bleeding or bruising (Tsegaye et al., 1999).
2.3.4 Mean platelet volume

This refers to the average size of platelets in a volume of blood. Mean platelet volume can be used as a indicator of the cardio vascular related diseases and also as a prognostic marker in cerebral vascular stroke (Chu et al., 2009; Dacie et al., 1991. It measures the average size of platelets in blood. The test results can be used to determine the platelet production in bone marrow or platelet destruction problems as the size of the cells dictates (Liu et al., 2012). Mean platelet volume is increase when there is increased platelet destruction as seen in inflammatory bowel disease, immune thrombocytopenic purpura (ITP), myeloproliferative diseases and pre-eclampsia (Giles 1981; Graham et al., 1987). Low MPV is due to impaired production as seen in aplastic anaemia (Chu et al.; 2010).Mean platelet volume can be used as an emerging risk marker for atherothrombosis (Qayyum et al., 2012).

2.5.5 Platelet distribution width

Platelet distribution width (PDW) can useful for distinguishing between reactive thrombocytosis and thrombocytosis associated with myeloproliferative disorder (Lewis, 2003). Together with mean platelet volume, platelet distribution width (PDW) can be useful in the differential diagnosis of aplastic anaemia and idiopathic thrombocytopenic purpura. An increased PDW is an indication for the anisocytosis of platelets and when used together with other parameters it helps in the differential diagnosis of thrombocytosis (Santimone et al., 2011).
CHAPTER THREE: METHODOLOGY

3.1 Study area

The study was conducted in the entire Nakuru County which is one of the forty seven Counties in Kenya (appendix I). Nakuru County is made up of eleven Sub Counties: Nakuru Town East, Nakuru Town West, Bahati, Rongai, Subukia, Kuresoi North, Kuresoi South, Gilgil, Naivasha, Njoro and Molo. However the Ministry of Health has merged the sub counties into four divisions to include Naivasha, Nakuru Central, Bahati and Molo, which the researcher used for this study. Nakuru County borders seven counties which include Laikipia, Kajiado, Baringo, Nyandarua Kericho, Narok, and Bomet. Nakuru Town East sub-county is the major administrative centre hosting the Counties headquarters offices.

Nakuru County covers an area of 7496.5 square kilometers and it lies at an altitude of 1850m above sea level. The county has a population of approximately 1, 603, 325 people according to the 2009 National Census with almost equal number of males and females. The County is located about 165 km from Nairobi and is a major agricultural centre due to its rich volcanic soil which accommodating nearly any kind of farming. The major cash crops are flowers, pyrethrum, coffee, tea and other staple foods such as maize and beans. Animal production is also population with large livestock farms such as Dalamere and Marula farms where a lot of milk originate from.

It is a cosmopolitan county, with its population originating from all the major tribes of Kenya. The Kikuyu and the Kalenjin are the dominant communities in Nakuru, making about 70% of the county’s population. Both communities are mainly engaged in farming, livestock rearing and trade business. Other communities such as Luo, Luhyia, Kamba,
Meru and Kisii are also present in Nakuru County especially in the urban centres. Majority of these people migrated here for business and employment and the government is the main employer in the county.

3.2 Study design

This haematological study utilized a cross sectional design. Blood samples were collected from volunteer donors from randomly selected institutions in Nakuru County, Kenya and were analyzed in the laboratory for determination of haematological measures.

3.3 Variables

3.3.1 Independent variables

Socio-demographic variables which includes sex, gender, and ethnicity and anthropometric measures such as bodyweight, height, and body mass index

3.3.2 Dependent variables

Erythrocytic parameters (red blood cell count, MCV, MCH, MCHC, PCV, RDW, reticulocyte count, absolute reticulocyte number, reticulocyte production index, and erythrocyte sedimentation rate) leukocytic (white blood cell count, absolute and differential neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts), and thrombocytic (platelet count, MPV, and PDW) indices.

3.4 Study population

The study population was adolescents (12-18 years) and adult (19-55 years) residents of Nakuru County. The participants also met all the inclusion criteria for participation into
the study. The prayer houses were selected as study sites owing to the diversity of people in these places in terms of ethnicity, gender, and the fact that all the targeted age groups were accessible at these sites. Secondary day school adolescents were particularly targeted from those prayer houses because the adolescents were able to obtain signed assent forms as soon as possible from the parents/guardians. Also day school students are most likely permanent residents in that particular place thus making them to be more eligible for participation in the study.

Adults were considered to be those of ages between nineteen to fifty five years old. The age of fifty five years is considered as that of healthy population who can participate in donating blood in Kenya. These adults could easily be found in the prayer houses where they were given a health talk in one venue and were able to come back for the result in the same venue conveniently. Adolescents were considered to be those of ages between twelve and eighteen years old (WHO, 2013). This age was chosen for our study since this age group could be found in the secondary schools which were the target sites. The prayer houses were considered as sample collection sites owing to the diversity of the people in terms of gender, age and ethnicity.
3.5: Inclusion and exclusion criteria

3.5.1: Inclusion criteria
The inclusion criteria included adolescents of age 12 to 18 years and adults of age of 19 to 55 years. The participants were also persons with no signs of acute or chronic illness, normal blood pressure, no history of drug taking, non smokers, those who provided informed consent or assent in case of a minor and also agree to participate in the study by signing a written consent form (APPENDIX II AND III).

3.5.2: Exclusion criteria
Study participants were excluded if they have had previous infection within six months (within 2 weeks, exhibiting febrile symptoms or on any medication, any chronic disease, any abnormal bleeding, pregnant women, and if they have had blood donation within the past six months and also if they were smokers. Peripheral blood film was examined and subjects excluded if any morphological abnormality such as hypochromasia, microcytosis and macrocytosis among others was noted. Participants were also excluded if the erythrocyte sedimentation rate (ESR) was more than 20 mm/hr by Westergren method and if they were positive for HIV 1 and 2, HBs antigen, hepatitis C antibody, syphilis, stool parasites, pregnancy and malaria tests. The participants who did not consent to the study were also excluded.

3.6 Sampling techniques
The number and the names of the churches were obtained from the National Council of Churches of Kenya, Nakuru County branch. A systemic sampling method was used to get the number of the required participants. All the churches in each subdivision which was
approximately fifteen (15) were all listed down and were then assigned numbers 1 to 15. To obtain a sampling fraction 15 was divided by 4 (the number of the mainstream churches) and the sampling fraction was therefore three (3) which the interval used to determine the number of churches to be used for the study and in this case five churches were used for each subdivision. The churches were selected by lottery method to determine the order in which sampling was to be done.

After selecting the sites, the research team visited the site and obtained permission for sample collection from the relevant authorities (prayer house leaders). This was done by explaining clearly the purpose of the visit, details of the research study, and any other information the authorities asked related to the study. Upon obtaining the permission from the relevant authorities, the team then set a date for the sample collection. A general health talk was conducted with special emphasize on HIV/AIDS testing and the willing participants were then issued with a consent or assent forms for adults and minors respectively for them to read and sign.

Three separate tents were erected, two for sample collection and one for relaxation and refreshments by the participants and also for observation in case of any serious discomfort after the bleeding procedure. In case of any serious complications, the medical doctor was consulted for proper management. The investigator then assigned a code for each participant and issued a card carrying the same code to the participant which they used to collect the results. The study participants were then selected through a rigorous evaluation by use of a structured questionnaire which was written in Kiswahili and later translated to English (appendix IV). For the clinical evaluation both weight and height
were taken for the body mass index (BMI) determination, temperature, and blood pressure among vital measurements obtained. This was done in every data collection site until at least one hundred and fifty participants’ samples were collected. Body mass index was also calculated (weight/height $^2$) and the participants with less than 18.5 kg/m$^2$ (malnourished) and more than 29.9 kg/m$^2$ (obese) were excluded from the study. Haemoglobin (Hb) was also estimated by use of a haemoglobinometer and those found to have haemoglobin of less than 8gm/dl which is considered as the haemoglobin threshold was excluded from the study (Ema, 2012)

### 3.7 Sample size determination

Sample size determination was based on the target population which was divided into two categories. Category one was based on adolescents (12-18 years) attending the recruitment centres which were the prayer houses with a population size of between 200 and 600 adolescents. In this category a population size of 600 people was used to determine the sample size. Category two was based on adults attending the prayer houses with a population of between 8000 and 10,000 people. In this category a population size of 10,000 people was used to determine the sample size. The following formula and sample size determination table by Bartlett et al. (2001), was used to determine the minimum number of study subject involved in each category (Appendix V)

$$n_0 = (t^2) (p) (q)/(d^2)$$

where $n_0$ = minimum sample size required

$t = 1.96$
(p)(q) = estimate of variance = 0.25

\[ d = \text{acceptable margin error for proportion being estimated} = 0.05 \]

Since the data for the study was categorical the sample size for each category was:

Category 1 = 235 and Category 2 = 370.

