ESTABLISHMENT OF ADULT REFERENCE INTERVALS FOR SELECTED BIOCHEMICAL ANALYTES IN ADULT RWANDAN POPULATION

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Establishment of Adult Reference Intervals for Selected Biochemical Analytes in Adult Rwandan Population

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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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DEDICATION

To all Rwandese blood donors.

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DEFINITION OF KEY TERMS

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

Reference population, a group consisting of all the reference individuals.

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

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

Reference distribution, the distribution of reference values.

Reference limit, a value derived from the reference distribution and used for descriptive purposes.

Reference interval, the interval between, and including, two reference limits.

Observed value, (patient laboratory test result), the value of a particular type of quantity, obtained by observation or measurement of a test subject (i.e., patient), to be compared with reference values, reference distributions, reference limits, or reference intervals.

LIST OF ABBREVIATIONS AND ACRONYNOMS

ALB: Albumin

ALP: Alkaline phosphatase

ALT: Alanine Aminotransferase

AST: Aspartate Aminotransferase

Bil-D: Bilirubin Direct

Bil-T: Bilirubin Total

Cl^-: Chloride ion

CREA: Creatinine

EPTRV: Expert Panel of Theory of Reference Values

GGT: Ɣ--glutamyl Aminotransferase

GLU: Glucose

ICSH: International Council for Standardization in Hematology

IFCC: International Federation of Clinical Chemistry

K^+: Potassium ion

Mg^{2+}: Magnesium ion

Na^+: Sodium ion

PO_4^{3-}: Phosphate ion

Rpm: Revolutions per minute

T-Prot: Total Protein

WHO: World Health Organization

ABSTRACT

Reference intervals are used to interpret laboratory reports in research and clinical settings. However, a small number of publications address reference intervals in Rwanda and they are limited in scope to some analytes and restricted geographically to some areas.

The aim of this study was to establish the reference values for Clinical chemistry analytes in selected Rwandan population. The present study was a cross-sectional prospective study that was carried out in National Center for Blood Transfusion, Rwanda and Kigali University Teaching Hospital, Laboratory Department, between 15th September and 10th December in 2014. 467 blood donors were enrolled and Clinical Chemistry tests were performed using Cobas Integra 400 Plus Chemistry analyzer. Median (Reference values: 2.5th and 97.5th percentiles) for male and female were, respectively: Direct Bilirubin 3.9 (2-6.9) and 3.9 (2.6-6.5) µmol/L; Total Bilirubin 10.3 (4.8-21.6) and 10.4 (5.9-17.3) µmol/L; Aspartate Aminotransferase, 27.8 (16.1-49.2) and 26.7 (16.8-45.1) U/L; Urea, 3.2 (1.3-5.8) and 3.1 (1.4-5.2) mmol/L; Glucose, 5.0 (3.2-7.7) and 4.6 (3.1-6.7) mmol/L; Total Proteins, 76.8 (68.2-87.7) and 76.9 (66.6-85.7) g/L; Albumin, 46.4 (39.7-55.5) and 46.7(40-54.5) g/L; Alanine Aminotransferase, 17.1 (7.2-36.2) and 16.0 (7.3-33.9) U/L; Ɣ-glutamyl transferase, 20.3 (8-75.6) and 21.1 (7.1-63.3) U/L; Alkaline Phosphatase, 74.3 (43.8-145.7) and 73.5 (50.3-135.4) U/L; Creatinine, 84.4 (65.2-107.1) and 81.1 (62.5-98.6) µmol/L; Sodium, 139.0 (134.5-145.5) and 141.0 (134.5-146.5) mmol/L; Potassium, .4.4 (3.7-5) and 4.3 (3.5-5.0) mmol/L; Chloride, 95.7 (89.9-104.2) and 99.3 (90.6-103.1) mmol/L; Magnesium, 0.9 (0.7-1.0) and 0.9 (0.7-1) mmol/L; Phosphate, 1.1 (0.8-1.5) and 1.2 (0.7-1.6) mmol/L. The results of this study on Clinical Chemistry parameters are similar to those published in other African countries, with variations due to the diet and geographical location.

Compared to other reference intervals established, reference values in our study presented remarkably low levels of urea which may be due to the diet low in proteins generally in Rwandan population. A large scale study is needed to establish local reference intervals.

CHAPTER ONE

INTRODUCTION

1.1. Background information

Health in our society has become a modern myth, a right for all, when health is difficult to define. According to World Health Organization (W.H.O.) “Health is a complete state of physical, mental or social well-being, not merely the absence of infirmity or disease.” (Siest *et al.*, 2013)

However, it is not possible to separate in the health concept, physical health and mental health, it is clear that the role of the Clinical Chemist is to know and appreciate, by biochemical equilibrium, the health status of a population but also of the individual. It is well-known that the biochemical information can, in many cases, tighten the first fruits of a pathophysiological condition, while the radiological findings, anatomical or clinical signs depend on lesions already established, and therefore late (Wane, 1985).

In health-related fields, a reference interval or reference interval describes the variations of a measurement or value in healthy individuals. It is a basis for a physician or other health professional to interpret a set of results for a particular patient (Saathoff *et al.*, 2008).

The standard definition for a reference interval basically originates in what is most prevalent in a reference group taken from the population. However, there are also optimal health intervals that appear to have the optimal health impact on people (Clinical and Laboratory Standard Institute [CLSI], 2008).

The standard definition of a reference interval for a particular measurement is defined as the prediction interval between which 95% of values of a reference group fall into, in such a way that 2.5% of a time a sample value will be less than the lower limit of this interval, and 2.5% of the time it will be larger than the upper limit of this interval, whatever the distribution of these values. Reference intervals that are given by this definition are sometimes referred to as standard ranges (CLSI, 2008).

Regarding the target population, if not otherwise specified, a standard reference intervals generally denotes the one in healthy individuals, or without any known condition that directly affects the intervals being established. These are likewise established using reference groups from the healthy population, and are sometimes termed normal, which may not be appropriate as not everyone outside the interval is abnormal, and people who have a particular condition may still fall within this interval (Waithaka *et al.*, 2009).

However, reference intervals may also be established by taking samples from the whole population, with or without diseases and conditions. Preferably, there should be specific reference intervals for each sub-group of the population that has any factor that affects the measurement, such as, specific intervals for each sex, age group, race or any other general determinant.

Preventive interventions for HIV, tuberculosis and malaria are being increasingly conducted in Africa and thus increasing the need of straddling the research infrastructure. In order to accurately assess what is healthy, reference intervals for standard laboratory tests in the target population are necessary (Karita *et al.*, 2009).

Common practice in Rwanda, both in hospitals and research laboratories is to use manufacturer's intervals for a given clinical laboratory assay system. Many of these assay systems are procured from Europe or the United States and use reference values based on their populations, which may not be representative of the Rwandan population. Numerous publications describe differences between clinical reference intervals in African populations compared to industrialized countries (Dosoo *et al.,* 2012; Eller *et al.,* 2008; J. B. Gahutu, 2013; J. Gahutu & Wane, 2006; Karita *et al.,* 2009; Kibaya *et al.,* 2008).

However, the use of improper reference intervals may falsely exclude otherwise volunteers who are eligible to participate in the research making the process of enrolment and execution more challenging (Eller *et al.,* 2008; Karita *et al.,* 2009). On the other hand, as the biological and environmental characteristics vary between populations, it is imperative to establish local reference intervals for clinical laboratories, which makes it possible to judiciously interpret laboratory results using reference intervals obtained from the local population and in the same environmental background (Eller *et al.,* 2008).

There is evidence that for some analytes and conditions, biological variability (BV) seen in healthy individuals mirrors that seen in sick individuals. Thus most BV data is collected in healthy subjects and then applied to unhealthy individuals. This is still a moot point, especially in setting of seriously ill individual where entirely different regulatory mechanisms may be operating (Badrick *et al.,* 2005).

The present study, therefore, aims to fill this gap by studying a set of serum biochemical parameters, chosen among the most relevant and the most requested by clinicians, namely: electrolytes among them Sodium (Na^+), Potassium (K^+), and Chloride (Cl^-), proteins among them Albumin (Alb) and Total Proteins (TP), Creatinine (Crea), Phosphate (PO_3^{2-}), Alanine

Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP), Ɣ-glutamyl Transferase (GGT), Total Bilirubin (T-Bil), Direct Bilirubin (D-Bil) and Glucose (Glu).

1.2. Statement of the Problem

Most clinical laboratories in developed countries are advanced in establishing reference intervals for laboratory parameters from their own local population. These reference intervals have been widely published in scientific journals (Sluss *et al.,* 2008), medical textbooks (Ichihara *et al.,* 2008) and internet. However little amount of literature exists on the reference intervals in Rwanda (Karita *et al.,* 2009; Gahutu & Wane, 2006, Gahutu, 2013), Furthermore, these are limited to hematologic and biochemical parameters and geographically to some areas in Rwanda.

Additionally most hospitals and research laboratories use the manufacturer reference interval which is not recommended. This study therefore aims at establishing the reference intervals for selected biochemical parameters in adult Rwandan population.

1.3. Justification of the Study

Clinical chemistry parameters normally show intra- and inter-individual variations. Intra-individual variations are due to changes over a long time, such as the circadian rhythm. Inter-individual variations are related to physiological differences linked to sex, age, and body mass index. Another source of environmental variation, particularly altitude, diet and geochemical. All those factors are known to influence the clinical laboratory parameters (Dosoo *et al.,* 2012; Gahutu, 2013; Zeh *et al.,* 2011).

Among populations biological and environmental characteristics vary, this makes it important to determine local reference intervals so that laboratory results can be interpreted using reference

intervals obtained from the local population living in the same environmental conditions (Karita *et al.,* 2009). It is therefore recommended that clinical laboratories should establish reference intervals for laboratory parameters from their own local population (CLSI, 2008). Only a few studies have addressed reference intervals in Rwanda (J. Gahutu & Wane, 2006; Karita *et al.,* 2009), and are limited in scope .

1.4. *Reference intervals in Rwanda.* Research Questions

1. What are the reference intervals for glucose, total protein, albumin, Alanine aminotransferase, Ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population?
2. Is there a gender based significant difference in the established reference intervals for glucose, total protein, albumin, Alanine aminotransferase, Ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population?
3. Is there an age based significant difference in the established reference intervals for glucose, total protein, albumin, Alanine aminotransferase, Ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population?
4. Are the established reference intervals for glucose, total protein, albumin, Alanine aminotransferase, Ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population different from those reported in the literature?

1.5. Objectives

1.5.1. General Objective

To establish the gender and age specific reference intervals for glucose, total protein, albumin, Alanine aminotransferase, ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population.

1.5.2. Specific Objectives

1. To establish reference intervals for glucose, total protein, albumin, Alanine aminotransferase, ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population.
2. To determine whether there are gender specific significant differences in the established reference intervals for glucose, total protein, albumin, Alanine aminotransferase, ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population.
3. To determine whether there are age specific significant differences in the established reference intervals for glucose, total protein, albumin, Alanine aminotransferase, ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population.
4. To determine whether the established reference intervals for glucose, total protein, albumin, Alanine aminotransferase, ɣ-glutamyltransferase, alkaline phosphatase, creatinine, sodium, potassium, chloride, magnesium, and phosphate in healthy adult Rwandan population differ from those reported in the literature.

CHAPTER TWO

LITERATURE REVIEW

2.1. Use of reference interval

Reference intervals are a set of values of a measured quantity of analytes obtained from a group of individuals or an individual in a defined state of health. They are very useful in the interpretation of laboratory data. A clinician or a researcher compares the given data from measured analytes with some reference intervals (Wimberley *et al.,* 1989). According to the Clinical and Laboratory Standards Institute (CLSI) and International Federation for Clinical Chemistry (IFCC) reference intervals are constructed from 95% of the healthy population, and recommend that each Laboratory establishes its reference values (Ichihara *et al.,* 2008).

A measured or observed laboratory test result from a person (usually a patient) is compared with a reference interval for the purpose of making a medical diagnosis, therapeutic management decision, or other physiological assessment. The interpretation of clinical laboratory data is, therefore, a comparative decision-making process. For this decision-making process to occur, reference values are needed for all tests in the clinical laboratory, and the provision of reliable reference intervals is an important task for clinical laboratories and diagnostic test manufacturers. The reference values most commonly used (known as "normal values" and sometimes "expected values") have traditionally been poorly defined and certainly not determined by a uniform process. It is now apparent that it is important to develop reference intervals using a more systematic process that takes into account the various influences on the measured laboratory test results (Lehmann *et al.,* 1989).

There are two types of reference intervals namely: the group based and subjects based reference intervals. In the group based reference intervals, the clinician estimates the probability that the subject (patient) from whom the given laboratory report was obtained belongs to a group of healthy subjects from whom the reference intervals were obtained. Therefore, group-based reference interval is used in the interpretation of laboratory report (CLSI, 2008). If the reference values are from the same subject when he/she was in a defined state of health, the clinician compares the present value with the past values to estimate whether the subject is still in the same state of health (Jan S. *et al.,* 2002).

Proper interpretation of quantitative laboratory results is based on the use of reference intervals established from healthy individuals. Several factors such as gender, age, sample type, analytical procedure, instruments and geographical location of the healthy individuals are known to influence the clinical laboratory parameters (Leclercq *et al.,* 1999). It is therefore recommended that clinical laboratories establish their own reference intervals for laboratory parameters based on healthy population.

In the developing countries, especially in Africa, the situation is changing since some regions reported reference intervals in scientific journals. These regions include Tanzania, Kenya, Central African Republic, Democratic Republic of Congo, Ethiopia and Ghana (Dosoo *et al.,* 2012; Eller *et al.,* 2008; J. B. Gahutu, 2013; Kibaya *et al.,* 2008).

Two major problems are encountered in establishing reliable reference intervals from a group of healthy subjects. One is involved in obtaining specimens from a sufficiently large number of healthy subjects of which CLSI (2008) recommended a minimum of 120 healthy subjects. The other obstacle is to ascertain that the factors involved in the preparation of the subjects and the

analytical procedures, noted during the establishment of reference values are the same factors present during day to day routine analysis of patient specimen .