### 3.8. Procedure for blood collection

Seven milliliters (mls) of blood samples was collected in ethylenediamine tetra acetic acid (EDTA) vacutainer tubes which were used for the analysis (appendix V). In order to minimize the diurnal variations all samples were collected between twelve noon and two o’clock. The blood samples were placed in ice cooler boxes and transported to the laboratory for analyses which was done within 4 hours of collection to minimize variations of the hematological parameters. A tourniquet was tied above the elbow to make the vein more visible and the site for venipuncture was cleaned using prep pads in 70 percent isopropyl alcohol. The vein was then entered with the needle parallel to and alongside the vein. After entry into the vein the tube was pushed into the holder and vacuum broken thereby the blood flowed freely into the Vacutainer tube.

Seven milliliters (mls) of blood samples was collected in ten milliliters ethylenediamine tetra acetic acid (EDTA) tubes, (purple top vacutainer tubes) which were used for analysis. The blood was adequately mixed by gently inverting the tube after which it was placed on the tube rack. The tourniquet was released and a sterile gauze pad placed over the point where the needle enters the skin. The needle was then withdrawn while placing pressure on the site and the patient was advised to extend the arm and maintain light
pressure on the gauze pad over the venipuncture site until the bleeding stopped and then the patient was released.

3.9 Procedure for urine and stool collection

Urine specimens were obtained from all female participants for pregnancy testing. This was done in order to find out whether they were pregnant so as to exclude them from the study as these are vulnerable groups and their results may cause variations and therefore interfere with the ranges. Stool samples were also collected from all participants to rule out parasitic infections that may cause variations in the blood parameters such as haemoparasites that causes anaemia. The participants were provided with the specimen containers for the urine and stool specimens respectively where they were given instructions on how to collect the right sample in the right manner. All samples were then transported to the laboratory for analysis.

3.10 Laboratory procedures

All samples were analyzed at Rift Valley Provincial General Hospital laboratory, Nakuru County. Rift Valley Provincial General Hospital is a level five hospital and the laboratory is currently undergoing accreditation. Upon arrival in the laboratory, all samples were analyzed according to the stipulated procedures. Haematological tests was analyzed using automated haematology analyzer (Quinttus, Boule Medical AB, Spånga, Sweden) which works on impedance principle for counting of the WBC, RBC and PLT and their indices. The instrument automatically counts and gives a printout result of erythrocytic parameters (RBC \(10^{12}/\text{L}\), Hb (g/dL), PCV (%), MCV (fl), MCH (pg), MCHC (g/dL)), and RDW %. Thrombocytic indices (PLT \(10^9/\text{L}\), PDW (fl), MPV (fl)) and leukocytic
indices (WBC count (10$^9$/L), neutrophils (10$^9$/L), lymphocytes (10$^9$/L), eosinophils (10$^9$/L), basophils (10$^9$/L), monocytic cells (10$^9$/L) and WBC differentials in %. An internal quality control was performed on the hematological equipment (Quinttus) every morning before any blood test was done using the quality control samples supplied by the manufacturers for that particular equipment. The analytical sessions in the current study were 22 in total. Quality control results for the analyzed parameters were within the specific assigned QC range of target value ± 2 standard deviations (SD). In case of any discrepancies a trouble shooting was performed until the testing passed. The results were also compared by using different haematology equipment (appendix VI).

The reticulocyte count was conducted manually by use of the new methylene blue staining technique and enumerated microscopically by use of Ehricks eye piece and were calculated using the formulae: Reticulocyte count = number of reticulocytes counted/number of erythrocytes counted multiplied by 100 (Prchal et al., 2010, National Committee for Clinical Laboratory Standards,1997).Erythrocyte sedimentation rate was performed by westergren method whereby 1mls of blood was drawn into the westergren tube after a dilution of with sodium citrate at the ratio of 4:1 and the level of red blood cell sedimentation was read after one hour and recorded in mm/hour.

### 3.10.1 Reticulocyte count procedure

Reticulocytes are usually stained by use of a supra vital stain where the reticulin fibres are demonstrated. The requirement includes New Methylene Blue, slide, spreader, EDTA blood, Ehricks eye piece, gauze, test tubes, microscope. Two drops of anticoagulated blood was placed in a test tube and two drops of New Methylene blue were added. There
was incubated for 30 minutes after which a thin blood film was prepared and the air dried. Examination was done using high power objective (100X). A Total of 1000 RBCs were counted using oil immersion lens and the number of reticulocytes were noted. Reticulocytes appeared greenish blue in colour with remnants if fibrin. The reticulocytes were reported as the percentage of total RBCs counted. Reticulocytes % = Reticulocytes / RBCs X 100, absolute Reticulocyte count = % reticulocytes X RBCs count and reticulocyte production index calculated = % reticulocytes X ( patient hematocrit / 45 is normal hematocrit ) which is normally = 1

A blood film was prepared which was stained with Giemsa method of staining the blood films in order to detect abnormalities of blood cells such as hypochromia), leukaemia, sickle cells, malaria parasites and other blood parasites and for those found positives, the participants were referred for further management

### 3.10.2 Giemsa staining

A thin film was made by placing a drop of blood at three quarter of a slide and then spread using a spreader. The thin film was then fixed with methanol for two minutes. Individual slides were placed on the staining rack, making sure that they are not touching each other. The Giemsa stain was gently poured onto the slides until they are totally covered for 15 minutes with 10% Giemsa solution. Internal quality control of the stain was used to indicate the optimum staining time. The slides were gently flooded with buffered water of 7.2 pH and leave for 2 minutes to differentiate. The slides were then placed in a drying rack to drain and dry. The slides were then examined using oil
emersion (10X) objective. The film was examined for cell abnormalities and for blood parasites.

3.10.3 Rapid pregnancy test

The requirements for pregnancy test (EUROMEDI EQUIP LTD, West Harrow U.K) include the urine specimen, Test device, desiccant, timer, disposable gloves and biohazard waste container. The urine specimen and controls were allowed to reach room temperature (15-30ºc) prior to testing. With the allow pointing towards the urine specimen, the test strips were immersed vertically in the urine specimen for at least 10seconds. The test strip was then removed and placed on a non-absorbent flat surface. The timer was started and the results were read after exactly 5 minutes. The results were termed positive if a line appeared in the control line region (C) and another one on the test (T) line region and negative if only one line appeared on the control(C) line region. If no line appears on the control region, the results are invalid. The probable causes of invalid test include insufficient specimen volume or incorrect technique and where it occurred the procedure was reviewed and the test repeated. If the problem persist the test kit was stopped and the manufactures were contacted (Catt et al., 1980, lenton et al., 1982).

Stool specimens were also collected from all participants to rule out parasitic infections that may cause anaemia hence variations in haematological reference intervals. The stool samples were then transported to the laboratory for analysis by wet method preparation.
samples were then taken to the laboratory and wet preparation method was used for preparation and examination of the sample.

Requirements for stool sample preparation included normal saline (0.85% NaCl), Lugol’s Iodine, glass slide, cover slips, pipettes, gloves and microscope. The patient’s sample was applied to a small area on a clean microscope slide and any gross fibers and particles were removed. Immediately before the specimen dried, 1 or 2 drops of lugos iodine was added with a pipette and mixed using an applicator stick. The specimen covered and examined for ova and cysts. The specimen was examined with the low power objective (10X) and low light and then by power 40 objectives. Any ova or cyst was reported and managed accordingly.