The variability of the laboratory results is well known in healthy humans or diseased (NCCLS, 2000). The sources of variation in healthy are numerous and are classified differently. A commonly accepted practice identifies two main groups of variations: The analytical variation and biological variation (Wane, 1985).

2.2. Commonly analyzed biochemical analytes

2.2.1. Total protein

Total protein is found in body fluids, that is, serum, cerebral spinal fluid, and urine. All protein molecules are pure polypeptide chains containing on average 16% by weight of nitrogen while the other contain non amino acids, e.g., lipids, carbohydrates and vitamins. Proteins are useful in proper functioning of body cells and enzymes (Wendy & Brickell, 2007). Functions of the protein are transportation of hormones, drugs, vitamins, lipids, and regulation of extra cellular fluids. Protein loss is mostly via glomerulus, but it is restricted by the size of the pore and the negative charge of the protein molecules.

2.2.2. Albumin

This is a protein specifically made by the liver and is easily and cheaply analyzed in the laboratory. It constitutes of 60 % total protein and globulins. Decreased levels of albumin are found in chronic liver disease (cirrhosis) and also nephritic syndrome where it is lost in urine. Low albumin results in oedema since the intravascular oncotic pressure is higher than the

pressure in extravascular space. Albumin diffuses through damaged membrane and is filtered by the kidney because of its molecular size of 65000 kD (Nagai *et al.,* 2016).

Serum albumin is mainly measured to investigate liver diseases protein energy malnutrition, disorders of water balance, nephritic syndrome, and protein losing gastrointestinal diseases.

2.2.3. Aspartate aminotransferase

A large amount of aspartate aminotransferase is found in liver, kidney, cardiac muscles and skeletal muscles. Elevated values of aspartate aminotransferase are associated with liver damage (Bishop *et al.,* 2013). After the liver cell damage, 24-36 hours are needed for a marked elevation to be noted, and the elevation takes 3-7 days to normalize. Aspartate aminotransferase to *alanine* aminotransferase ratio values are useful in differentiating liver damage causes. Increase in aspartate aminotransferase alone is not specific to liver damage since the enzyme can be released from other organs (Sohn *et al.,* 2013).

2.2.4. Alanine aminotransferase

Most alanine aminotransferase is found in liver with small amount found in other organs. Elevated levels are found in liver infected with hepatitis. The alanine aminotransferase levels in serum and plasma are increased before any presentation (Sull *et al.,* 2009). Some drugs such as lipid reducing and anti-diabetic drugs elevates the *a*lanine aminotransferase levels thus the need to monitor them before making any decision in the laboratory (Sorbi *et al.,* 1999).

2.2.5. Bilirubin

Bilirubin is formed from the breakdown of erythrocytes and other haem-containing proteins such as myoglobin and cytochromes. The haem (iron porphyrin) of the hemoglobin molecule is

separated from the globin and the haem is converted mainly in the spleen to biliverdin which is reduced to bilirubin. This bilirubin is referred to as unconjugated (indirect) bilirubin. It is not soluble in water and cannot be excreted in the urine. It is bound to albumin and transported in the blood to the liver. In the liver cells; the enzyme glucuronosyltransferase conjugates glucuronic acid to bilirubin forming bilirubin glucuronides (mainly diglucuronides) which is soluble and excreted in urine (Kozaki *et al.,* 1998).

This refers to both unconjugated and conjugated bilirubin. Conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several diseases, in which the bilirubin conjugation is impaired cause similar elevations of circulating unconjugated bilirubin. Bile tract obstruction or damage to hepatocellular structure also causes increases in levels of both direct and indirect bilirubin in the circulation (Shcherbinina, 2007).

2.2.6. Direct bilirubin

In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract**.** An increase in conjugated bilirubin is highly specific for disease of the liver or bile ducts. Hepatocellular injury or cholestasis is suspected when more than 50% of total bilirubin is conjugated bilirubin (Mohamadnejad *et al.,* 2003).

2.2.7. Alkaline phosphatase

Enzyme is mainly derived from the liver, bones, and in lesser amounts from intestines, placenta, kidneys and leukocytes. Where the liver has obstructive disease, alkaline phosphatase is the first enzyme to be elevated, while if the disease is due to damage of liver cells, the aminotransferase

will be markedly increased. This makes enzyme analysis useful to distinguish the disease condition, cholestatic and hepatocellular, respectively (Liu *et al.,* 2016).

Under normal conditions, the alkaline phosphatase is increased due to bone growth, healing of a bone that was broken or rickets disease. Germ cell tumors and inflammatory bowel (ulcerative colitis) disease also produce alkaline phosphate that leaks in to the blood stream.

2.2.8. Urea

Urea is the major nitrogen containing metabolic product of protein catabolism, accounting for more than 75% of non-protein eventually excreted. Urea cycle is used to synthesis urea from amino nitrogen derived from ammonia carried out by hepatic enzymes. The rate of synthesis depends on intake of exogenous nitrogen and endogenous protein catabolism. Up to 90% of urea is excreted by the kidney while the rest is eliminated via gastrointestinal tract. Urea and serum creatinine determinations aid in the differential diagnosis of the three types of azotemia: pre-renal, renal and post-renal. Elevations in blood urea nitrogen concentration are seen in inadequate renal perfusion, shock, diminished blood volume (pre-renal causes), chronic nephritis, nephlosclerosis, tubular necrosis, glomerular-nephritis (renal causes) and urinary tract obstruction (post-renal causes). Urea is used to detect any abnormality of the kidneys. Urea nitrogen in plasma and serum levels is shown to be higher in men than women (Raimann *et al.,* 2016).

2.2.9. Creatinine

Serum creatinine is an important indicator of renal health because it is an easily-measured by-product of muscle metabolism. Creatinine itself is an important biomolecule because it is a major by-product of energy usage in muscle, through biological system involving creatine,

phosphocreatine (creatine phosphate) and adenosine triphosphate. Creatine is synthesized in the liver from the methylation of glycocyamine. This then is transported through blood to the other organs, muscle, and brain where through phosphorylation, becomes the high energy compound phosphocreatine. Creatinine is filtered out of the blood by the kidneys and there is little or no tubular reabsorption of creatinine. In case of faulty filtering of the kidney, creatinine blood levels rise. Creatinine levels in blood and urine may be used to calculate the creatinine clearance, which reflects the glomerular filtration rate. The glomerular filtration rate is clinically important because it is a measurement of renal function (Xin *et al.*, 2004). Elevated blood creatinine level is observed with marked damage to functioning nephrons, this makes it unsuitable for detecting early-stage kidney disease (Wendy & Brickell, 2007).

2.2.10. Potassium

Potassium serum values are critical to normal physiology of the adrenal, heart, renal functions and the maintenance of blood and urine pH. Potassium is viewed in relation to the other electrolytes and can be classified as high or low levels of potassium that are associated with disorders such as renal dysfunction, metabolic acidosis, and bradycardia (Bakris *et al.*, 2015). Under functioning of adrenal cortex, diabetes, massive tissue destruction and respiratory dysfunction and low potassium level are as a result of diarrhea or vomiting, over function of the adrenal cortex, anemia, use of diuretics, paralysis, hypertension, metabolic alkalosis and anaerobic state (Krijthe *et al.*, 2013).

2.2.11. Sodium

Blood sodium level represents a balance between the sodium and water in the food and drinks consumed and the amount in urine. Small amounts of sodium are lost through stool and sweat.

Sodium is analyzed in case of recent injury, surgery, or serious illness, consumption of large or small amounts of salt or fluid, treatment with water pills (diuretics) or certain other medications such as aldosterone and giving of intravenous fluids (Terry, 1994).

Abnormal sodium levels are hypernatremia and hyponatremia. Hypernatremia occurs due to: Cushing syndrome, diabetes insipidus, hyperaldosteronism, increased fluid loss due to excessive sweating, diarrhea, use of diuretics, or burns, too much salt or sodium bicarbonate in the diet, use of certain medicines, including birth control pills, corticosteroids, laxatives, lithium, and non-steroidal ant-inflammatory drugs such as ibuprofen or naproxen. A lower than normal sodium occurs in case of Addison's disease, dehydration and vomiting (Bishop *et al.*, 2013).

2.2.12. Chloride

Chloride is a type of electrolyte that is needed by the human body for metabolism and keeping the body's acid-base balance. Chloride ions also have important physiological roles such as, in the central nervous system where the inhibitory action of glycine and some of the action of Gamma-Amino butyric acid relies on the entry of chloride ions into specific neurons (Wendy & Brickell, 2007). Also, the chloride-bicarbonate exchanger biological transport protein relies on the chloride ion to increase the blood's capacity of carbon-dioxide, in the form of the bicarbonate ion as the blood passes through oxygen-consuming capillary beds (Terry, 1994).

2.2.13. Inorganic phosphorous

Phosphorus is a major intracellular anion in mammals, of which 85% is in the skeleton in the hydroxyapatite phase and the remaining 15% is in soft tissues. Almost all of the phosphorus found in the extracellular fluid space is in the form of inorganic phosphate. The majority of the phosphate in the body is in the organic form as a complex with carbohydrates, lipids, and

proteins. Phosphorus is an essential element in the cellular structure, cytoplasm, and mitochondrion for enzymatic processes in glycolysis, ammoniagenesis and oxidative phosphorylation for energy from the formation of adenosine triphosphate from adenosine diphosphate. It also influences oxygen-carrying capacity of hemoglobin. The normal serum phosphorus concentration is 1.12-1.45 mmol/L and this fluctuates with age and it is lower in adults than in children (Wendy & Brickell, 2007).

Disorders of phosphorus are hyperphosphatemia and hypophosphatemia. These are caused by cellular shifts of phosphate. Phosphate dysfunction is as a result of dietary intake, gastro intestinal and renal status. Hyperphosphatemia occurs in the presence of renal insufficiency with a decrease in phosphate excretion. In chronic renal failure, hyperphosphatemia is seen when the glomeruli filtrate rate has fallen below 25 ml/min.

Hypophosphatemia occurs when the serum concentration drops to less than 2.5 mg/dl. Phosphorus depletion is characterized by reduction in total body phosphorus stores. However, hypophosphatemia (mild, moderate, or severe) can occur without phosphate depletion. The common causes of hypophosphatemia are transcellular shift of phosphorus (from extracellular volume to either soft tissues or bones) increased phosphate excretion, poor dietary intake and diarrhea. Deficiency of vitamin D will lead to rickets in childhood and osteomalacia in adults with poor phosphate absorption (Goździalska *et al.*, 2016).

2.2.14. Ɣ-glutamyl transferase

Gamma-glutamyl transferase is found in the cell membranes of many tissues, such the kidneys, bile duct, pancreas, gallbladder, spleen, heart, brain, and seminal vesicles. Ɣ-glutamyl transferase

is involved in the transfer of amino acids across the cellular membrane and leukotriene metabolism.

High levels of serum Ɣ-glutamyl transferase activity are found in diseases of the liver, biliary system, and pancreas. In this respect, similar to alkaline phosphatase is useful in detecting disease of the biliary tract. The two markers correlate well, though there is conflicting data about whether Ɣ-glutamyl transferase has better sensitivity, but generally, alkaline phosphatase is the first test for biliary disease (Williams *et al.,* 2016). The main value of Ɣ-glutamyl transferase is verifying that alkaline phosphate elevations are due to biliary disease.

The enzyme Ɣ-glutamyl transferase is also elevated by large quantities of alcohol ingestion. Isolated elevation or disproportionate elevation compared to other liver enzymes (such as alkaline phosphatase or alanine amino transferase) may indicate alcohol abuse or alcohol liver disease. Determination of total serum Ɣ-glutamyl transferase activity alone is not specific to alcohol intoxication. Where Ɣ-glutamyl transferase alone is found elevated, this indicates excess alcohol consumption of up to 3 or 4 weeks prior to the test. Drugs such as barbiturates and phenitoin, congestive heart failure may also result to increase in Ɣ-glutamyl transferase (Wang *et al.,* 2016).

2.3. Quality control assessment

Quality control involves all the procedures that are followed from collection of specimen, analysis, reporting, and dispatch of the results. This is essential to countercheck the quality of test performed. This in return assists in detection and ratification of different procedures that are responsible for the errors (Jaafar *et al.,* 2010). External quality control is performed once in a

while where the control material is from outside the laboratory setting from where the values are performed and these compared with what is received from the laboratory after using the same sample material which usually is lyophilized (Kubono, 2004). In this study, internal quality control was followed to the letter that is proper collection of blood samples, to avoid hemolysis, transportation from the field to the laboratory in a cooled ice box, separation of the serum, refrigeration and the standard operating procedure on analysis.

Analytical stages cover the principle of the test method, reagents, standard control material and equipment used. Quality control (internal and external) procedures are used to detect and minimize errors in the performance of tests. This in turn ensures the results are reliable, precise and accurate (Cheesbrough, 2009). Control is evaluated by use of quality control chart that (Levy Jennings control chart) was prepared daily. Control values within ± 2 SD is a good sign and the results that are produced are reliable and therefore can be reported with confidence.

Developing countries have been found to have problems in issuing accurate results. This has necessitated the building of reliable quality control systems in order to report excellent results assured of quality and proper documentation of test carried out on quality control material (Jaafar *et al.*, 2010).

Reliability and reproducibility of laboratory results have been found to be affected by the environment, laboratory materials, specimen handling, personnel, test methods, equipment, reading and reporting. To check on the above, internal and external quality procedures are employed (Albetkova et al., 2011). External quality control is an objective system of assessing laboratory ability to produce reliable results. Participation of external quality assessments should always be regarded as additional to internal quality control (Wendy & Brickell, 2007).

CHAPTER THREE

METHODOLOGY

3.1. Study area

Samples used in this study were collected from the following sites in Rwanda: Central Province (Kigali), Northern Province, Southern Province, Eastern Province, and Western Province.

Figure 3. 1: Rwanda Google map accessed on 13 September, 2014

3.2. Study design

This was a cross sectional prospective study.

3.3. Study population

Blood donors were recruited in rural area, schools and urban cities in five provinces from September to December 2014.