Further routine screening was conducted where the blood was tested for HIV/AIDS, the first testing kit being determine (Alere Medical Co., Ltd, Chiba, JAPAN) and the confirmatory test being the first response (Premier Medical Corporation Private Limited, Gujarat, INDIA), hepatitis B virus, hepatitis C surface antigen (EUROMEDI EQUIP LTD, West Harrow U.K), presence of syphilis (Guangzhou Wondfo Biotech Co., LTD. Science city, CHINA) and pregnancy test(EUROMEDI EQUIP LTD, West Harrow U.K) by use of rapid test kits as directed by the manufacturers of that kit. The stool specimen was examined by wet preparation method whereby it was emulsified using normal saline and then placed on a microscopic slide for examination for parasites that may cause anaemia such as hookworms. All positive samples results to any of these tests were discarded and the samples which were negative were utilized for the study.
3.10.5 The HIV rapid tests

The current HIV/AIDS WHO algorithm involves two test kits which are the ‘determine’, as the first kit and the first response test kit which is used for confirmation if the determine results are positive. The requirements includes test device, desiccant, pipettes, assay buffer, alcohol swabs, capillary blood, needles, syringes, timer, disposable gloves, and biohazard waste container. For the ‘determine’, test the test device and the specimen transfer device were removed from foil pouch and were placed on a flat, clean and dry surface. 50 µl of whole blood was applied to the sample pad using specimen transfer device and One drop of chase buffer was added to the sample pad.

The formation of the colour bands was observed and the results interpreted after 15 minutes. Results were declared positive if red bars appeared at the region for the control (labeled “C”) and the region for patients sample (labeled “P”) on the strip. Any visible red bar in the (“P”) was interpreted as positive. If one red bar appeared at the (“C”) of the strip and no red bar on the (“P”) the results were termed as negative. If no red bar appeared at (“C”) of the strip and even if a red bar appears at the (“P”) of the strip, the results were termed as invalid and a repeat was done (CLSI, 2002, O’Connel et al., 2006).

The first response HIV 1-2 o card test kit components were brought to room temperature. The test device and the specimen transfer device were removed from foil pouch and were place on a flat, clean and dry surface. 20µl of whole blood was added to the sample well using specimen transfer device. 35µl of the assay buffer was added to the sample well. The formation of the colour bands was observed and the results interpreted after exactly
15 minutes. Results were declared non-reactive if one color line appeared at the control line. If two color lines appeared at the control line “C” and the other at the test line HIV-1 “1” the specimen was reactive for antibodies to HIV-1. A faint line was also interpreted as a reactive line. If two color lines appeared at the control line “C” and the other at the test line HIV-2 “1” and HIV-2 “2” the specimen was reactive for antibodies to HIV-2 and HIV-2. A faint line was also interpreted as a reactive line. If three colour lines appeared at the control line “C” and the other two at the test line HIV-1 “1” the specimen was reactive for antibodies to HIV-1. A faint line was also interpreted as a reactive line. If there is no colour at control line “C” at the end of the incubation period, result was termed as invalid. The result was also invalid if a color band appeared only at one line “1” and/or “2”. If there is high background coloring and incomplete migration along the test strip, the results are invalid (CLSI, 2002, O’ Connel et al., 2006).

3.10.6 One step syphilis test

The Requirements for one step syphilis test includes test device, desiccant, pipettes, assay buffer, alcohol swabs, capillary blood, needles and syringes, timer, disposable gloves and biohazard waste container. The specimen and all the reagents were brought to room temperature before the testing was done. The test device and the specimen transfer device were placed on a flat surface in readiness for the procedure where µl of whole blood was applied to the sample device using specimen transfer device. One drop of dilution buffer was added to the sample pad and the results were interpreted after exactly 15 minutes. The results were positive if coloured appeared in both control region and the test region indicating positive result for antibodies of Treponema Pallidum in the specimen. If the coloured band was visible only in the control region and non in the test region it indicated
the concentration for antibodies of Treponema Pallidum in the specimen was below zero or below detectable limit. The test was invalid if there was no visible band or there was a visible band only in the test region. In this case the test was repeated using a new test kit and if still invalid the manufacturers of the test kit were contacted (Zhang et al., 2002, George et al., 1998).

3.10.6 Hepatitis B surface antigen and C antigen test

This is a rapid test for the detection of hepatitis infections in blood specimen. The test utilizes the principle of immunochromatography. As the sample flow the membrane, the coloured anti hepatitis B surface antigen conjugate react with the HBsAg in the sample where reaction moves towards the test region and is immobilized by the HBsAg antibodies and the formation of a pink colour indicates a positive result. The requirements includes test device, desiccant, pipettes, assay buffer, alcohol swabs, capillary blood, needles and syringes, timer, disposable gloves, biohazard waste container. All reagents and samples were left for a while to warm in room temperature. The dipstick was then dipped into the plasma specimen submerging the area below the MAX line. The dipstick was removed after 10 seconds and laid on a flat clean, dry surface. The results were then read within fifteen minutes. The results were positive if distinct pink-purple bands appeared on the dipstick and negative if only one band appeared on the dipstick. The results were invalid if there was no control band and the test was then repeated with new dipstick (Caldwell, 1977).
3.10.6 **Erythrocyte sedimentation rate estimation**

When anticoagulated whole blood is allowed to stand in vertical position in a standard tube for a stipulated time, the RBCs settle out from the plasma and the rate of the settling is measured in millimeters of clear plasma present after one hour (mm/hr). The requirements include anticoagulated blood (citrated), Westergren tube, Westergren stand, Rubber bulb (sucker). The blood was well mixed and blood was drawn into the tube upto 0 mark with the help of rubber bulb. Excess blood was wiped out blood from bottom of the tube with cotton and the tube was set upright in stand. The tube was left undisturbed for 1 hour and the results were read the after exactly 1 hour. After all the analysis were completed the residual samples such as blood, stool and urine were placed in the disposable plastic bags and transported by a trained personnel to the incineration site for disposal. All procedures and protocols adhered to the standard operating procedures.
3.11. Ethical Considerations

Scientific, ethical approval and authorization of the study was obtained from Kenyatta University (ethics review committee), the National committee of science and technology and the Ministry of health, Nakuru County (serial number 3970). Consent to obtain the samples obtained from the participants and for the minors; assent was sought first from the minor and then from the guardian both of whom signed the assent forms (one for the minor and the other one for the parents/guardian). Post counseling for HIV positive results was provided by a well trained counselor when coming back for the results at the sample collection site where a private tent was erected and a referral made for appropriate care and treatment. All results were communicated to the guardian/parents and the participants at a set date whereby every participant went back for the results at the same venue where the samples were collected from. The results and any other information regarding the study were held in total confidentiality both by the investigator and Kenyatta University administration by placing them in lockable cabinets and were only accessed by authorized persons.

3.12 Data analysis and presentation

Data generated from the laboratory assays was dual entered and was analyzed statistically using a Statistical Package for Social Sciences (SPSS version 21). The data was grouped according to age and gender with adolescents aging between 13 to 18 years and 19 to 55 years for the adults. The measure of the central tendency was the median, where the 2.5 and 97.5 percentiles specified the 95% reference interval for the data. Further a parametric method (ANOVA) was used to establish the reference intervals (Rhoads, 2007b). Comparisons in the hematological measures across the study groups were
performed using the ANOVA test. Subsequently, a post-hoc analysis between-group comparisons were performed on the haematological measurements (RBC count, Hb, HCT, MCH, MCHC, ESR RETIC count WBC, lymphocyte, neutrophil, monocyte, eosinophil and basophil count and the WBC differentials) variables that were statistically significant ($P<0.05$) across the groups in the ANOVA analyses. All tests were two-tailed and an alpha-value of 5% was used to determine the statistical inferences.
CHAPTER FOUR: RESULTS

4.1: Socio-demographic characteristics of the study participants

The number of the participants recruited in to the study were 627 who included the adults (male, n=252; female, n=134) and adolescents (male, n=128; female, n=113). The socio-demographic characteristics of the study participants are summarized in Table 4.1. Consistent with the recruitment criteria, the media and range (median, range) was significantly different among the adult and adolescents genders (adults: males, median, 22.0; range (20.0-53.0); females, median, 22.0; range (19.0-50.0); adolescents: males, median, 17.0, range, (13.0-18.0); females, median, 17.0, range (13.0-18.0); \( P<0.0001 \)). Among the study groups, Bantu ethnic group comprised 62-70%; Nilotes were 36-38% and Cushites constituted less than 5% of the study participants.