3.3.1. Inclusion criteria

1. To be of Rwandese nationality;
2. To be in good physical and mental health;
3. To have no history of disease in the preceding six months;
4. Not been on any medication in the preceding six months;
5. To have been in the region for at least the preceding six months;
6. To be between 17 to 60 years of age;
7. To belong to either male or female gender.

3.3.2. Exclusion criteria

1. To have had any disease in the preceding six months;
2. To be in menses period for females;
3. To be using hormonal contraception;
4. Lactating women;
5. Pregnant women.

3.4. Sampling procedure

Four hundred and sixty seven blood donors, 93 in Northern, Southern, Western and Eastern provinces each, and 95 in Central province, all in rural areas, schools and urban cities were sampled for this study. Each blood donor who fulfilled all criteria (Appendix 1, 2, 3) as set by NCBT was given a questionnaire and a consent form for participation in this study (Appendix 5, 6).

Two blood specimens were withdrawn from each donor in a plain and Sodium Fluoride tubes as set in the Standard Operating procedure (SOP) (Appendix 4).

3.5. Sample collection

Whole blood was collected from blood donors into two tubes, on the occasion of blood donation, the donor being in supine position and at complete physical rest (Appendix 4). Phlebotomists were trained and explained clearly about sample requirements.

3.6. Laboratory procedures

3.6.1. Specimens type

Whole blood was collected into two tubes, in 5 ml plain tube without anticoagulant for serum chemistry and in Sodium fluoride tube specifically for blood glucose levels determination.

3.6.2. Specimens handling and storage

Specimens after collection were allowed to clot at room temperature and put in a cool box for transportation. Blood cells were separated from serum and put in code labelled serum vials, and

analyzed within 10 hours. Those which were not analyzed were kept in a fridge at 4 to 8^{o}C and analyzed the following day.

3.6.3. Analysis of specimens

Laboratory analysis was performed at Kigali University Teaching Hospital, in Clinical chemistry service, using Cobas Integra 400 Plus, a chemistry analyzer (Roche Diagnostics Ltd, Switzerland). Cobas Integra 400 Plus is a chemistry analyzer using four measuring methods, absorbance photometry for measuring enzymes and substrates, turbidimetry for measuring specific proteins and drugs of abuse, fluorescence polarimetry for measuring therapeutic drugs and thyroid tests, Ion-Selective Electrode potentiometry for measuring Sodium, Potassium, Chloride and Lithium. The same analyzer was used to measure blood concentrations for all the analytes in this study. All tests were done according to the laboratory SOPs, equipment and reagent manufacturer's instructions.

3.6.4. Methods used for analytes measurement

a. Direct Bilirubin

Diazo method

Conjugated bilirubin and δ-bilirubin (direct bilirubin) react directly with 3, 5-dichlorophenyl diazonium salt (R1) in acid buffer (SR) to form the red-colored azobilirubin.

Bilirubin + 3, 5-DPD $\longrightarrow$ azobilirubin

The color intensity of the red azo dye formed is directly proportional to the direct (conjugated) bilirubin concentration and can be determined photometrically. It is determined by measuring the absorbance at 552/659 nm (Roche company, 2013).

Pipetting parameters

		Diluent (H2O)
R1	120 µL	–
SR	24 µL	–
Sample	7 µL	2 µL
Total volume	153 µL	

b. Total Bilirubin

Colorimetric assay

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with a diazonium ion (R1) in a strongly acidic medium (SR).

Bilirubin + diazonium ion $\xrightarrow{\text{acid}}$ azobilirubin

The color intensity of the red azo-dye formed is directly proportional to the total bilirubin in the sample and can be determined photometrically. It is determined by measuring the absorbance at 552/659 nm (Roche company, 2014).

Pipetting parameters

		Diluent (H2O)
R1	110 µL	0
SR	24 µL	0
Sample	2 µL	8 µL
Total volume	144 µL	

c. Albumin

Colorimetric assay with endpoint method

At a pH of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromocresol green (BCG) R1, an anionic dye (SR), to form a blue-green complex.

Albumin + BCG $\xrightarrow{\text{pH 4.1}}$ Albumin-BCG complex

The color intensity of the blue-green color is directly proportional to the albumin concentration in the sample. It is determined by monitoring the increase in absorbance at 583 nm (Roche company, 2011).

Pipetting parameters

		Diluent (H2O)
R1	100 µL	0
SR	20 µL	10
Sample	2 µL	20 µL
Total volume	152µL	

d. Total Protein

Colorimetric assay

Divalent copper (R1) reacts in alkaline solution with protein peptide bonds to form the characteristic purple-colored biuret complex. Sodium potassium tartrate (SR) prevents the precipitation of copper. Hydroxide and potassium iodide prevents auto-reduction of copper.

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{Alkaline pH}} \text{Cu-protein complex}$$

The color intensity is directly proportional to the protein concentration. It is determined by measuring the increase in absorbance at 552 nm (Roche company, 2012).

Pipetting parameters

		Diluent (H2O)
R1	90 µL	0 µL
SR	32 µL	0 µL
Sample	2 µL	28 µL
Total volume	152µL	

e. Alanine aminotransferase

Method according to the International Federation of Clinical Chemistry (IFCC), with pyridoxal-5'-phosphate.

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate (R1). The pyruvate formed is reduced by NADH in a reaction catalyzed by lactate dehydrogenase (LDH) to form L-lactate and NAD^+. Pyridoxal phosphate (SR) serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

L-alanine + 2-oxoglutarate $\xrightarrow{ALT}$ pyruvate + L-glutamate

Pyruvate + NADH + H^+ $\xrightarrow{LDH}$ L-lactate + NAD^+

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance at 340 nm (Roche company, 2014).

Pipetting parameters

		Diluent (H2O)
R1	59 µL	10 µL
SR	32 µL	9µL
Special diluent(SD)	18 µL	0 µL
Sample	11µL	8 µL
Total volume	132µL	

f. Aspartate aminotransferase

Method according to the International Federation of Clinical Chemistry (IFCC), but without pyridoxal-5'-phosphate. AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate (R1) to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), SR, to form NAD^+.

L-Aspartate + 2-oxoglutarate $\xrightarrow{AST}$ oxaloacetate + L-glutamate

Oxaloacetate + NADH + H^+ $\xrightarrow{MDH}$ L-malate + NAD^+

The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance at 340 nm (Roche company, 2009).

Pipetting parameters

		Diluent (H2O)
R1	40 µL	29 µL
SR	17 µL	9µL
Sample	11µL	26 µL
Total volume	132µL	

g. Ɣ- glutamyltransferase

Enzymatic colorimetric assay

Ɣ-glutamyltransferase transfers the γ-glutamyl group of L-γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine (R1, SR).

L-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine $\xrightarrow{GGT}$ L-γ-glutamyl-glycylglycine + 5-amino-2-nitrobenzoate

The amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT activity in the sample. It is determined by measuring the increase in absorbance at 409 nm (Roche company, 2014).

Pipetting parameters

		Diluent (H2O)
R1	25 µL	35 µL
SR	20 µL	20 µL
Sample	3 µL	20 µL
Total volume	123µL	

h. Urea

Kinetic test with urease and glutamate dehydrogenase

Urea is hydrolyzed by urease to form ammonium and carbonate. In the second reaction 2-oxoglutarate reacts with ammonium in the presence of glutamate dehydrogenase (GLDH) and the coenzyme NADH (R) to produce L-glutamate. In this reaction two moles of NADH are oxidized to NAD^+ for each mole of urea hydrolyzed.

$$\text{Urea} + 2\ H_2O \xrightarrow{\text{Urease}} 2\ NH_4^+ + CO_3^{2-}$$

$$NH_4^+ + \text{2-oxoglutarate} + \text{NADH} \xrightarrow{\text{GLDH}} \text{L-glutamate} + \text{NAD+} + H_2O$$

The rate of decrease in the NADH concentration is directly proportional to the urea concentration in the specimen. It is determined by measuring the absorbance at 340 nm (Roche company, 2012).

Pipetting parameters

		Diluent (H2O)
R	50 µL	95 µL
Sample	2 µL	98 µL
Total volume	245µL	

i. Creatinine

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution (SR), creatinine forms a yellow-red complex with picrate (R1). The rate of dye formation is proportional to the creatinine concentration in the specimen.

$$\text{Creatinine} + \text{picric acid} \xrightarrow{\text{alkaline pH}} \text{yellow-red complex}$$

It is determined by measuring the absorbance at 512-831 nm (Roche company, 2015).

Pipetting parameters

		Diluent (H2O)
R1	13 µL	71 µL
Sample	10 µL	20 µL
SR	17 µL	16 µL
Total volume	147 µL	

j. Electrolytes (Na^+, K^+, Cl^-)

The electrolyte module uses flow-through ion selective electrodes and a reference electrode with an open liquid junction. Each electrode has a membrane or capillary that is sensitive to a particular type of ion (Roche company, 2015).

k. Magnesium

In alkaline solution (R2), magnesium forms a purple complex with xylidyl blue (R1), diazonium salt. The magnesium concentration is measured photometrically via the decrease in the xylidyl blue absorbance. It is determined by measuring the absorbance at 505-600 nm (Roche company, 2009).

Pipetting parameters

		Diluent (H2O)
R1	97 µL	0 µL
R2	97 µL	0 µL
Sample	3 µL	16 µL

l. Phosphate (Inorganic)

Endpoint method with sample blanking.

Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula $(NH_4)_3[PO_4\ (MoO_3)_{12}]$ with ammonium molybdate (R1) in the presence of sulfuric acid (SR).

Phosphate + ammonium molybdate $\xrightarrow{H_2SO_4}$ ammonium-phosphomolybdate

The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration. It is determined by measuring the increase in absorbance at 340 nm (Roche company, 2013).

Pipetting parameters

		Diluent (H2O)
R1	90 µL	0 µL
SR	38 µL	0 µL
Sample	2.5 µL	27.5 µL
Total volume	158 µL	

3.7. Data management and analysis

Socio-demographic and medical information was obtained from the questionnaires and included gender, age, demographic location, and results of transmissible disease from blood donors files. Results from the laboratory tests were entered into a hard cover register and a password protected Microsoft Excel database. Samples were identified by a unique study number for confidentiality.

All calculations to determine the reference values were based on Clinical and Laboratory Standards Institute/International Federation of Clinical Chemistry (CLSI/IFCC, the Former National Committee of Clinical Laboratory Standards) guidelines document on defining, establishing and verifying reference intervals in Clinical Laboratory (CLSI, 2008).

2.5^{th} and 97.5^{th} percentiles were calculated non-parametrically, after removing identified outliers in each subgroup by using Reed-Dixon method of identifying the outliers (Dixon, 2013; Reed et al., 1971). The extreme values were retained in the distribution if $D/R<0.33$, where D is the

absolute difference between the most extreme distribution and the next value and R is the interval (maximum-minimum), as recommended by CLSI/IFCC guidelines document.

Differences between genders and age groups were evaluated using Wilkoxon-Mann-Whitney test and when $p<0.05$, it was considered as a statistically significant difference between groups. All statistical analyses were carried out using SPSS v.20 (IBM Corporation, 2012).

Table 3.1: Literature based reference intervals.

ANALYTES	Gender	Reference intervals from Roche Diagnostic Company 2012 Cobas Integra 400 Plus	
METABOLISM		**Lower**	**Upper**
BIL-D (µmol/L)	Male	-	≤5.0
	Female	-	≤5.0
BIL-T (µmol/L)	Male	-	≤24.0
	Female	-	≤15.0
GLU (mmol/L)	Male	3.0	5.6
(Fasting)	Female	3.2	5.7
SERUM PROTEINS			
TP (g/L)	Male	66.0	87.0
	Female	*N/A.*	*N/A*
ALB (g/L)	Male	32.0	52.0
	Female	*N/A*	*N/A*
ENZYMES			
ALP (U/L)	Male	40.0	129.0
	Female	35.0	104.0
ALT (U/L)	Male	10.0	50.0
	Female	10.0	35.0
AST (U/L)	Male	0	40.0
	Female	0	32.0
GGT (U/L)	Male	8.0	61.0
	Female	5.0	36.0
KIDNEY FUNCTION			
CREA (µmol/L)	Male	44.0	80.0
	Female	62.0	106.0
UREA (mmol/L)	Male	2.8	8.1
	Female	*N/A*	*N/A*
ELECTROLYTES			
Na^+ (mmol/L)	Male	135.0	145.0
	Female	*N/A*	*N/A*
K^+ (mmol/L)	Male	3.5	5.5
	Female	*N/A*	*N/A*
Cl^- (mmol/L)	Male	95.0	105.0
	Female	*N/A*	*N/A*
Mg^+ (mmol/L)	Male	0.7	1.1
	Female	*N/A*	*N/A*
PO_3^{2-} (mmol/L)	Male	0.8	1.5
	Female	*N/A*	*N/A*

N/A: No data available

CHAPTER FOUR

RESULTS

4.1. Overview

The subjects used in the statistical analysis were 467, with 333 males and 134 females (representing 71.3 % and 28.7%, respectively), between the ages of 19 and 58 years, with a mean age of 32.6 years for males and 29 years for females.

Before analyzing the obtained data, outliers were removed as recommended by Reed and Dixon in CLSI essential guidelines for establishing reference intervals. The number of outliers removed is indicated in the brackets for males and females respectively as following: Albumin (0, 0), Alkaline Phosphatase (5, 1), Alanine Aminotransferase (1, 0), Aspartate Aminotransferase (1, 0), Creatinine (0, 0), γ-glutamyl transferase (13, 2), Magnesium (0, 0), Phosphate (6, 1), Total Proteins (1, 0), Urea (1, 0), Sodium (1, 0), Potassium (0, 0), Chloride (0, 0), Bilirubin Direct (0, 0), Bilirubin Total (0, 0), Glucose (0, 0).

The reference values were constructed using 2.5^{th} and 97.5^{th} percentiles as lower and upper limits at 95% confidence interval in accordance with CLSI guidelines for determining reference intervals. The medians for gender and age groups were statistically compared using Mann-Whitney test. $p< 0.05$ was considered statistically different.

4.2. Serum concentration according to sex of the participants

Gender difference for each analyte was determined to assess whether combined or gender-specific reference intervals should be established.

Tables 4.1, 4.2 and 4.3 show combined or sex specific reference values for each parameter based on the p-values for the difference between male and female participants. The Tables also indicate

the number of combined and sex specific participants used for determining the reference values for each parameter which were all above the minimum sample size (N = 120) suggested by CLSI (CLSI, 2008).

The serum concentration of Albumin (p=0.637), Alkaline Phosphatase (p=0.553), Alanine Aminotransferase (p=0.41), Aspartate Aminotransferase (p=0.242), Ɣ-glutamyl Transferase (p=0.726), Magnesium (p=0.204), Phosphate (p=0.216), Total Protein (p=0.595), Urea (p=0.435), Potassium (p=0.297), Bilirubin Direct (p=0.852), Bilirubin Total (p=0.396), Glucose (p=0.078) shown that there is no significant difference between gender, (p-> 0.05.

Results showed a statistically significant difference in gender, whereby males had significantly higher serum concentration values for creatinine of 65.2-107.1 µmol/L against 62.5-98.6 µmol/L for females (p< 0.001), serum concentration of sodium higher in females with 134.5-146.5 mmol/L against 134.5-145.5 for males (p<0.013) and serum concentration of chloride higher in females with 90.6-103.1 mmol/L against 89.9-104.2 for males (p<0.028).

4.3. Serum concentration according to the age group of the participants

The age groups were made in two different groups, participants below 30 years, and participants above 30 years.

Comparing age groups, subjects aged below 30 years had a significantly lower serum concentration values of total proteins of 62.9-86.5 g/L against 68.6-87.7 g/L for subjects aged above 30 years (p<0.002), serum concentration of albumin of 37.1-55.2 g/L against 40.9-55.5 for subjects aged above 30 years (p<0.022), serum concentration of alkaline phosphatase of 43.3-133.1 U/L against 44.6-152.0 U/L for subjects aged above 30 years (p<0.001), serum concentration of sodium of 134.5-146.0 mmol/L against 134.5-146.5 mmol/L for subjects aged

above 30 years ($p<0.015$), serum concentration of bilirubin Direct of 1.8-7.0 g/L against 2.3-6.8 µmol/L for subjects aged above 30 years ($p<0.025$).

The serum concentration of alanine aminotransferase ($p=0.878$), aspartate aminotransferase ($p=0.178$), creatinine ($p=0.397$), Ɣ-glutamyltransferase ($p=0.123$), magnesium ($p=0.603$), phosphate ($p=0.389$), urea ($p=0.283$), potassium ($p=0.154$), chloride ($p=0.102$), bilirubin total ($p=0.145$) and glucose ($p=0.089$) showed that there is no statistically significant effect of the age, since the *p*-values are greater than 0.05.

Tables 4.4., 4.5., and 4.6. show combined or age specific reference values for each parameter based on the p-values for the difference between participants with 30 years and below and participants with above 30 years. The Tables also indicate the number of combined and age specific participants used for determining the reference values for each parameter which were all above the minimum sample size (N = 120) suggested by CLSI (NCCLS, 2000).

Table 4.1: Renal and liver function tests reference intervals and gender differences

Percentiles are higher and lower limits of reference intervals. They are used to establish reference intervals used in interpretation of laboratory result. Table 4.1 indicates the established renal and liver function reference intervals for both genders while separate and combined. The values are given both the median and the percentiles. The number of subjects is indicated under the column labelled N. Reference interval is given as the interval between 2.5 and 97.5 of the percentile.

	Male				Female				All participants					
Analyte (Unit)			**Percentiles**				**Percentiles**				**Percentiles**			
	N	**Median**	**2.5th**	**97.5th**	**N**	**Median**	**2.5th**	**97.5th**	**N**	**Median**	**2.5th**	**97.5th**	**Z-Value**	**P-Value**
BIL-D (µmol/L)	333	3.9	2.0	6.9	134	3.9	2.6	6.5	467	4.0	2.1	6.9	-0.186	0.852
BIL-T (µmol/L)	333	10.3	4.8	21.6	134	10.4	5.9	17.3	467	10.4	5.0	21.6	-0.849	0.396
AST (U/L)	332	27.8	16.1	49.2	134	26.7	16.8	45.1	466	27.6	16.4	49.2	-1.169	0.242
UREA (mmol/L)	332	3.1	1.3	5.8	134	3.1	1.4	5.2	466	3.1	1.3	5.8	-0.781	0.435
GLU (mmol/L)	333	5.0	3.2	7.7	134	4.6	3.1	6.7	467	4.8	3.1	7.7	-1.760	0.078

Table 4.2: Liver function tests reference intervals in genders

Percentiles are higher and lower limits of reference intervals. They are used to establish reference intervals used in interpretation of laboratory result. Table 4.2 indicates the established liver function reference intervals for both genders while separate and combined. The values are given both the median and the percentiles. The number of subjects is indicated under the column labelled N. Reference interval is given as the interval between 2.5 and 97.5 of the percentile. Significant differences were considered at $p < 0.05$.

	Male				Female				All participants					
Analyte (Unit)			**Percentiles**				**Percentiles**				**Percentiles**			
	N	**Median**	**2.5th**	**97.5th**	**N**	**Median**	**2.5th**	**97.5th**	**N**	**Median**	**2.5th**	**97.5th**	**Z-Value**	**P-Value**
TP (g/L)	332	76.8	68.2	87.6	134	76.9	66.6	85.7	466	76.8	68.0	87.6	-0.532	0.595
ALB (g/L)	333	46.4	39.7	55.5	134	46.7	40.0	54.5	467	46.5	39.8	55.4	-0.472	0.637
ALT (U/L)	332	17.1	7.2	36.2	134	16.0	7.3	33.9	466	16.9	7.4	36.2	-0.824	0.41
GGT (U/L)	320	20.3	8.0	75.6	132	21.1	7.1	63.3	452	20.4	7.6	75.2	-0.350	0.726
ALP (U/L)	328	74.3	43.8	145.7	133	73.5	50.3	135.4	461	74.3	44.2	145.7	-0.593	0.553

Table 4.3: Renal function tests and electrolytes reference intervals and differences in genders

Percentiles are higher and lower limits of reference intervals. They are used to establish reference intervals used in interpretation of laboratory result. Table 4.3 indicates the established renal function and electrolytes reference intervals for both genders while separate and combined. The values are given both in the median and the percentiles. The number of subjects is indicated under the column labelled N. Reference interval is given as the interval between 2.5 and 97.5 of the percentile. The bolded values indicate significant difference between sexes at $p<0.05$ (Creatinine, Sodium and Chloride).

	Male				**Female**				**All participants**					
Analyte (Unit)			**Percentiles**				**Percentiles**				**Percentiles**			
	N	**Median**	**2.5th**	**97.5th**	**N**	**Median**	**2.5th**	**97.5th**	**N**	**Median**	**2.5th**	**97.5th**	**Z-Value**	**P-Value**
CREA (µmol/L)	333	**84.4**	**65.2**	**107.1**	134	**81.1**	**62.5**	**98.6**	467	**83.3**	**64.5**	**106.3**	**-3.258**	**0.001**
Na^+ (mmol/L)	332	**139.0**	**134.5**	**145.5**	134	**141.0**	**134.5**	**146.5**	466	**140.0**	**134.5**	**146.5**	**-2.472**	**0.013**
K^+ (mmol/L)	333	4.4	3.7	5.0	134	4.3	3.5	5.0	467	4.4	3.7	5.1	-1.044	0.297
Cl^- (mmol/L)	333	**95.7**	**89.9**	**104.2**	134	**99.3**	**90.6**	**103.1**	467	**97.5**	**90.3**	**104.1**	**-2.199**	**0.028**
Mg^+ (mmol/L)	333	0.9	0.7	1.0	134	0.9	0.7	1.0	467	0.9	0.7	1.0	-1.270	0.204
PO_3^{2-} (mmol/L)	327	1.1	0.8	1.5	133	1.2	0.7	1.6	460	1.2	0.8	1.6	-1.237	0.216

Table 4.4: Liver and renal function tests reference intervals and differences in age groups

Percentiles are higher and lower limits of reference intervals. They are used to establish reference intervals used in interpretation of laboratory result. Table 4.4 indicates the established liver and renal function tests reference intervals for both age groups while separate and combined. The values are given both in the median and the percentiles. The number of subjects is indicated under the column labelled N. Reference interval is given as the interval between 2.5 and 97.5 of the percentile. The bolded values indicate significant difference between sexes at $p<0.05$ (Bilirubin Direct).

	<= 30				31+				All participants					
Analyte (Unit)			**Percentiles**				**Percentiles**				**Percentiles**			
	N	**Median**	**2.5th**	**97.2th**	**N**	**Median**	**2.5th**	**97.2th**	**N**	**Median**	**2.5th**	**97.2th**	**Z-Value**	**P-Value**
BIL-D (µmol/L)	193	**3.6**	**1.8**	**7.0**	274	**3.9**	**2.3**	**6.8**	467	**4.0**	**2.1**	**6.9**	**-2.244**	**0.025**
BIL-T (µmol/L)	193	9.7	4.2	21.4	274	10.7	5.8	23.0	467	10.4	5.0	21.6	-1.458	0.145
UREA (mmol/L)	193	2.9	1.3	5.8	273	3.0	1.4	4.8	466	3.1	1.3	5.8	-1.075	0.283
AST (U/L)	192	26.7	17.1	45.6	274	28.3	16.1	49.3	466	27.6	16.4	49.2	-1.347	0.178
GLU (mmol/L)	193	4.7	3.1	6.7	274	4.8	2.6	8.2	467	4.8	3.1	7.7	-1.701	0.089

Table 4.5: Liver function tests reference intervals and differences in age groups

Percentiles are higher and lower limits of reference intervals. They are used to establish reference intervals used in interpretation of laboratory result. Table 4.5 indicates the established liver function reference intervals for both age groups while separate and combined. The values are given both in the median and the percentiles. The number of subjects is indicated under the column labelled N. Reference interval is given as the interval between 2.5 and 97.5 of the percentile. The bolded values indicate significance difference between sexes at $p<0.05$ (Total Proteins, Albumin, and Alkaline Phosphatase).

	<= 30				31+				All participants					
Analyte (Unit)			**Percentiles**				**Percentiles**				**Percentiles**			
	N	**Median**	**2.5th**	**97.2th**	**N**	**Median**	**2.5th**	**97.2th**	**N**	**Median**	**2.5th**	**97.2th**	**Z-Value**	**P-Value**
TP (g/L)	192	**75.9**	**62.9**	**86.5**	274	**77.4**	**68.6**	**87.7**	466	**76.8**	**68.0**	**87.6**	**-3.035**	**0.002**
ALB (g/L)	193	**45.9**	**37.1**	**55.2**	274	**46.8**	**40.9**	**55.5**	467	**46.5**	**39.8**	**55.4**	**-2.288**	**0.022**
ALT (U/L)	193	17.4	6.9	37.4	273	16.8	7.9	34.2	466	16.9	7.4	36.2	-0.153	0.878
GGT (U/L)	189	19.5	7.6	70.7	263	22.2	6.1	76.1	452	20.4	7.6	75.2	-1.542	0.123
ALP (U/L)	189	**68.8**	**43.3**	**133.1**	272	**76.6**	**44.6**	**152.0**	461	**74.3**	**44.2**	**145.7**	**-3.250**	**0.001**

Table 4.6: Renal function tests and electrolytes reference intervals and differences in age groups

Percentiles are higher and lower limit of reference intervals. They are used to establish reference intervals used in interpretation of laboratory result. Table 4.6 indicates the established renal function tests and electrolytes reference intervals for both age groups while separate and combined. The intervals are given as both the median and the percentiles. The number of subjects is indicated under the column labelled N. Reference interval is given as the interval between 2.5 and 97.5 of the percentile. The bolded values indicate significance difference between sexes. Significant difference is considered at $p < 0.05$. (Sodium).

	<= 30				31+				All participants					
Analyte (Unit)			**Percentiles**				**Percentiles**				**Percentiles**			
	N	**Median**	**2.5th**	**97.2th**	**N**	**Median**	**2.5th**	**97.2th**	**N**	**Median**	**2.5th**	**97.2th**	**Z-Value**	**P-Value**
CREA (µmol/L)	193	83.7	66.3	104.6	274	82.7	62.7	105.9	467	83.3	64.5	106.3	-0.846	0.397
Na^+ (mmol/L)	192	**139.0**	**134.5**	**146.0**	274	**141.0**	**134.5**	**146.5**	466	**140.0**	**134.5**	**146.5**	**-2.425**	**0.015**
K^+ (mmol/L)	193	4.3	3.6	5.1	274	4.4	3.7	5.1	467	4.4	3.7	5.1	-1.426	0.154
Cl^- (mmol/L)	193	95.4	89.2	103.5	274	98.0	90.5	105.1	467	97.5	90.3	104.1	-1.636	0.102
Mg+ (mmol/L)	193	0.9	0.7	1.0	274	0.9	0.7	1.0	467	0.9	0.7	1.0	-0.521	0.603
PO_3^{2-} (mmol/L)	190	1.2	0.7	1.5	270	1.2	0.8	1.2	460	1.2	0.8	1.6	-0.862	0.389