The median (range) heights were similar across the study groups [adults: females, 1.7 (1.5-1.8); males, 1.7 (1.5-1.8); adolescents: females, 1.7 (1.5-1.9); males, 1.7 (1.5-1.9); \( P=0.668 \)]. Likewise, weight [adults: females, 63.8 (47.7-79.4); males, 64.2 (44.2-79.6); adolescents: females, 64.7 (43.7-88.8); males, 63.9 (43.7-88.8); \( P=0.412 \)] and BMI [adults: females, 23.0 (18.5-26.4); males, 23.0 (18.5-25.6); adolescents: females, 23.0 (18.5-25.4); males, 22.7 (18.5-25.4); \( P=0.960 \)] were not significantly different amongst the groups. Ninety (90-91%) of the adult groups and 89-90% of the adolescent groups had at least secondary level education with less than 11% of the individuals in the study groups having primary or no education \( (P<0.0001) \) (Table 4.1).
The median (range) heights were similar across the study groups [adults: females, 1.7 (1.5-1.8); males, 1.7 (1.5-1.8); adolescents: females, 1.7 (1.5-1.9); males, 1.7 (1.5-1.9); P=0.668]. Likewise, weight [adults: females, 63.8 (47.7-79.4); males, 64.2 (44.2-79.6); adolescents: females, 64.7 (43.7-88.8); males, 63.9 (43.7-88.8); P=0.412] and BMI [adults: females, 23.0 (18.5-26.4); males, 23.0 (18.5-25.6); adolescents: females, 23.0 (18.5-25.4); males, 22.7 (18.5-25.4); P=0.960] were not significantly different amongst the groups. Ninety (90-91%) of the adult groups and 89-90% of the adolescent groups had at least secondary level education with less than 11% of the individuals in the study groups having primary or no education (P<0.0001) (Table 4.1).
### Table 4.1: Social-demographic characteristics of the study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Adolescents</th>
<th>Adults</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( \text{Male, } n=128 )</td>
<td>( \text{Female, } n=113 )</td>
<td>( \text{Male, } n=252 )</td>
</tr>
<tr>
<td>Age, years</td>
<td>17.0 (13.0-18.0)</td>
<td>17.0 (13.0-18.0)</td>
<td>22.0 (20.0-53.0)(^b)</td>
</tr>
<tr>
<td>Ethnicity, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bantu</td>
<td>79 (69.7)</td>
<td>70 (61.9)</td>
<td>159 (63.1)</td>
</tr>
<tr>
<td>Nilotes</td>
<td>48 (37.5)</td>
<td>41 (36.3)</td>
<td>91 (36.1)</td>
</tr>
<tr>
<td>Cushites</td>
<td>1 (0.8)</td>
<td>2 (1.8)</td>
<td>2 (0.8)</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.7 (1.5-1.9)</td>
<td>1.7 (1.5-1.9)</td>
<td>1.7 (1.5-1.8)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>63.9 (43.7-88.8)</td>
<td>64.7 (43.7-88.8)</td>
<td>64.2 (44.2-79.6)</td>
</tr>
<tr>
<td>BMI, ( \text{kg/m}^2 )</td>
<td>22.7 (18.5-25.4)</td>
<td>23.0 (18.5-25.4)</td>
<td>23.0 (18.5-25.6)</td>
</tr>
<tr>
<td>Education, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertiary</td>
<td>33 (25.8)</td>
<td>35 (31.0)</td>
<td>112 (44.4)</td>
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<tr>
<td>Secondary</td>
<td>82 (64.1)</td>
<td>66 (58.4)</td>
<td>115 (45.6)</td>
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<td>11 (9.7)</td>
<td>10 (4.0)</td>
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<tr>
<td>None</td>
<td>1 (0.8)</td>
<td>1 (0.9)</td>
<td>15 (6.0)</td>
</tr>
</tbody>
</table>

Data are presented as medians and range for continuous variables and number and proportion of subjects for categorical variables.

\(^a\)\(^b\) \( P < 0.0001 \) vs. male and female adolescents, respectively.
4.2 Erythrocytic reference intervals for adults and adolescents

Males had higher median for [RBC, 5.8 × 10^{12}/L versus 5.0 × 10^{12}/L; HB, 15.1 g/dl versus 13.1 g/dl; HCT ,46.9% versus 42.3%; MCH, 86.1 Pg versus 85.5 Pg; MCHC, 32.7% versus 32.2%] than females for adults and RBC, 5.3× 10^{12}/L versus 4.8× 10^{12}/L; HB, 14.1 gm/dl versus 13.2 gm/dl; HCT, 43.1% versus 41.6%; MCH, 85.8 Pg versus 85 2 Pg; MCHC, 32.5% versus 31.9%] than female for adolescents. The values for the RBC, HB, HCT, MCH and MCHC were statistically different ($p <0.05$) as shown in table 2.

Subsequent post hoc analysis indicated statistical differences within study groups between male versus female for RBC, Hb, HCT and MCHC ($P<0.0001$) for both adults and adolescents. Differences were also observed for RBC count, HB, HCT and MCHC by age with male adults having significant higher values than male adolescents ($P<0.0001$). The values for the females were similar. The medians for MCV, RDW and ESR were similar and there was no statistical difference by gender or age (Table 4.2). In contrast to the erythrocytic measures, the reference values for the reticulocyte indices were not significantly different amongst adult and adolescent or males and females (retic count, $P=0.116$; retic index, $P=0.093$; absolute reticulocyte number, $P=0.177$; and reticulocyte production index, $P=0.093$ (Table 4.3).
Table 4. 2 Erythrocytic reference intervals for adults and adolescents

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adolescents</th>
<th>Adults</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male, n=128</td>
<td>Female, n=113</td>
<td>Male, n=252</td>
</tr>
<tr>
<td>RBC×10^12/L</td>
<td>5.3 (4.0-6.4)</td>
<td>4.8 (3.5-6.1)\textsuperscript{f}</td>
<td>5.5 (5.0 – 5.9)\textsuperscript{d}</td>
</tr>
<tr>
<td>Hb, g/dl</td>
<td>15.1 (12.2-18.0)</td>
<td>13.2 (10.7-15.7)\textsuperscript{f}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15.1 (12.1-17.3)\textsuperscript{d}</td>
<td>13.1(10.4-15.8)\textsuperscript{a,c}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hct, %</td>
<td>46.1 (39.6-53.0)</td>
<td>41.6 (29.0-54.8)\textsuperscript{f}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>43.9 (32.7-53.3)\textsuperscript{d,e}</td>
<td>43.3 (32.6-53.1)\textsuperscript{a,c}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>85.8 (65.5-106.3)</td>
<td>85.2 (59.6-109.0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>86.1 (76.1-94.5)</td>
<td>85.5 (68.0-104.2)</td>
<td>0.484</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>28.7 (23.4-34.0)</td>
<td>30.6 (19.6-30.5)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>28.4 (16.6-40.5)\textsuperscript{d}</td>
<td>29.1 (18.6-39.6)</td>
<td>\textbf{0.034}</td>
</tr>
<tr>
<td>MCHC, %</td>
<td>32.5 (29.4-35.6)</td>
<td>31.9 (23.5-31.9)\textsuperscript{f}</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32.7 (29.1 -35.9)\textsuperscript{d}</td>
<td>32.2 (29.2-35.8)\textsuperscript{b}</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>RDW, %</td>
<td>17.3 (7.8-27.8)</td>
<td>17.3 (11.3-24.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>17.3 (12.0-23.8)</td>
<td>17.2 (13.8-21.2)</td>
<td>0.555</td>
</tr>
<tr>
<td>ESR, mm/hr</td>
<td>5.8 (0.0-17.0)</td>
<td>3.5 (0.0-17.1)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.0 (0.0-18.0)</td>
<td>4 (0.0-16.7)</td>
<td>\textbf{0.091}</td>
</tr>
</tbody>
</table>