Table 4.7: Comparison of established reference intervals to those in literature

Analytes (Unit)	Gender	Established reference intervals		Northern province-Rwanda		Central Province-Rwanda		Central part of Ghana		Ugandan adult blood bank donors		Kenya		Case records of Massachusetts General Hospital		Mbeya, Tanzania	
		Lower	Upper	Lower	Upper	lower	upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
BIL-D (µmol/L)	Male	2.0	6.9	2.0	7.0	0.4	8.8	0.9	4.1	1.71	8.5	1.3	9.0	1.7	5.1	0.93	8.43
	Female	2.6	6.5	-	-	-	-	0.8	4.0	0.0	6.8	0.8	6.7	-	-	0.70	5.83
BIL-T (µmol/L)	Male	4.8	21.6	3.0	29.0	2.9	37.0	3.8	32.0	6.8	44.4	5.6	41.9	5.1	17	6.0	42.0
	Female	5.9	17.3	-	-	-	-	2.7	26.6	5.1	32.5	4.4	26.8	-	-	4.5	31.3
TP (g/L)	Male	68.2	87.6	65.0	85.0	58.0	88.0	46.7	86.4	65.0	89	-	-	55.0	80.0	67.2	85.2
	Female	66.6	85.7	-	-	-	-	55.2	86.9	68.0	90	-	-	-	-	65.8	85.5
ALB (g/L)	Male	39.7	55.5	34.0	54.0	35.0	52.0	32.7	49.8	39.0	55	29.0	52.0	35.0	55.0	37.06	50.72
	Female	40.0	54.5	-	-	-	-	33.5	50.4	37.0	52	28.0	50.0	-	-	35.57	49.27
ALP (U/L)	Male	43.8	145.7	27.0	122.0	48.0	164.0	101.0	353.0	42.0	159	13.0	201.0	-	-	45.4	170.4
	Female	50.3	135.4	-	-	-	-	82.0	293.0	47.0	160	5.0	227.0	-	-	45.3	155.0
ALT (U/L)	Male	7.2	36.2	12.0	43.0	8.0	61.0	8.0	54.0	7.2	43.3	0.0	39.0	0.0	35.0	9.1	55.3
	Female	7.3	33.9	-	-	-	-	7.0	51.0	5.3	39.9	0.0	34.0	-	-	6.7	44.9
AST (U/L)	Male	16.1	49.2	16.0	47.0	14.0	60.0	17.0	60.0	13.2	35.9	6.0	40.0	0.0	35.0	15.2	53.4
	Female	16.8	45.1	-	-	-	-	13.0	48.0	11.4	28.8	3.0	37.0	-	-	13.5	35.2
GGT (U/L)	Male	8.0	75.6	9.0	77.0	-	-	9.0	71.0	8.7	70.7	-	-	-	-	9.3	120.8
	Female	7.1	63.3	-	-	-	-	6.0	53.0	8.0	41.3	-	-	-	-	7.3	51.8
CREA (µmol/L)	Male	65.2	107.1	44.0	97.0	47.0	109.0	56.0	119.0	53.1	106.2	59.0	127.0	0.0	133.0	48.0	96.0
	Female	62.5	98.6	-	-	-	-	47.0	110.0	44.2	79.6	54.0	122.0	-	-	40.0	81.0
UREA (mmol/L)	Male	1.3	5.8	-	-	-	-	0.9	6.2	-	-	3.2	12.6	3.6	7.1	-	-
	Female	1.4	5.2	-	-	-	-	0.9	5.4	-	-	2.7	12.8	-	-	-	-
Na+ (mmol/L)	Male	134.5	145.5	137.0	147.0	-	-	135.0	151.0	111.0	153.0	141.8	152.1	136.0	145.0	133.6	143.1
	Female	134.5	146.5	-	-	-	-	135.0	150.0	117.0	151.0	140.3	155.3	-	-	133.4	142.3

K+ (mmol/L)	Male	3.7	5.0	3.3	5.0	-	-	3.6	5.2	3.4	4.8	3.0	5.3	3.5	5.0	3.8	55
	Female	3.5	5.0	-	-	-	-	3.4	5.1	3.4	4.8	3.1	5.1	-	-	-	-
Cl- (mmol/L)	Male	89.9	104.2	100.0	112.0	-	-	101.0	115.0	96.0	104.6	100.4	110.8	98.0	106.0	97.9	108.3
	Female	90.6	103.1	-	-	-	-	103.0	113.0	97.4	104.5	101.1	113.4	-	-	97.8	107
Mg+ (mmol/L)	Male	0.7	1.0	-.	-	-	-	-	-	0.4	1.0	-	-	0.8	1.2	0.7	1.0
	Female	0.7	1.0	-	-	-	-	-	-	0.4	1.0	-	-	-	-	0.7	1.0
Phos (mmol/L)	Male	0.8	1.5	-	-	-	-	0.8	2.0	-	-	-	-	-	-	0.7	1.5
	Female	0.7	1.6	-	-	-	-	0.8	1.8	-	-	-	-	-	-	0.7	1.5
GLU (mmol/L)	Male	3.2	7.7	-	-	-	-	-	-	3.0	5.6	2.8	6.8	4.2		2.9	5.3
	Female	3.1	6.7	-	-	-	-	-	-	3.2	5.7	2.6	7.0-	-	-	3.3	5.1

4.4. Quality control report for the studied analytes

Table 4.7. : Quality control for liver and renal function tests under study

Quality assurance was maintained daily in the analytical process by using predetermined liver function test values from pathological and normal values as shown in Table 4.7., where all values were within acceptable limits as indicated by % CV values for assigned QC and study QC report shown in the Table 4.7 below.

Analyte (unit)	QC Type	Assigned QC Report		Study QC Report	
		Mean	% CV	Mean	% CV
Direct bilirubin (µmol/l)	PPU	41.5	0.5	43	0.43
	PNU	16.2	0.9	17.1	0.82
Total bilirubin (µmol/l)	PPU	87.5	1.6	84.6	1.73
	PNU	19.5	3.4	23	1.3
Aspartate aminotransferase (U/L)	PPU	198	1.5	184	1.9
	PNU	39.2	1.7	44.5	1.5
Urea (mmol/L)	PPU	4.08	3.9	3.9	3.6
	PNU	31	2.8	34.7	1.9
GLU (mmol/L)	PPU	14	1.9	14.7	1.7
	PNU	6.55	1.9	7.25	1.8

PPU (Quality control for pathological parameters). PNU (Quality control for normal parameters, %CV-Coefficient variation.

Table 4.8. : Quality control for liver function tests under study

Quality assurance was maintained daily in the analytical process by using predetermined liver function test values from pathological and normal values as shown in Table 4.8., where all values were within acceptable limits as indicated in by % CV values for assigned QC and study QC report shown in the Table 4.8 below.

Analyte (unit)	QC Type	Assigned QC Report		Study QC Report	
		Mean	% CV	Mean	% CV
Total Protein (g/L)	PPU	92	1.5	98.1	1.7
	PNU	52.6	1	48.3	0.8
Albumin (g/L)	PPU	30.8	3.07	28	3.7
	PNU	48.8	4.1	49.1	3.9
Alanine aminotransferase (U/L)	PPU	40	2	38	2.57
	PNU	132	1.3	121	1.1
GGT (U/L)	PNU	40.6	2	42.1	1.7
	PPU	210	1.3	235	1.1
Alkaline phosphate (U/L)	PPU	230	2.8	221	2.6
	PNU	81.8	2.8	78.46	3.3

PPU (Quality control for pathological parameters). PNU (Quality control for normal parameters, %CV-Coefficient variation.

Table 4.9. : Quality control for renal function tests and electrolytes under study

Quality assurance was maintained daily in the analytical process by using predetermined renal function tests and electrolytes values from pathological and normal values as shown in Table 4.9., where all values were within acceptable limits as indicated by % CV values for assigned QC and study QC report shown in the Table 4.9 below.

Analyte (unit)	QC Type	Assigned QC Report		Study QC Report	
		Mean	% CV	Mean	% CV
Creatinine (µmol/L)	PPU	323	1.3	341	1.7
	PNU	65.6	2.8	73.1	2.5
Sodium (mmol/L)	PPU	-	-	-	-
	PNU	135.5	1.7	138.2	2.1
Potassium (mmol/L)	PPU	-	-	-	-
	PNU	4.8	2.8	4.1	1.8
Chloride (mmol/L)	PPU	-	-	-	-
	PNU	89	2.9	94	3.6
Magnesium (mmol/L)	PPU	1.73	0.02	1.81	0.15
	PNU	0.891	1	0.93	1.1
Phosphate (mmol/L)	PPU	-	-	-	-
	PNU	1.2	3.9	1.25	3.5

PPU (Quality control for pathological parameters). PNU (Quality control for normal parameters, %CV-Coefficient variation.

CHAPTER FIVE

DISCUSSION

Reference Intervals are defined as a set of a measured quantity of an analyte obtained from a group of individuals or an individual in a defined state of health. They are used by the clinicians in the interpretation of clinical laboratory data. International Federation of Clinical Chemistry (IFCC) recommends that the reference intervals be constructed from 95% of a reference population of healthy individuals. This study was aimed at establishing the reference intervals for the biochemical parameters in adult Rwandan population to serve as standards for the interpretation of laboratory results during screening and follow-ups in clinical trials and routine healthcare.

This study provides the established clinical chemistry reference intervals for adults 19-58 years for both males and females in Rwanda derived from healthy individuals. Out of 467 participants recruited, the number of males (333) was quite high compared to that of females (134); each group exceeded the minimum of 120 participants for nonparametric estimates required for 95% reference interval determination as recommended by National Committee for Clinical Laboratory Standard (NCCLS, 2000). Emphasis was laid on external and internal quality control methods which ensured accuracy and precision in addition to following all the set standard operating procedure of Kigali University Teaching Hospital (Appendix 4).

The results of this study show that the measured biochemical parameters Albumin, Alkaline Phosphatase, Sodium, Potassium, Magnesium, and Phosphate concentrations are comparable with the intervals established by Roche Diagnostics Company, in Germany, 2012.

Bilirubin Direct, Bilirubin Total, Aspartate Aminotransferase and Creatinine are slightly higher than the limits established by consensus by the Roche Diagnostics. Alanine Aminotransferase, Urea and Chloride are below the established limits by consensus by the manufacturer. However there are variations in findings with other studies which may be due to differences in the age and gender of the study population, diet and environmental differences (Table 4.1., 4.2, 4.3).

The results of this study confirmed the results reported in a study conducted in Northern Province (Gahutu, 2013), and show similar results as the previous study (Gahutu & Wane, 2006) in Rwandan students in Northern Province (Table 4.7).

The significantly higher values of the reference intervals for creatinine and chloride in male compared to female, and the higher values of the reference values for sodium in female compared to male indicates sex differences in these clinical chemistry parameters. Sex differences in creatinine have been known to exist due to differences in muscle mass (Table 4.3). Similar findings have been reported in adult black populations of Kampala, Uganda; Kenya; Tanzania; Huye, Rwanda; Kintampo, Ghana and adult USA populations (Roche Company, 2015; Dosoo et al., 2012; Eller et al., 2008; Gahutu & Wane, 2006; Kibaya et al., 2008; Saathoff et al., 2008; Waithaka, 2009).

Significant differences in gender in the reference interval for total protein and albumin (Table 4.2) could be attributed to the size; however, this difference may not have any clinical significance (Mussap & Plebani, 2004). The sex difference in the reference interval values for serum total protein observed in this study are in contrast to that reported for the American

population (Roche Diagnostics, 2015) where males and females have common reference intervals but agrees with the findings of a Rwandan study (Gahutu & Wane, 2006).

Serum concentration of Total Bilirubin and Direct Bilirubin, Total proteins and Albumin show high values in age of 30 years and below comparing to that of above 30 years individuals (Table 4.5) confirming Gahutu (2013) and Wane (1985).

Sex differences in the total bilirubin and direct bilirubin values could be partly due to influence of sex hormones. These findings are in agreement with those of similar studies done in Uganda (Eller *et al*. 2008) for adults. Notably higher values of liver enzymes (Table 4.6) compared to this study for both males and females have been reported in Kenya (Kibaya et al., 2008; Waithaka, 2009). Bilirubins, glucose, electrolytes values are comparable with the reported values in other study (Eller et al., 2008; Karita et al., 2009; Kibaya et al., 2008; Waithaka et al., 2009), except low values in serum concentration of chloride in this study. This study shows low levels of urea in females compared to that in males (Table 4.1), and shows a slight decrease with age (Table 4.5), and is in conformity with a study done in Congo by Wane (1985). Serum concentration of urea in this study is low compared to the results published in other studies (Table 4.7). This decline may be due to a diet low in animal proteins common practice in Rwanda.

Literature-based reference intervals show similar results (Sluss *et al.,* 2008) with differences in higher serum concentrations of Total Bilirubin, Creatinine, Urea, Chloride and Alkaline Phosphatase for both males and females (Table 3.1, 4.7).

Variations have been observed in other studies in the East African region (Karita *et al.*, 2009; Palacpac *et al.*, 2014; Saathoff *et al.*, 2008) which may be attributed to differences in age and living environment.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The results of this study on Clinical Chemistry parameters are similar to those published in other African countries, with variations due to the diet and geographical location.

This study has shown that a strict adherence to reference intervals developed from industrialized countries could qualify many healthy Rwandans as pathological cases, and also exclude them from participating in clinical trials.

Compared to other reference intervals established, reference values in this study presented remarkably low levels of urea which may be due to the diet low in proteins generally in Rwandan population.

This study not only defined the reference intervals of healthy Rwandans attending NCBT in CHUK, but also enhanced laboratory capacity of Clinical Chemistry service as it was conducted under the guidelines of Good Clinical and Laboratory Practice, thus preparing the site to Clinical trials as a center of research.

6.2. Recommendations

The reference values for most analytes determined in this study vary from the same population, this indicates that there is need for use of sex and age established reference values that are applicable to specific populations rather than take a set of reference values determined for one population and apply it on another population. As such it is recommended that;

1. Using reference intervals developed in industrialized countries, a quite large number of our participants would have been misclassified. Thus, Physicians need to take into consideration the population limitations and variations while attending the patients in our region.

2. Subjects in this study also represent volunteers that would participate in clinical trials. We highlight the need of developing local reference values especially in younger age groups as their participation in clinical trials is solicited.

3. Similar studies should be carried out to establish reference values for other biochemical parameters not done in this study like hormonal profiles and arterial blood gas parameters.
4. Future studies should target children less than 18 years and the elderly population more than 60years who were not covered by the study.
5. Future studies should incorporate for example fasting blood samples for glucose and lipid profiles since this was not done in the current study design. Fasting is a requirement in determining fasting blood glucose, this is essential as fasting blood sugar levels are supposed to be lower than random sugar levels and they are the bases of determining the

diabetic candidates, while for lipid profiles fasting is essential as this gives the actual value if the subject unlike when sample is taken at random, thus elevating the lipid profile values.

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APPENDICES

Appendix 1: Blood donor medical questionnaire

BLOOD DONOR NUMBER	DONATION NUMBER

BLOOD DONORMEDICAL QUESTIONNAIRE

Tick where applicable ☒
Please answer the following questions correctly. This was help to protect you and the patient who receives your blood. Thank you!

Names:

……..……

Sex: Male ☐ Female ☐

Date of Birth: ……..….. Place of Birth (District-Sector):

……

Occupation: …….

Place of Residence

District: Cell:

……..……..… ……..…

Sector:…….. Village (Umudugudu):

… ……..…

Telephone: E-mail:

……..……..… ……..……

Are you married? Yes☐ No☐

Have you donated blood previously Yes☐ No☐ , If yes, how many times?.....

What is the date of the last donation:…… ……Where?

……

Did you have any discomfort during/after donation? Yes ☐ No ☐

BLOOD DONOR DECLARATION

I understand that I should not donate blood if:

- I consume drugs or use illegal intravenous drugs.
- I have HIV AIDS
- I have or have had sex with a partner of the same sex (Even using condom)
- My sexual partner has HIV/AIDS, hepatitis or any other Sexually Transmitted Infection.

I understand that I should wait for 6 months to donate blood if:

- I have had sex with a person who is not my spouse even using condoms
- I don't trust my partner even if I use condoms
- I have not got any HIV/AIDS test before marriage

QUESTIONS TO THE BLOOD DONOR (TICK THE APPROPRIATE ANSWER): ☒

Today

EVER HAD

		YES	NO
1	Are you feeling healthy and well?		
2	Are you taking any medication?		
3	Do you have any wound		

In the past 48 hours

1	Have you taken Aspirin?		

In the past 3 months

1	Have you had Malaria?		

In the past 6 months have you had

1	Weight loss?		
2	Repeated diarrhea?		

DO YOU HAVE OR HAVE YOU

1	Heart Disease?		
2	Kidney Disease?		
3	Cancer?		
4	Epilepsy?		
5	Asthma?		
6	Diabetes?		
7	Tuberculosis?		
8	Abnormal bleeding tendency?		
9	Hepatitis B?		
10	Hepatitis C?		

3	Swollen glands?		
4	Continuous low – grade fever?		
5	Tattooing?		
6	Ear piercing?		
7	Dental Extraction?		
8	Surgery or circumcision?		
9	Blood transfusion?		
10	Endoscopy?		

11	HIV/AIDS		
12	Syphilis?		
13	Gonorrhea?		

For female blood donors only

1	Are you pregnant?		
2	Do you have a child who is under 12 months		
3	Have you had abortion in the last 6 months?		

I am not donating to receive an HIV test:

I agree to have my blood tested for HIV, Hepatitis B& C, and Syphilis, and haemostatic tests (APTT, PT, and Coagulation factor and inhibitors levels) and any other test that is required to ensure my blood is safe to give to another person and for research purpose. I know that someone may be infected and have a negative test. I understand that I was be notified of my test results whatever they are.

I have carefully read education materials and answered all the questions truthfully. I understand that not being honest while answering questions on this form is a serious matter and a lie could harm another person. I understand the eventual side effects of my donation.

I agree that the National Center for Blood Transfusion may use my blood as needed. I have had an opportunity to ask questions and all my questions were answered.

_____ _____ _____

Date **Blood donor Signature** **Name and Signature of the Nurse**

MINI-PHYSICAL EXAMINATION (Filled by Investigator):

	Value	Initials
Scale Wt		
Pulse		
Hb		
BP		
T˚		

		Initials
Start time		
Stop time		
Quantity to bedrawn		
Blood donnor reaction		
Reaction management		

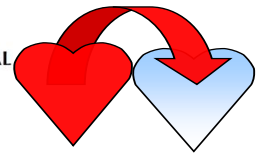
Comments :_____
_____
_____

Eligible ☐ Non-eligible ☐
Reason_____

Appendix 2: Whole blood collection standard operating procedure

RWANDA BIOMEDICAL CENTER

A Healthy People. A Wealthy Nation



MEDICAL PRODUCTION AND PROCUREMENT | NATIONAL CENTRE FOR BLOOD TRANSFUSION (MPP/NCBT)

STANDARD OPERATING PROCEDURE (SOP)

WHOLE BLOOD COLLECTION

Authors:

- **GAHONGAYIRE Félicite, Phlebotomist**
- **UTAMULIZA Clarisse, Donor Selection Officer**
- **MUJIJIMA Chantal, Phlebotomist**
- **NAMAHORO Yvette, Donor Selection Officer**
- **HAKIZIMANA Dieudonné, Phlebotomist**

Effective Date:	**Copy №:**

Approvals and Authorization				
Approved By	**Title**	**Names**	**Signature**	**Date**
	Process owner/Head of Department:			
	Quality Officer:			
	Medical Director:			
Authorized By	Head of NCBT Division:			

Document history

Version	Date	Author(s)	Main Changes
1.0	18-03-2011	- DUSHIME Gratia - UMURAZA Laetitia	N/A
2.0	09-07-2012	- NAMAHORO Yvette - MUJIJIMA Chantal - GAHONGAYIRE Félicité - HAKIZIMANA Dieudonne - UTAMULIZA Clarisse	- Format change - Addition of steps in the procedure

Purpose

This procedure provides guidance on how to collect whole blood units from a donor while providing a safe and comfortable environment for the donor during the donation process.

Scope

This procedure is applicable to all NCBT centers collecting blood. It is applicable to the fixed site as well as in mobile session. All Donor selection Officers and Phlebotomists in NCBT must follow this SOP.

Related procedures/ documents

1. COL_SOP_15_Use and maintenance of the collection scale/mixer
2. FRM_DON_SOP_01A_V02.0 (DMQ Kinyarwanda)
3. FRM_DON_SOP_01B_V02.0 (DMQ English)
4. FRM_DON_SOP_01C_V02.0 (DMQ French)
5. COL_SOP_06_Disinfection of the Venipuncture site
6. DON_SOP_05_Management of donor reactions
7. COL_SOP_09_Post donation care
8. COL_SOP_08_Packaging Blood Units and Samples for transport

Materials and reagents needed

1. Clips
2. Plastic hemostat
3. Scissors
4. Test tube racks
5. Containers for sharps (safety box)
6. Waste bin for non-sharp biohazard wastes
7. Scale/Mixer
8. Adjustable automatic donor scales
9. Beds for phlebotomy
10. Adhesive tapes
11. Labeled blood bags and tubes
12. Hand gripper
13. Hand sealer clips
14. Tube stripper
15. Pens
16. Gauzes
17. Gauzes containers
18. Tray
19. Trolley

Refer to the safety plan

Safety requirements

Sample preparation requirements N/A

Reagent preparation requirements N/A

Sample preparation N/A

Donor reception	**Step**	**Action**
	1	Greet the donor cordially
	2	Tell the donor to lie down on the collection bed
	3	Ask the donor his/her names
	4	Compare the initials on the blood bag and tubes with the donor names. If the donation number and names are non-concordant, consult the donor registration officer for correction.
	5	Briefly explain to the donor the donation process by emphasizing on the following: • The venipuncture action (not harm) • The volume to be collected
	6	Reconcile the numbers and donor names on the tubes, bags, DMQ
Verification of the integrity of collection bags and tubes	**Step**	**Action**
	1	Verify the expiration date of the bags and the tubes
	2	Inspect the bag for any defects and discoloration.
	3	Apply pressure to check for leaks
	4	Verify the integrity of the bag and the appearance of the solution.
	5	If the bags appearance is abnormal, find a new bag from the donor registration officer and make sure for the same identification as previous one.
	6	Put the bag and its satellite bags to the scale mixer or Adjustable automatic donor scale in accordance with the procedure of use of the scale,

Selection of the vein	**Step**	**Action**
	1	Perform arm inspection and choose the arm on which the venipuncture was be performed Consider the followings: a) Best position for venipuncture b) Observation from arm inspection (Scar, malformation, skin diseases) c) Donor preference d) Availability and accessibility of the vein
	2	Apply and fix the tourniquet approximately 5-10 cm above antecubital area
	3	Ask donor to squeeze hand squeezer.
	4	Palpate donor's veins
	5	Select the best vein in order of preference: a) 1st choice: Median cubital b) 2nd choice: Cephalic c) 3rd choice: Basilic
	6	Repeat Step 1-5 above on another arm if the vein is not found on the first arm.
	7	Consult experienced staff if a suitable vein cannot be identified.
Disinfection of the venipuncture site	**Step**	**Action**
	1	Disinfect the venipuncture site in accordance with the procedure for disinfection of the venipuncture site (COL_SOP_06_ Disinfection of the Venipuncture site).
Performing phlebotomy using blood bag without diversion pouch	**Step**	**Action**
	1	Clamp the tubing of the bag at 10-15 cm next to the needle of the bag by a plastic hemostat
	2	Remove the needle protective cover and check the status of the needle before use
	3	Anchor the vein by holding the arm below the prepared area
	4	Point the needle, bevel up, in the direction of the vein. **Note**: Perform the venipuncture as soon as possible to prevent contamination of donor arm and material.

	5	Firmly pierce the skin at about a 15 – 20 degree angle and advance the needle horizontally into the vein.
	6	Open hemostat and verify blood is flowing into the tubing. If not flowing adequately. Refer to section below on Troubleshooting
	7	Stabilize the needle by holding the tubing to the arm of the donor and secure using an adhesive tape.
	8	Cover the venipuncture site with sterile gauze square and fix it with an adhesive tape.
	9	Record immediately the start collection time on the Donor Medical Questionnaire (DMQ)
	10	Loosen tourniquet slightly.
	11	Ask the donor to open and close hand every 10 to 12 seconds during collection.
	12	If no mixer is being used, gently mix the blood and anticoagulant by inverting the bag at least three times as it flows into the bag and periodically (approximately every 1 minute) throughout the collection process. A complete end-to-end mixing of the bag is required.
	13	Monitor donor and blood flow: For slow running units, assess troubleshooting needs. Refer to Troubleshooting section.
	14	If donor appears to have a reaction, refer to Management of Donor Reactions SOP. **Note:** Keep the donor under observation throughout the donation process. The donor should never be left unattended during or immediately after donation
Ending the phlebotomy when using blood bag without diversion pouch	**Step**	**Action**
	1	Note: The scale mixer end collection if the drawn quantity is achieved Clamp tubing near the diversion using a clip and hand sealer clips
	2	Record the end time on the DMQ
	3	Clamp the tubing using a hemostat and cut using scissors
	4	Draw sample for 2 tubes (one EDTA tube and one plain tube) from the tubing in donor vein. Mix the EDTA tube by gently inverting it at least 3 times.

	5	Release the tourniquet and remove the previously fixed gauze and tape from the venipuncture site.
	6	Find gauze, hold it on the donor arm above the venipuncture site and remove the needle.
	7	Ask the donor to raise the arm and hold the gauze firmly with the other hand
	8	Put the safety protective cover to the needle gently
	9	Don't recover the needle with the removed cover
	10	Discard the remaining tubing into the Biohazard container for sharps
	11	Immediately after discontinuing the phlebotomy, strip the tubing on the blood unit as completely as possible (at least twice) into the bag, starting at seal.
	12	Mix the blood in the collected unit by inverting bag several times to mix thoroughly and perform this step at least 3 times.
	13	Keep the collected units at room temperature for at least 2 hours to allow blood to cool. Do not put blood units directly into the containers with ice packs
Performing Phlebotomy using blood bag with diversion pouch	**Step**	**Action**
	1	Close white pinch clamp for 2 tubules of the needle and diversion pouch of the bag by a plastic hemostat provided with the bag
	2	Remove needle cover and check the status of the needle before use.
	3	Anchor the vein by holding the arm below the prepared area.
	4	Point the needle, bevel up, in the direction of the vein.
	5	Firmly pierce the skin at about a 15-20 degree angle and advance the needle horizontally into the vein.
	6	Open hemostat of the diversion pouch to let the first 10 mL to flow into the diversion pouch.
	7	Verify blood is flowing into the tubing-If not flowing adequately, refer to section below on Troubleshooting.
	8	Stabilize the needle by holding the tubing to the arm of the donor and secure using an adhesive tape.

	9	Cover the venipuncture site with sterile gauze square and fix it with an adhesive tape.
	10	Record immediately the start collection time
	11	Loosen tourniquet slightly.
	12	When the Diversion pouch is full, close white pinch clamp and tighten the loose knot in the tubing between the sample collection pouch and the intersection with the main tubing.
	13	Break snap-open closure completely to allow blood to flow to the primary collection bag by: a) Holding the snap-open closure with both hands b) Bending in two directions. An audible click was be heard in each direction.
	14	Take 2 samples by attaching the vacutainer tube to adapter of the Diversion pouch and mix sample thoroughly if EDTA tube is used.
	15	If not using a scale mixer, gently mix the blood and anticoagulant as it flows into the bag and periodically (approximately every 1minute) throughout the collection. A complete end-to-end mixing of the bag is required.
	16	Ask the donor to open and close hand every 10 to 12 seconds during collection.
	17	Monitor donor and blood flow: For slow running units, assess troubleshooting needs. Refer to Troubleshooting section.
	18	If donor appears to have a reaction, refer to Management of Donor Reactions SOP. **Note:** Keep the donor under observation throughout the donation process. The donor should never be left unattended during or immediately after donation
Ending the phlebotomy when using blood bag with diversion pouch	**Step**	**Action**
	1	End the phlebotomy following the steps 1 to 13 above for ending the phlebotomy when using blood bag without diversion pouch except step 4 for drawing sample for 2 tubes from the tubing in donor vein.

Discontinue Phlebotomy **in Emergency**	**Step**	**Action**
	1	Note: Discontinue a donor collection procedure in an emergency situation, i.e., donor has a donor reaction during collection. Clamp tubing near needle hub.
	2	Release and remove the tourniquet.
	3	Fold gauze in half and place over phlebotomy site.
	4	Remove the adhesive tape from tubing and hold the tubing near the needle steady.
	5	Remove needle from donor's arm
	6	Immediately apply pressure to site and react according the situation
	7	Apply a bandage to the puncture site arm and give post donation instructions
	8	Refer to the appendix "COL_SOP_07_Whole Blood Collection_A04_V02.0(Phlebotomy Exceptions)
	9	Notify the team leader or designee.