Data presented are medians and reference values (mean ± 2SD). n, number of subjects. RBC, red blood cell; Hb, haemoglobin; Hct, haematocrit; MCV, mean cell volume; MCH, mean cell haemoglobin; MCHC, mean cell haemoglobin concentration; RDW, red cell distribution width; ESR, erythrocyte sedimentation rate. \textsuperscript{a}P<0.05 versus male adults; \textsuperscript{b}P<0.001 versus female adolescents; \textsuperscript{c}P<0.0001 versus male adolescents; \textsuperscript{d}P<0.05 versus female adolescents; \textsuperscript{e}P<0.01 versus male adolescents; and \textsuperscript{f}P<0.0001 versus male adolescence.
**Table 4.3** Reference intervals for reticulocyte indices

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adolescents</th>
<th>Adults</th>
<th></th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male, n=128</td>
<td>Female, n=113</td>
<td>Male, n=252</td>
<td>Female, n=134</td>
<td></td>
</tr>
<tr>
<td>Retic count, %</td>
<td>0.2 (0.0-2.2)</td>
<td>0.2 (0.0-2.9)</td>
<td>0.2 (0.0-0.8)</td>
<td>0.2 (0.0-1.0)</td>
<td>0.116</td>
</tr>
<tr>
<td>Retic index, %</td>
<td>0.2 (0.0-2.2)</td>
<td>0.2 (0.0-1.3)</td>
<td>0.2 (0.0-0.8)</td>
<td>0.2 (0.0-0.1)</td>
<td>0.093</td>
</tr>
<tr>
<td>ARN ( \times 10^9 )/L</td>
<td>11.0 (10.3-30.0)</td>
<td>9.4 (12.6-32.0)</td>
<td>8.8 (9.7-15.9)</td>
<td>9.0 (9.8-18.8)</td>
<td>0.177</td>
</tr>
<tr>
<td>RPI</td>
<td>0.2 (0.0-2.0)</td>
<td>0.2 (0.0-2.4)</td>
<td>0.2 (0.0-0.8)</td>
<td>0.2 (0.0-1.1)</td>
<td>0.093</td>
</tr>
</tbody>
</table>

Data presented are medians and reference values (mean ± 2SD). Retic, reticulocyte; ARN, absolute reticulocyte number; RPI, reticulocyte production index.
4.3 Leukocytic reference intervals for adults and adolescents

The median and the reference levels for the absolute monocytes counts were higher in females [monocyte, $0.4 \times 10^9$ versus $0.3 \times 10^9$] than males adolescent and $0.4 \times 10^9$ versus $0.3 \times 10^9$] than male adults. The levels were statistically significance ($p < 0.05$). Consequent post hoc analysis indicated differences within study groups between males versus females in both adults and adolescents for monocyte count ($P<0.0001$) with females having higher values.

Age differences were also noted with female adolescents having higher values than the female adults ($P<0.0001$). No differences were noted among the males. Higher eosinophil counts were also noted in males [Eosinophils, $0.1$ (0.0-0.6) versus $0.1$ (0.0-1.0) female adults and $0.1$ (0.0-0.3) versus $0.1$ (0.0-0.2) than female adolescents] and the values shows statistical differences ($P<0.001$). Further analysis observed differences within groups among male and female adolescents ($P<0.0001$) with males indicating higher values but no differences noted for the adults. Age differences were also noted with male adolescents having higher median and reference values than the male adults for the absolute eosinophil count. There was however no gender or age differences were observed for WBC, lymphocyte, neutrophil and basophil counts (Table 4.4).

The females indicated higher median and reference differential count levels for [lymphocytes, 43.8 % versus 42.8 %; monocytes; 6.5% versus 6.3%; basophils, 0.2% versus 0.1%] than male adults and [lymphocytes, 44.7 % versus 41.6 %, ; monocytes, 6.5 % versus 6.2 %; basophils, 0.2% versus 0.1%] than male adolescents and the values were statistically significant ($p < 0.05$). Post hoc indicated statistical differences within
study groups with females having higher median and higher reference values for a lymphocytes, monocytes than the males adults \((P<0.05)\) and male adolescents \((P<0.0001)\). Age differences were also noted for lymphocytes, monocytes and basophils with female adults having lower values than the female adolescents \((P<0.0001)\). Also noted were higher reference values for basophils in male adults versus male adolescents \((P<0.0001)\) (Table 4.5).
Table 4.4 Reference intervals for leukocytic absolute counts for adults and adolescents

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adolescents</th>
<th>Adults</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male, n=128</td>
<td>Female, n=113</td>
<td>Male, n=252</td>
</tr>
<tr>
<td>Absolute counts×10^9/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>5.4 (2.8-8.2)</td>
<td>5.0 (2.5-7.7)</td>
<td>5.7 (2.6-8.0)</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>2.1 (1.5-3.5)</td>
<td>2.2 (0.9-3.7)</td>
<td>2.1 (1.2-3.2)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.1 (0.3-4.3)</td>
<td>2.3 (0.5-4.5)</td>
<td>2.1 (0.4-4.2)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.4 (0.0-1.2)</td>
<td>0.4 (0.0-1.3)</td>
<td>0.3 (0.4-1.2)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0.1 (0.0-0.6)</td>
<td>0.1 (0.0-1.0)</td>
<td>0.1 (0.0-0.3)</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.0 (0.0-0.9)</td>
<td>0.0 (0.0-0.1)</td>
<td>0.0 (0.0-0.1)</td>
</tr>
</tbody>
</table>

Data presented are median and reference value (mean ±2SD). WBC, white blood cell. ^aP<0.05 versus male adults; ^bP<0.0001 versus female adolescents; ^cP<0.0001 versus male adolescents; ^dP<0.05 versus female adolescents; ^eP<0.01 versus male adolescents; and ^fP<0.0001 versus male adolescents.
Table 4.5 Reference intervals for leukocytic differential counts for adults and adolescents

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adolescents</th>
<th>Adults</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male, n=128</td>
<td>Female, n=113</td>
<td>Male, n=252</td>
</tr>
<tr>
<td></td>
<td>Differential counts (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>41.6 (22.0-59.6)</td>
<td>44.7 (29.6-60.8)</td>
<td>42.7 (24.4-60.8)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>45.5 (26.7-64.3)</td>
<td>46.9 (27.5-65.9)</td>
<td>47.9 (27.9-68.9)</td>
</tr>
<tr>
<td>Monocytes</td>
<td>6.2 (1.3-11.7)</td>
<td>6.5 (1.2-12.2)</td>
<td>6.5 (2.7-10.7)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>2.2 (0.0-8.6)</td>
<td>2.0 (0.0-7.2)</td>
<td>1.8 (0.0-8.2)</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.1 (0.0-0.7)</td>
<td>0.2 (0.0-1.2)</td>
<td>0.1 (1.0-1.4)</td>
</tr>
</tbody>
</table>

Data presented are median and reference value (mean ±2SD). WBC, white blood cell. aP<0.05 versus male adults; bP<0.0001 versus female adolescents; cP<0.0001 versus male adolescents; dP<0.05 versus female adolescents; eP<0.01 versus male adolescents; and fP<0.0001 versus male adolescents.
4.4 Thrombocytic reference intervals