Inspection of the Venipuncture site and Application of Bandage

SITUATION	ACTION
If bleeding has stopped	Place the tape to the used gauze if no bleeding occurred.
If bleeding occurred,	Change the gauze before applying the tape
If bleeding continues,	1. Elevate arm again for approximately 1 minute, 2. Apply pressure to phlebotomy site, 3. Apply new gauze and tape once bleeding stops.
If bleeding persists after arm is wrapped and verbal donor consent for treatment is obtained,	1. Apply ice pack , 2. Allow donor to hold ice on the phlebotomy site, 3. Remove ice after approximately 2 minutes and check donor's arm to verify bleeding has stopped, 4. Continue ice pack until bleeding stops, Note: Ice should never be "wrapped" onto donor's arm,
If a hematoma develops,	Refer to the procedure for management of donor reactions

Post Phlebotomy Instructions

Step	Action
1	Keep the donor on donor bed for at least 5 minutes after donation
2	Instruct the donor to keep the bandage on for 2 hours once bleeding stops.
3	Instruct the donor to refrain from strenuous activities such as mountain climbing, running marathons and flying an airplane for at least 72 hours following the donation
4	Advise the donor that smoking or alcohol intake within 6 hours after donating may cause dizziness
5	Advise the donor that If He/ She get dizzy, to sit down and put the head between his/her knees or lie down with his/her legs elevated
6	Instruct the donor to eat well at the next meal
7	Instruct the donor to drink extra fluids, twice the normal intake
8	Advise the donor that normal activities may be resumed when He/She is finished with the refreshments

	9	Advise the donor that If He/ She develops fever, chills or diarrhea within 24 hours, after donating please to call the NCBT "Hotline" number at 1011
	10	Thank the donor and direct the donor in the refreshment area(Refer to Post donation care procedure)
Limitations (or Notes), if applicable	1. Blood collection must only be done using sterile, single use pyrogen free blood collection bags and using a closed system. If the collection bag sterility is questionable do not use it. 2. For phlebotomy exceptions Refer to the Phlebotomy Exceptions Appendix. 3. Routinely gloves are not used during phlebotomy, if you have got any skin scratches make sure to wear gloves while performing phlebotomy and change them between donors. 4. Use hand sanitizer in accordance with procedure.	
References	AABB Technical manual, 17th Edition AABB standards for Blood Banks and Transfusion Services, 27th edition	

Appendix 3: Consent form

CONSENT FORM FOR PARTICIPATION IN RESEARCH STUDY

JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY

ESTABLISHMENT OF ADULT REFERENCE VALUES FOR SOME BLOOD ANALYTES COMMONLY REQUESTED

Information Sheet

Purpose of the Study. As part of the requirements for Masters of Medical Laboratory Sciences in Clinical Chemistry at JKUAT, I have to carry out a research study. The study is concerned with Establishment of reference values for some blood analytes commonly requested.

What will the study involve? The study will involve your participation will involve following the instructions given and blood will be withdrawn (about 5 ml), after blood donation.

Why have you been asked to take part? You have been asked because you are specifically suitable to provide data for my study.

Do you have to take part? No. Participation is voluntary. And after signing a consent form, you are still having the option of withdrawing before the study commences (even if you have agreed to participate) or discontinuing after data collection has started. It is also allowed for afterthoughts to withdraw within two weeks of participation and ask to have their data destroyed. **Will your participation in the study be kept confidential?** Yes. I will ensure

that no clues to your identity appear in the thesis. Any extracts from what you say that are quoted in the thesis will be entirely anonymous.

What will happen to the information which you give? The data will be kept confidential for the duration of the study. On completion of the thesis, they will be retained for a further six months and then destroyed.

What will happen to the results? The results will be presented in the thesis. They will be seen by my supervisors, a second marker and the external examiner. The thesis may be read by future students on the course. The study may be published in a research journal.

What are the possible disadvantages of taking part? I don't envisage any negative consequences for you in taking part. It is possible that after blood withdraws you may feel some discomfort on the site of puncture which is temporally (some minutes).

What if there is a problem I will discuss with you how you found the experience and how you are feeling. If you subsequently feel distressed, you should contact, me, Mr. Robert RUTAYISIRE, Phone#:+250788881791, email:robertrutayisire@gmail.com.

Who has reviewed this study? KHI Ethics Committee, CHUK Ethics Committee Approval must be given before studies like this can take place.

Any further queries? If you need any further information, you can contact me, Mr. Robert RUTAYISIRE, Phone#:+250788881791, email:robertrutayisire@gmail.com.

If you agree to take part in the study, please sign the consent form overleaf.

CONSENT FORM

I……agree to participate in Mr. Robert RUTAYISIRE's research study.

The purpose and nature of the study has been explained to me in writing.

I am participating voluntarily.

I understand that I can withdraw from the study, without repercussions, at any time, whether before it starts or while I am participating.

I understand that I can withdraw permission to use the data within two weeks of the interview, in which case the material will be deleted.

I understand that anonymity will be ensured in the write-up by disguising my identity.

I understand that disguised extracts from my interview may be quoted in the thesis and any subsequent publications if I give permission below:

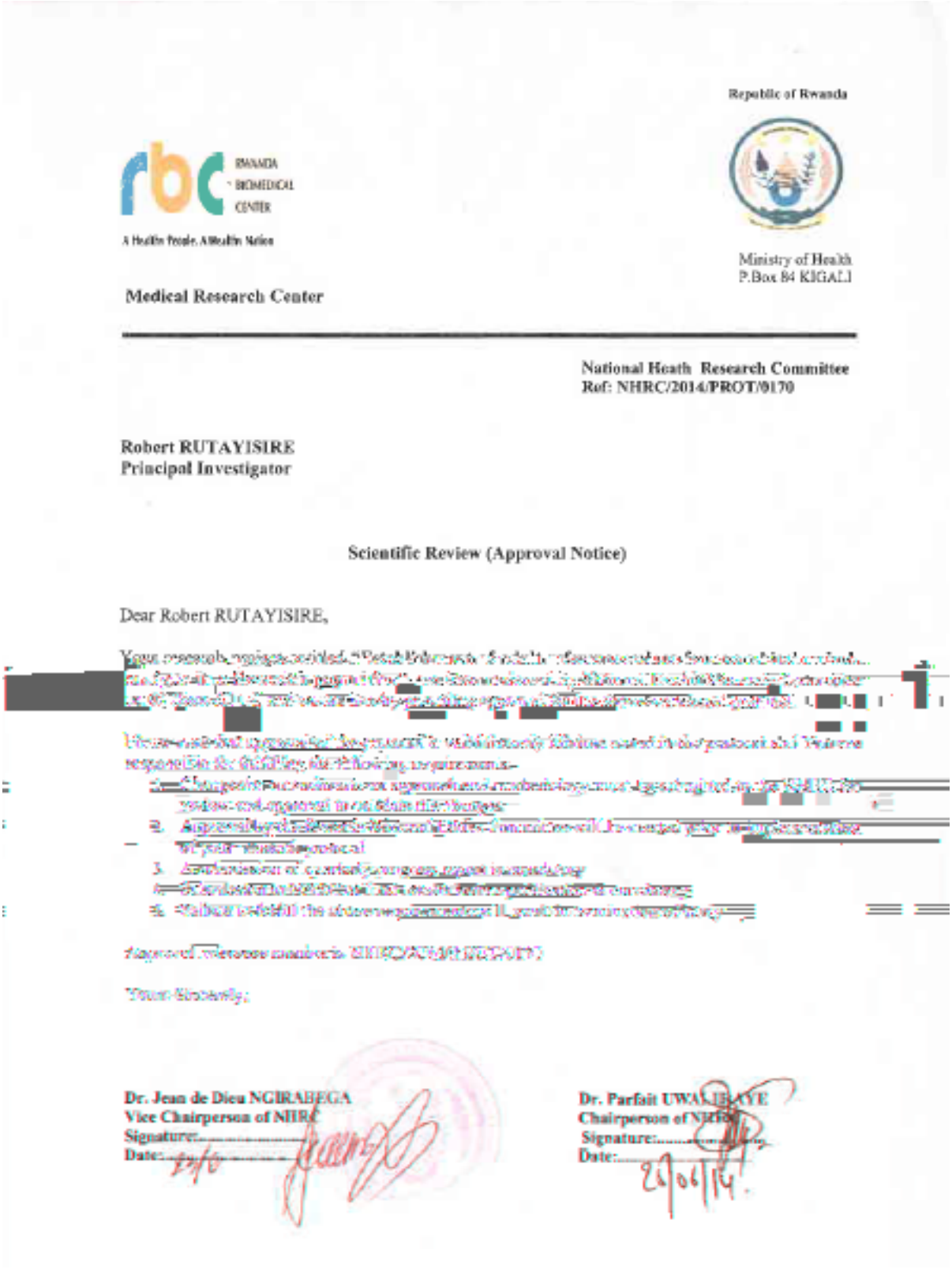
Please tick one box.

I agree to quotation/publication of extracts from my interview ☐

I do not agree to quotation/publication of extracts from my interview ☐

Signature……. Date…….

Appendix 4: Ethical Approval by National Health Research committee



Republic of Rwanda

RWANDA BIOMEDICAL CENTER

A Healthy People, A Healthy Nation

Ministry of Health
P.Box 84 KIGALI

Medical Research Center

National Heath Research Committee
Ref: NHRC/2014/PROT/0170

Robert RUTAYISIRE
Principal Investigator

Scientific Review (Approval Notice)

Dear Robert RUTAYISIRE,

[redacted]

Dr. Jean de Dieu NGIRABEGA
Vice Chairperson of NHRC
Signature:
Date: 24/6

Dr. Parfait UWAMUNGU
Chairperson of NHRC
Signature:
Date: 24/06/14

Appendix 5: Protocol approval by CHUK Ethics committee

CENTRE HOSPITALIER UNIVERSITAIRE

UNIVERSITY TEACHING HOSPITAL

Quality Health Care
Training & Research

Ethics Committee / Comité d'éthique

July 21, 2014 **Ref.: EC/CHUK/115/14**

Review Approval Notice

Dear **Robert Rutayisire,**

Your research project: ***"Establishment of adult reference values for some biochemical analytes in a Rwandan population at Kigali University Teaching Hospital"***

During the meeting of the Ethics Committee of Kigali University Teaching Hospital (KUTH) that was held on 21/07/2014 to evaluate your protocol of the above mentioned research project, we are pleased to inform you that the Ethics Committee/CHUK has approved your protocol.
You are required to present the results of your study to KUTH Ethics Committee before publication.

PS: Please note that the present approval is valid for 12 months.

Yours sincerely,

DR. STEPHEN RULISA
HEAD - DEPARTMENT OF CLINICAL RESEARCH
CHUK

Dr. Stephen Rulisa
The President, Ethics Committee,
Kigali University Teaching Hospital

<<University teaching hospital of Kigali Ethics committee operates according to standard operating procedures (Sops) which are updated on an annual basis and in compliance with GCP and Ethics guidelines and regulations>

B.P. :655 Kigali- RWANDA Tél Fax : 00 (250) 576638E-mail : chuk.hospital@chukigali.org

Appendix 6: Permission to carry out the study by NCBT

RWANDA BIOMEDICAL CENTER

A Healthy People, A Wealthy Nation

RWANDA BIOMEDICAL CENTER/ NATIONAL CENTRE FOR BLOOD TRANSFUSION (RBC/BIOS/NCBT)

Kigali, on 03/09/2014

N° 46 /RBC/BIOS/NCBT/2014

Mr RUTAYISIRE Robert

Dear Robert,

Re: Your research project: "Establishment of adult reference value for some biochemical analytes in a Rwanda population at RBC/BIOS/NCBT Division"

Reference is made to your letter dated on 26/08/2014 requesting for a permission to carry out your research study which will be submitted in partial fulfilment of the requirements for the award of Master degree of Medical Laboratory Sciences in the College of Health Sciences/JMU.

I hereby inform you that your request has been granted.

Your are required to present the result of your study to RBC/BIOS/NCBT before publication.

Best regard,

HEAD OF NATIONAL BLOOD TRANSFUSION CENTER
RWANDA BIOMEDICAL CENTER

Dr KATABE Swaibu
Ag Division Manager RBC/BIOS/NCBT

National Centre for Blood Transfusion RBC/NCBT P.O. Box 4729, Telephone: 252 570 408, Website: www.cnts.gov.rw
E-mail: info@cnts.gov.rw

Appendix 7: Published paper

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ESTABLISHMENT OF ADULT REFERENCE VALUES FOR SOME BIOCHEMICAL ANALYTES IN A RWANDAN POPULATION

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ABSTRACT

Objectives: **To establish the reference values for some routinely performed biochemical analytes in CHUK.**

Design: **Cross-sectional descriptive study.**

Settings: **National Center for Blood Transfusion, Rwanda and Kigali University Teaching Hospital, Laboratory Department. This study was conducted during the period between 15th September 2014 and 23rd February in 2015.**

Subjects: **Blood donors donating blood at National Center for Blood Donation, and recruited by mobile teams across the country.**

Results: **Median (Reference values:2.5th and 97.5th percentiles) for male and female respectively: Bilirubin Direct,3.9(2-6.9) and 3.9(2.6-6.5) µmol/L; Bilirubin Total,10.3(4.8-21.6) and 10.4(5.9-17.3) µmol/L; Aspartate Aminotransferase, 27.8(16.1-49.2) and 26.7(16.8-45.1) U/L;Urea,3.2(1.3-5.8) and 3.1(1.4-5.2)mmol/L; Glucose, 5.0(3.2-7.7) and 4.6(3.1-6.7) mmol/L; Total Proteins, 76.8(68.2-87.7) and 76.9(66.6-85.7)g/L; Albumin, 46.4(39.7-55.5) and 46.7(40-54.5) g/L; Alanine Aminotransferase, 17.1(7.2-36.2) and 16.0(7.3-33.9) U/L; Gamma Glutamyltransferase, 20.3(8-75.6) and 21.1(7.1-63.3) U/L; Alkaline Phosphatase, 74.3(43.8-145.7) and 73.5(50.3-135.4) U/L; Creatinine, 84.4(65.2-107.1) and 81.1(62.5-98.6) µmol/L; Sodium, 139.0(134.5-145.5) and 141.0(134.5-146.5) mmol/L; Potassium, .4.4(3.7-5) and 4.3(3.5-5.0) mmol/L; Chloride, 95.7(89.9-104.2) and 99.3(90.6-103.1) mmol/L; Magnesium, 0.9(0.7-1.0) and 0.9(0.7-1) mmol/L; Phosphate, 1.1(0.8-1.5) and 1.2(0.7-1.6) mmol/L.**

Conclusion: **The results of our study on Clinical Chemistry parameters are similar to those published in other African countries, with variations due to the diet and geographical location. This study has shown that a strict adherence to reference ranges developed from industrialised countries could qualify many healthy Rwandans as pathological cases, and also exclude them from participating in clinical trials. Compared to other reference ranges established, reference values in our study presented remarkably low levels of urea which may be due to the diet low in proteins generally in Rwandan population.**

INTRODUCTION

In health-related fields, a reference range or reference interval describes the variations of a measurement or value in healthy individuals. It is a basis for a physician or other health professional to interpret a set of results for a particular patient (Saathoff *et al.*, 2008). The use of improper reference ranges may false or exclude otherwise volunteers who are eligible to participate in the research making the process of enrolment and execution more challenging(Kanita et

al.,2009; Eller *et al.*, 2008). On the other hand, as the biological and environmental characteristics vary between populations, it is imperative to establish local reference intervals for clinical laboratories, which makes it possible to judiciously interpret laboratory results using reference intervals obtained from the local population and in the same environmental background(Eller *et al.*, 2008). The present study, therefore, aims to fill this gap by studying a set of serum biochemical parameters, chosen among the most relevant and the most commonly requested by our clinicians.