Females had higher median levels [absolute platelet counts, $272.0 \times 10^9/L$ versus $228.0 \times 10^9/L$ than males for adult and $324.0 \times 10^9/L$ versus $296 \times 10^9/L$] than males for adolescents. The post hoc analysis further illustrated that the values for absolute platelet count for the adults were statistically significance ($P < 0.0001$) with females indicating higher values. Adult males also showed higher reference values than the male adolescents ($P < 0.0001$). MPV also illustrated higher medium levels in females [MPV, $5.8 \text{ fl}$ versus $4.2 \text{ fl}$] than males for adolescent ($P < 0.05$). Further analysis indicated statistical significance within study groups between female adolescents and male adolescents ($P < 0.0001$) with females having higher values. Age differences were noted with male adults versus male adolescents for MPV ($P < 0.05$) with male adults having higher values. The medians PDW ($P < 0.73$) were similar and there was no statistical difference by gender or age (Table 4.6).
### Table 4.6: Thrombocytic reference intervals

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Adolescents</th>
<th>Adults</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male, n=128</td>
<td>Female, n=113</td>
<td>Male n=252</td>
</tr>
<tr>
<td>Platelets×10^9L</td>
<td>228.0 (107.0-405.0)</td>
<td>272.0 (97.0-430.0)(^c)</td>
<td>296.0 (104.0-436.0)(^{b,c})</td>
</tr>
<tr>
<td>MPV, fl</td>
<td>4.2 (3.9-9.1)</td>
<td>5.8 (5.3-10.0)(^e)</td>
<td>5.9 (4.6-10.2)(^c)</td>
</tr>
<tr>
<td>PDW, fl</td>
<td>14.9 (12.4-17.4)</td>
<td>14.4 (12.7-21.8)</td>
<td>14.9 (11.8-18.0)</td>
</tr>
</tbody>
</table>

Data presented are median and reference value (**mean ± 2SD**). PLT, platelet; PDW, platelet distribution width; MPV, mean platelet volume; \(^a\)\(P<0.05\) versus male adults; \(^b\)\(P<0.0001\) versus female adolescents; \(^c\)\(P<0.0001\) versus male adolescents; \(^d\)\(P<0.05\) versus female adolescents; \(^e\)\(P<0.05\) versus male adolescents.
CHAPTER FIVE: DISCUSSION, CONCLUSIONS & RECOMMENDATIONS

5.1 Discussion

Haematological reference interval values are important in clinical care and research and therefore appropriate interpretation of these values will aid in the management of the population from which they have been derived (Kibaya et al., 2008; Tugune et al., 1995; Solberg et al., 2001). The data for these haematological values are scarce in most African countries including Kenya and clinician have continued using published reference intervals from Europe and America despite indications of difference between the Caucasian and blacks (Horn et al., 2003; Kibaya et al., 2008; Tugune et al., 1995).

The study showed that the reference intervals for the haemoglobin, RBC count, haematocrit and mean cell haemoglobin concentration (MCHC) in female adults were lower compared to male adults and adolescents but were higher relative to female adolescents. The MCV, ESR, Reticulocytes and reticulocyte indices were similar by gender or age. There were findings of significance gender differences for the red blood cell indices (HB, HCT, RBC, MCH and MCHC) and the results concurred with other studies both in western countries and also African countries (Koram et al., 2007; Mwinga et al., 2009). The differences are associated with nutritional with prevalence of iron deficiency anaemia in women probably as a result of menstrual blood loss as well as hormonal influences on haemopoiesis where estrogen lowers haemoglobin by hemodilution and testosterone increases the plasma volume (Lewis, et al., 2006; Zeh et al., 2012).
Age differences for the RBC, HCT and MCHC was also observed among male participants, with adults having higher levels compared to male adolescents in the current study which are consistence with other African studies (Zeh et al., 2011). Similar results were also reported in a Caucasian adolescents study (Romeo et al., 2009). This difference may be associated the presence androgen hormone which is found in larger amount in older males than in the young males. Increased levels in older males are also associated with an increase in the size and mass of muscle fibers hence an increase in the number of circulating red blood cells (Zeh et al., 2012). The females indicated no age difference for the said parameters which may support the above idea. Other RBC parameters such as MCV, RDW, RETIC count and ESR parameters showed neither gender nor age related statistical differences unlike other studies which showed differences in some parameters such as MCV (Zeh et al., 2011). The differences in these parameters from other studies may be due to the methodology used such as the manual methods and microscopy used for reticulocyte count and westergren method in the estimation of erythrocyte sedimentation rate instead of automation.

The haematological reference intervals for RBC parameters in the current study are higher in comparison to other Kenyan haematological studies. The differences may be attributed to many factors such as methodology, type of study population used and environmental factors among others. The current study for instance investigated helminthes infestations and malaria which causes chronic blood loss while the other tests did not. Also some regions like Kisumu where one study was performed is a malaria endemic zone which accounts for low Hb levels in the population. The Nakuru County where the current study was carried out is a rich agricultural area and therefore a
balanced diet may account for the higher RBC values compared to Kericho and Kisumu where food crop farming is not a major occupation. Altitude can also affect these hematologic parameters and has been associated with reduced red blood cell components (Wintrobe et al., 1999) which may account for differences in reference values in the Kenyan studies. These study areas are located in different geographical zones with Kisumu County in lowest altitude hence low values for haemoglobin than Nakuru County.

The results for all RBC parameters were lower in comparison with the commonly used European and American reference interval values. Studies in Kenya indicated similar observations for the red blood cell parameters (Kibaya et al., 2008; Zeh et al., 2011), Uganda (Luganda et al., 2004), Ethiopia (Saathoff et al., 2008) and Tanzania (Tsegaye et al., 1999). The reason for these reduced RBC components is attributed to lower dietary iron intake, genetic polymorphism like sickle cell trait, chronic blood loss, and chronic exposure to endemic parasites, environmental and ethnic factors which may not have been tested in these studies and has been shown to be common for African populations. However the African reference values also differs from country to country. The methods of data collection such as diurnal variation, time for the samples analysis after blood collection among others may have contributed to these discrepancies. One Ugandan study had its samples analyzed after twelve hours while the current study analyzed the samples after four hours. The Ugandan samples were also collected in a wide range of time between nine am to four pm morning which may be influenced by diurnal variations. An Eastern and Central African study did not perform malaria or helminthes infections which of course affects the haemoglobin levels.
Low levels of RBC parameters below the internationally accepted levels are often diagnosed as anaemia. Anaemia is usually a healthy problem which is usually corrected by transfusion. Anaemia is the reduced oxygen carrying capacity of blood and haemoglobin is a major marker in determining whether there is anaemia or not and whether transfusion is required. Transfusion of blood products is important as a life saver and therefore accurate laboratory results are paramount. Transfusion may also pose some risks to the patient such as exposure to infections including the HIV/AIDS, and hepatitis and therefore correct interpreting the laboratory reference values based on a particular population as the haematological values is important. Haemoglobin concentration is an important indicator for assessments of immune status and response to antiretroviral treatment in individuals infected with human immunodeficiency virus (HIV) and is also used in clinical trials and therefore is very crucial since participants may be wrongly treated or left out in clinical trials.

The median and the reference levels for the absolute monocytes counts were higher in females than male adolescent and male adults. Age differences were also noted with female adolescents having higher values than the female adults. No differences were noted among the males previous studies however indicates no gender or age differences (Zeh et al., 2011; Kibaya et al., 2008; Karita et al., 2009). This is purported to be as a result of endemic parasitic infections or exposure to environmental allergens (Abdulkarir 1999; Luganda, et al., 2004). Higher eosinophil counts were also noted in males than female adults and female adolescents. Age differences were also noted with male adolescents having higher median and reference values than the male adults for the absolute eosinophil count. Eosinophil counts in the current are similar with those from
the Caucasian population. This may be due to the fact that some parasites such as malaria and hookworms were investigated in the current study and those participants found infected were excluded from the study. The values differs with other African populations studies which shows increased eosinophil counts in both genders compared to the western values reference intervals (Zeh et al., 2011; Karita et al., 2009). The results are attributed to prevalence of parasitic infections such as schistosomiasis, helminthes and malaria (Saathoff et al., 2008; luganda et al., 2004) and the failure to test for the infections in most studies.

The females indicated higher median and reference differential count levels for lymphocytes, neutrophils, Monocytes and basophils, than male adults and male adolescents. This is consistent with others studies which indicates higher levels in female for the WBC parameters (luganda et al., 2004). Age differences were also noted for lymphocytes, neutrophils, Monocytes and basophils differential counts with female adults having lower values than the male adults while the values for the adolescents were similar. Also noted were higher reference values for neutrophils and basophils in male adults versus male adolescents. Higher levels of neutrophil counts in women is associated with high levels of estrogen hormone in younger women as they have shown to decrease with age (Zeh et al., 2011). Mechanisms by which black population have lower values of neutrophils is still not clear but hypothesis of an excess of marginated neutrophils pool is often proposed but a recent study did not confirm it (Phillips et al., 2000). Variation in lymphocyte counts is from region to region with some African populations showing lower reference values than others (Bussmann et al., 2004). Age-related have also been observed with adolescents indicating higher levels for lymphocytes than the adults.
However, the differences are not significantly. These variations in the WBC parameters could are associated to chronic infections and therefore locality is important when interpreting lymphocyte counts (Romeo et al., 2009; Zeh et al., 2011).

Lower WBC parameter reference values for the African populations have been reported in relation to the western values (Zeh et al., 2011) and among blacks in the U.S.(Eller et al., 2008). The current study intervals for the WBC parameters would mean that the study population would be said to have had adverse effects due to the low values of most parameters. The decisions to initiate and continue, or change antiretroviral therapy regimen are determined using the lymphocyte subsets such as CD4 count and therefore correct lymphocyte reference intervals for a particular population will be appropriate to avoid patient mismanagement.

The current study indicated that females had higher median levels for absolute platelet and MPV count than males for both adults and adolescents. Also the male adults had higher reference values than the male adolescents for these parameters. Platelet counts were lower in the current study when compared to the Western populations consistent in several African studies (Bain 1984; Tsegaye et al., 1999). The causes are however not known but factors such as dietary, environmenta,malaria and genetics have been indicated as the probable cause (Gill et al., 1979; Bain 198; Ngowi et al., 2009). Gender differences have been noted in platelet counts and MPV with higher values in females than males (Table 4. 5) which concurs with other studies regardless of ethnicity (Tikly et al., 1987; Bain et al., 1996). This has been attributed to hormonal influences (Tikly et al., 1987).
A surgical patient require platelet counts of 50,000/mm³ and this criterion is based on western population whose values have proved to be higher than African values including this study. The difference in lower platelet parameters reference interval between the current study and other similar studies warrants consideration when interpreting platelet counts either for the patients or in clinical research. Generally the values obtained in this study are lower than the commonly used reference values which mean that these people could be referred as abnormal. Possible explanations for the variation of the results may include the criterion for the selection of study subjects which excluded those with abnormal results especially for the haemoglobin and the screening of some parasites in the current study which was not performed in other studies. Also there could have been bias in the selection of the study subjects where only those deemed health after a thorough medical examination were included in the study. These differences stress the need for reference interval values for a specific population.
5.2 Conclusions

1. There was variation among the data sets for haemoglobin, RBC count, HB, HCT, MCH and MCHC across gender and age groups with female indicating lower levels than the males. In addition, adolescents had increased levels than the adult for these parameters.

2. The WBC count, neutrophils, monocytes, and lymphocytes levels had lower levels compared to those on currently used guidelines with adolescents having higher reference values than the adults. However, the rest of the WBC parameters were within those that are currently used guidelines.

3. Platelet parameters varied across gender and age with females showing higher values than the males for both adults and adolescents.

5.3 Recommendations

1. The study showed low RBC parameters reference intervals indicating a need to review the cut-off like in blood transfusion. Also RI for adolescent were different from those of adults expressing a need to include them in clinical care.

2. The study also showed the need to adjust the WBC parameters reference intervals to suit the local population for their in patients management and in clinical trials.

3. The reference intervals for the platelet may also be adjusted for proper client management particularly in bleeding disorders where they may seem low and yet they are normal.

4. The endemic diseases, nutritional, genetic and environmental factors which were not factored in this study but are known to influence the haematological reference intervals be taken into account in future studies.
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World Health Organisation, 2013


APPENDICES

Appendix I: A map showing Nakuru County
Appendix II: Informed Consent

My name is Rose Nyambura Gachie. I am a PHD student from Kenyatta University. I am conducting a study on “Haematological reference intervals among adolescents and adults in Nakuru County, Kenya”. The samples will be analyzed at Rift Valley Provincial General Hospital (RVPGH) laboratory, Nakuru County. The information will be used by the Ministry of Health to improve diagnosis and management of haematological disorders in all the health facilities in Nakuru County.

Procedures to be followed

Participation in this study requires that I ask you some questions and also examine you in order to establish your suitability for collection of blood for determination of haematological reference intervals. Blood samples will be taken from you for use in this study and the results will be communicated to you upon testing through the same channel. I will record the information from you in a questionnaire. You have the right to refuse participation in this study and your decision will not change the care you will receive from any health facility in Nakuru County at other time.

Please remember that participation in the study is voluntary. You may ask questions related to study at any time. You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from Rift Valley Provincial Hospital or any other organization now or in the future.
Discomforts and risks

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. The interview may end approximately half an hour and you may also stop the interview at any time you may wish to do so. You may experience slight pain, swelling (hematoma), or slight dizziness after the blood is drawn which will soon disappear. However if this happens there will be a medical personnel to take care of them or any other discomfort. These are not serious and they will not have any consequences in future.

Benefits

If you participate in this study, you will benefit from being screened for any haematological disorder or blood born infections and if you are found to have any problem, you will be referred for treatment and management accordingly. Your results and the results from other participants will also provide us with important information that may assist in the proper diagnosis and management of patients with haematological disorders. The results from this study may also be utilized by the Ministry of health for the screening of the entire community for haematological disorders in order to maintain a health population in the County.

Reward

If you agree to participate in this study, no rewards will be given since it is a voluntary process. However at the end of the bleeding procedure, a refresher such as soft drinks or milk will be provided and bus fair not exceeding Ksh 300 will be refunded upon production of a receipt.
Confidentiality

The interviews and examinations will be conducted in a private setting at a chosen venue. Your name will not be recorded on the questionnaire. The questionnaires will be kept in a locked cabinet for safe keeping at Kenyatta University and everything will be kept private.

Contact information

If you any questions you may contact Dr. Tom Were 1. On 0720326127 or Dr. Stanley Waithaka 0722362719 or the Kenyatta University Ethical Review Committee Secretariat on kuerc@ku.ac.ke.

The consent form

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. The participation in this study is entirely voluntary. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and any decision will not change the care to be received from any health facility in Nakuru County today or from any other hospitals at any other time.

Name of Participant…………………………………………………..

____________________  __________________________
Signature or Thumb print       Date
Appendix III: The assent form for the minor and consent form (for parents/guardian)

The above information regarding my son’s/daughter’s participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. The participation in this study is entirely voluntary. I understand that my son/my daughter will still get the same care and medical treatment whether she/he decide to leave the study or not and any decision will not change the care to be received from any health facility in Nakuru County or from any other hospitals at any other time.

Name of guardian/parent…………………………………………………

Signature or Thumb print  Date

The assent form (for the minor)

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. The participation in this study is entirely voluntary. I understand that i will still get the same care and medical treatment whether s i decide to leave the study or not and any decision will not change the care to be received from any health facility in Nakuru County or from any other hospitals at any other time.

Name of minor…………………………………………………..

Signature or Thumb print  Date

Investigator’s Statement
I, the undersigned have explained to the volunteer/guardian in a language she/he understands, the procedures to be followed in the study and the risks and benefits involved.

Name of interviewer---------------------------------------------------------------

Interviewers signature--------------------------  Date-----------------------------
Appendix IV: Questionnaire

1. Sample ID-----------------------------

2. Name-----------------------------------

3. Telephone No------------------------

4. Age/years---------------------------

5. Sex----------------------------------

6. Height (Meters)-----------------------

7. Weight (Kilograms)---------------------

8. Ethnicity----------------------------

9. Religion-----------------------------

10. Occupation--------------------------

11. Duration of stay in Nakuru County (≥3 years)

12. Do you feel sick? Yes/No

13. Have you been sick recently? Yes/No If yes

14. When? And what was the condition of your sickness? -----------------------------

15. Are there any inherited health disorders in your family? Yes/No, if yes details?----

16. Are you taking any prescribed Medication? Yes/No, If yes

17. For what ailment ?-----------------------------------------------

18. Are you under a doctor’s care presently? Yes/ No?

19. If yes, details?-----------------------------------------------

20. Do you suffer from any of the following diseases? Answer yes or no

High blood pressure, diabetes mellitus Yes / No, any allergy, epilepsy, Stomach ulcers, tuberculosis and pneumonia
21. Do you use Tobacco? Yes/No

22. Do you smoke? yes or no

23. Do you drink alcoholic beverages? Yes/No

FOR WOMEN

24. Presently are you menstruating? Yes/No If yes, date of last period?------------------

25. Are you breast feeding? Yes/No

26. Are you pregnant? Yes/No
Appendix V: Bartlett’s table for the determination of minimum sample size

<table>
<thead>
<tr>
<th>Population size</th>
<th>Sample size</th>
<th>Continuous data (margin of error = .03)</th>
<th>Categorical data (margin of error = .05)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>alpha = .10</td>
<td>alpha = .05</td>
<td>alpha = .01</td>
</tr>
<tr>
<td>100</td>
<td>46</td>
<td>55</td>
<td>68</td>
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<td>200</td>
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<td>300</td>
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<td>900</td>
<td>76</td>
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<tr>
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<td>77</td>
<td>106</td>
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</tr>
<tr>
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<td>79</td>
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<td>183</td>
</tr>
<tr>
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<td>83</td>
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</tr>
<tr>
<td>4,000</td>
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</tr>
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<td>83</td>
<td>119</td>
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</tr>
<tr>
<td>8,000</td>
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<td>119</td>
<td>209</td>
</tr>
<tr>
<td>10,000</td>
<td>83</td>
<td>119</td>
<td>209</td>
</tr>
</tbody>
</table>

NOTE: The margins of error used in the table were .03 for continuous data and .05 for categorical data. Researchers may use this table if the margin of error shown is appropriate for their study; however, the appropriate sample size must be calculated if these error rates are not appropriate. Table developed by Bartlett, Kotrlik, & Higgins.
### Appendix VI: Internal Quality Control values for haematological indices

<table>
<thead>
<tr>
<th>Parameter (unit)</th>
<th>Assigned QC values</th>
<th>Study QC values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>session</td>
<td>Mean</td>
</tr>
<tr>
<td>RBC, 10^{12}/l</td>
<td>22</td>
<td>4.12-4.53</td>
</tr>
<tr>
<td>HGB, g/dl</td>
<td>22</td>
<td>12.9-13.9</td>
</tr>
<tr>
<td>HCT, %</td>
<td>22</td>
<td>39.2-45.2</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>22</td>
<td>84.5-94.5</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>22</td>
<td>27.0-30.0</td>
</tr>
<tr>
<td>MCHC, g/dl</td>
<td>22</td>
<td>28.8-34.8</td>
</tr>
<tr>
<td>RDW %</td>
<td>22</td>
<td>12.4-20.4</td>
</tr>
<tr>
<td>WBC ×10^9/l</td>
<td>22</td>
<td>7.50-8.70</td>
</tr>
<tr>
<td>Mono×10^9/l</td>
<td>22</td>
<td>0.00-0.50</td>
</tr>
<tr>
<td>Neu ×10^9/l</td>
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<td>3.60-6.20</td>
</tr>
<tr>
<td>Eos ×10^9/l</td>
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<td>0.00-0.20</td>
</tr>
<tr>
<td>Baso ×10^9/l</td>
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<td>25.3-35.3</td>
</tr>
<tr>
<td>Lym diff, %</td>
<td>22</td>
<td>201-261</td>
</tr>
<tr>
<td>PLT 10^9/l</td>
<td>22</td>
<td>6.5-8.6</td>
</tr>
<tr>
<td>PDW, (fl</td>
<td>22</td>
<td>34.9-44.9</td>
</tr>
</tbody>
</table>
Appendix VII

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

NACOSTI/P/14/7330/4179

Rose Nyambura Gachie
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on "Haematological reference intervals among adolescents and adults in Nakuru County, Kenya," I am pleased to inform you that you have been authorized to undertake research in Nakuru County for a period ending 1st December, 2015.

You are advised to report the County Commissioner and the County Director of Education, Nakuru County before embarking on the research project.

On completion of the research, you are required to submit two hard copies and one soft copy in pdf of the research report/thesis to our office.

Said Hussein
FOR: DIRECTOR-GENERAL/CEO

Copy to:
The County Commissioner
Nakuru County.

The County Director of Education
Nakuru County.

Date:
8th January, 2015
Appendix VIII

THIS IS TO CERTIFY THAT:
MS. ROSE NYAMBURA GACHIE
of KENYATTA UNIVERSITY, 0-20100
NAKURU, has been permitted to conduct
research in Nakuru County

on the topic: HAEMATOLOGICAL
REFERENCE INTERVALS AMONG
ADOLESCENTS AND ADULTS IN NAKURU
COUNTY, KENYA

for the period ending:
1st December, 2015

Applicant’s Signature

Permit No. : NACOSTI/P/14/7330/4179
Date Of Issue : 8th January, 2015
Fee Received : Ksh 2,000

Secretary
National Commission for Science,
Technology & Innovation

CONDITIONS

1. You must report to the County Commissioner and
   the County Education Officer of the area before
   embarking on your research. Failure to do that
   may lead to the cancellation of your permit.
2. Government Officers will not be interviewed
   without prior appointment.
3. No questionnaire will be used unless it has been
   approved.
4. Excavation, filming and collection of biological
   specimens are subject to further permission from
   the relevant Government Ministries.
5. You are required to submit at least two (2) hard
   copies and one (1) soft copy of your final report.
6. The Government of Kenya reserves the right to
   modify the conditions of this permit including
   its cancellation without notice.

REPUBLIC OF KENYA

National Commission for Science,
Technology and Innovation

RESEARCH CLEARANCE
PERMIT

Serial No. A

CONDITIONS: see back page
Appendix IX

REPUBLIC OF KENYA
NAKURU COUNTY GOVERNMENT
DEPARTMENT OF HEALTH SERVICES

CHIEF OFFICER, HEALTH SERVICES
NAKURU COUNTY
P.O. BOX 2060
NAKURU

Date: 22nd January, 2015.

Ref No: Gen/VOL.II/(383)

Rose Nyambura Gachie
Rift Valley Provincial General Hospital
P O Box 71
NAKURU

RE: PERMISSION TO CONDUCT HEMATOLOGICAL REFERENCE RESEARCH IN NAKURU COUNTY BY ROSE N. GACHIE

I received your request to conduct the above named research in Nakuru County as to the fulfillment of your PHD Study at Kenya University.

This office has gone through your research proposal and permission from the Ethics Review Committee of your Institution found it to be in order.

You are hereby granted the permission to proceed with the Study and share your findings with the County Department of Health.

[Signature]

Dr. Samuel Mwaura
Chief of Health Services
NAKURU COUNTY
Ref. No. C.C.IR.EDU 12/1/2 VOL.1/123

19TH January, 2015

Deputy County Commissioners,
NAKURU NORTH
NJORO
MOLO
RONGAI
GILGIL
SUBUKIA
KURESOI
NAIVASHA
NAKURU TOWN

RE: RESEARCH AUTHORIZATION - ROSE NYAMBURA GACHIE

The above named student has been given permission to carryout research on “Haematological reference intervals among adolescents and adults” in your Sub Counties.

Kindly give her the necessary assistance.

NDERITU T.W.
FOR: COUNTY COMMISSIONER
NAKURU COUNTY