MATERIALS AND METHODS

Samples: The sites dispatched in five provinces of Rwanda have been used to collect samples: Central Province (Kigali), Northern Province, Southern Province, Eastern Province, and Western Province. This was a cross sectional prospective study.

Blood donors were recruited in rural area, schools and urban cities in five provinces during the study period.

Three hundred and eighty four blood donors, 93 in Northern, Southern, Western and Eastern provinces each, and 95 in Central province, all in rural areas, schools and urban cities were sampled for this study. Each blood donor who fulfilled all criteria as set by NCBT was given a questionnaire and a consent form for participation in this study.

Whole blood was collected into two tubes, in 5 ml plain tube without anticoagulant for serum chemistry and in Sodium fluoride tube specifically for blood glucose levels determination.

Samples after collection were allowed to clot at room temperature and put in a cool box for transportation. After clotting at room temperature, the blood cells were separated from the serum by centrifugation at 5000 rounds for 5 minutes, and serum was put in code labeled serum vials, and analysed within 10 hours. Those which were not analysed were kept in a fridge at -21°C and analysed the following day.

Laboratory analysis was performed at Kigali University Teaching Hospital, using Cobas Integra 400 Plus, a chemistry analyser (Roche Diagnostics Ltd, Switzerland). All tests were done according to the laboratory SOPs, equipment and reagent manufacturer's instructions.

Statistical analysis: Socio-demographic and medical information was obtained from the questionnaires and included gender, age, demographic location, and results of transmissible disease from blood donors files. Results from the laboratory tests were entered into a hard cover register and a password protected Microsoft Excel database. Samples were identified by a unique study number for confidentiality. Results were analysed using SPSS version 20 (IBM corporation, 2012). All calculations to determine the reference values were based on Clinical and Laboratory Standards Institute/International Federation of Clinical Chemistry(CLSI/IFCC)(The Former National Committee of Clinical Laboratory Standards) guidelines document on defining, establishing and verifying reference intervals in Clinical Laboratory(Edward A.et al., 2000).

2.5^{th} and 97.5^{th} percentiles were calculated non-parametrically, after removing identified outliers in each subgroup by using Reed-Dixon method of identifying the outliers(Dixon, 2013; Reed, Henry, & Mason, 1971).The extreme values were retained in the distribution if $D/R = 0.33$,where D is the absolute difference between the most extreme distribution and the next value and R is the range(maximum-minimum), as recommended by CLSI/IFCC guidelines document.

Differences between genders and age groups were evaluated using Wilkoxon-Mann-Whitney test and when $p = 0.05$, it was considered as a statistically significant difference between groups. All statistical analyses were carried out using SPSS v.20 (IBM Corporation, 2012).

Ethical consideration: This study was ethically approved by Rwanda Biomedical Center division of Medical Research Committee, Ethical approval from Rwanda National Ethics Committee, Ethical Review Committees of National Center for Blood Transfusion and Kigali University Teaching Hospital before excursion.

RESULTS

The subjects used in the statistical analysis were 467, with 333 males and 134 females (representing 71.3 % and 28.7%, respectively). The study participants had a mean age of 32.6 years.

Before analysing obtained data, outliers were removed. An outlier was any value distant from the other values or outside the range of ±3SD. An outlier may be a result of variability in measurement or an experimental error. All outliers were removed as recommended by Reed *et al.* and Dixon in CLSI essential guidelines for establishing reference intervals. The number of outliers removed is indicated in the brackets for males and females respectively as following: Albumin (0, 0), Alkaline Phosphatase (3, 1), Alanine Aminotransferase (1, 0), Aspartate Aminotransferase (1, 0), Creatinine (0, 0), Gamma Glutamyl Transferase (13, 2), Magnesium (0, 0), Phosphate (6, 1), Total Proteins (1, 0), Urea (1,

0), Sodium (1, 0), Potassium (0, 0), Chloride (0, 0), Bilirubin Direct (0, 0), Bilirubin Total (0, 0), Glucose (0, 0).

The reference values were constructed using 2.5th and 97.5th percentiles as lower and upper limits at 95% confidence interval in accordance with CLSI (formerly NCCLS) guideline for determining reference intervals. The medians for males and females and age groups were statistically compared using Mann-Whitney test. P< 0.05 was considered statistically different. Serum concentration difference of the analyte was determined to assess whether age group specific reference ranges or reference ranges for the whole population regardless the age should be established. Results showed a statistically significant difference in gender, whereby males have significantly higher serum concentration values of Creatinine of 65.2-107.1 µmol/L against 62.5-98.6 µmol/L for females (p = 0.001), serum concentration values of Sodium higher in females with 134.5-146.5 mmol/L against 134.5-145.5 for males (p = 0.013) and serum concentration values of Chloride higher in females with 90.6-103.1 mmol/L against 89.9-104.2 for males (p = 0.028), since their p-values are less than 0.05(Table 1.,2.,3.). Comparing age groups, subjects aged of 30 years and below have a significantly lower serum concentration values of Total Proteins of 62.9-86.5 g/L against 68.6-87.7 g/L for subjects aged above 30 years (p = 0.002), serum concentration of Albumin of 37.1-55.2 g/L against 40.9-55.5 for subjects aged of above 30 years (p = 0.022), serum concentration of Alkaline Phosphatase of 43.3-133.1 U/L against 44.6-152.0 U/L for subjects aged of above 30 years (p = 0.001), serum concentration of Sodium of 134.5-146.0 mmol/L against 134.5-146.5 mmol/L for subjects aged of above 30 years (p = 0.015), serum concentration of Bilirubin Direct of 1.8-7.0 g/L against 2.3-6.8 µmol/L for subjects aged of above 30 years (p = 0.025)(Table 4.,5.,6.).

Table 1

Reference values of clinical chemistry analytes under study and differences in genders

Analytes (Unit)	Male				Female				All participants					
		Percentiles				Percentiles				Percentiles				
	N	Median	2.5th	97.5th	N	Median	2.5th	97.5th	N	Median	2.5th	97.5th		
Renal and Liver function tests													Z - Value	P - Value
Bil-D (µmol/L)	333	3.9	2.0	6.9	134	3.9	2.6	6.5	143	4.0	2.1	6.9	-0.186	0.852
Bil-T (µmol/L)	333	10.3	4.8	21.6	134	10.4	5.9	17.3	143	10.4	5.0	21.6	-0.849	0.396
AST (U/L)	332	27.8	16.1	49.2	134	26.7	16.8	45.1	436	27.6	16.4	49.2	-1.169	0.242
UREA (mmol/L)	332	3.1	1.3	5.8	134	3.1	1.4	5.2	436	3.1	1.3	5.8	-0.781	0.435
GLU (mmol/L)	333	5.0	3.2	7.7	134	4.6	3.1	6.7	143	4.8	3.1	7.7	-1.760	0.078
Liver function tests														
TP (g/L)	332	76.8	68.2	87.6	134	76.9	66.6	85.7	436	76.8	68.0	87.6	-0.532	0.595
ALB (g/L)	333	46.4	39.7	55.5	134	46.7	40.0	54.5	437	46.5	39.8	55.4	-0.472	0.637
ALT (U/L)	332	17.1	7.2	36.2	134	16.0	7.3	33.9	436	16.9	7.4	36.2	-0.824	0.41
GGT (U/L)	320	20.3	8.0	75.6	132	21.1	7.1	63.3	349	20.4	7.6	75.2	-0.350	0.726

ALP (U/L)	328	74.3	43.8	145.7	133	73.5	50.3	135.4	431	74.3	44.2	145.7	-0.593	0.553
Renal function tests and electrolytes														
CREA (µmol/L)	333	84.4	65.2	107.1	134	81.1	62.5	98.6	437	83.3	64.5	106.3	-3.258	0.001
Na^+ (mmol/L)	332	139.0	134.5	145.5	134	141.0	134.5	146.5	142	140.0	134.5	146.5	-2.472	0.013
K^+ (mmol/L)	333	4.4	3.7	5.0	134	4.3	3.5	5.0	143	4.4	3.7	5.1	-1.044	0.297
Cl^- (mmol/L)	333	95.7	89.9	104.2	134	99.3	90.6	103.1	143	97.5	90.3	104.1	-2.199	0.028
Mg^+ (mmol/L)	333	0.9	0.7	1.0	134	0.9	0.7	1.0	321	0.9	0.7	1.0	-1.270	0.204
PO_3^{2-} (mmol/L)	327	1.1	0.8	1.5	133	1.2	0.7	1.6	430	1.2	0.8	1.6	-1.237	0.216

Table 1. indicates renal and liver function tests and electrolytes for both genders while separate and combined. The values are given as both the median and the percentiles. Reference range is given as the range between 2.5 and 97.5 of the percentile. The number of subjects is indicated under the column labelled N. The bolded values indicate significance difference between sexes. The sex difference is significant at $p < 0.05$(Creatinine, Sodium and Chloride).

Table 2

Reference values of clinical chemistry analytes under study and differences in age groups

Analytes (Unit)	<=30				31+				All participants					
		Percentiles				Percentiles				Percentiles				
	N	Median	2.5th	97.2th	N	Median	2.5th	97.2th	N	Median	2.5th	97.2th	Z-Value	P-Value
Renal and liver function tests														
BIL-D (µmol/L)	193	3.6	1.8	7.0	274	3.9	2.3	6.8	467	4.0	2.1	6.9	-2.244	0.025
BIL-T (µmol/L)	193	9.7	4.2	21.4	274	10.7	5.8	23.0	467	10.4	5.0	21.6	-1.458	0.145
UREA (mmol/L)	193	2.9	1.3	5.8	273	3.0	1.4	4.8	466	3.1	1.3	5.8	-1.075	0.283
AST (U/L)	192	26.7	17.1	45.6	274	28.3	16.1	49.3	466	27.6	16.4	49.2	-1.347	0.178
GLU (mmol/L)	193	4.7	3.1	6.7	274	4.8	2.6	8.2	467	4.8	3.1	7.7	-1.701	0.089
Liver function tests														
TP (g/L)	192	75.9	62.9	86.5	274	77.4	68.6	87.7	466	76.8	68.0	87.6	-3.035	0.002
ALB (g/L)	193	45.9	37.1	55.2	274	46.8	40.9	55.5	467	46.5	39.8	55.4	-2.288	0.022
ALT (U/L)	193	17.4	6.9	37.4	273	16.8	7.9	34.2	466	16.9	7.4	36.2	-0.153	0.878
GGT (U/L)	189	19.5	7.6	70.7	263	22.2	6.1	76.1	452	20.4	7.6	75.2	-1.542	0.123
ALP (U/L)	189	68.8	43.3	133.1	272	76.6	44.6	152.0	461	74.3	44.2	145.7	-3.250	0.001
Renal function tests and electrolytes														
CREA (µmol/L)	193	83.7	66.3	104.6	274	82.7	62.7	105.9	467	83.3	64.5	106.3	-0.846	0.397

Na+ (mmol/L)	192	139.0	134.5	146.0	274	141.0	134.5	146.5	466	140.0	134.5	146.5	-2.425	0.015
K+ (mmol/L)	193	4.3	3.6	5.1	274	4.4	3.7	5.1	467	4.4	3.7	5.1	-1.426	0.154
Cl- (mmol/L)	193	95.4	89.2	103.5	274	98.0	90.5	105.1	467	97.5	90.3	104.1	-1.636	0.102
Mg+ (mmol/L)	193	0.9	0.7	1.0	274	0.9	0.7	1.0	467	0.9	0.7	1.0	-0.521	0.603
PO32- (mmol/L)	190	1.2	0.7	1.5	270	1.2	0.8	1.2	460	1.2	0.8	1.6	-0.862	0.389

Table 2. indicates renal and liver function tests and electrolytes for both age groups while separate and combined. The values are given as both the median and the percentiles. Reference range is given as the range between 2.5 and 97.5 of the percentile. The number of subjects is indicated under the column labelled N. The bolded values indicate significance difference between age groups. The age group difference is significant at $p < 0.05$(Bilirubin Direct, Total Proteins, Albumin, Alkaline Phosphatase and Sodium).

DISCUSSION

Reference ranges are used by the clinicians in the interpretation of clinical laboratory data. They are defined as a set of a measured quantity of an analyte obtained from a group of individuals or an individual in a defined state of health. International Federation of Clinical Chemistry (IFCC) recommends that the reference ranges be constructed from 95% of a reference population of healthy individuals. This study was aimed at establishing the reference ranges for the biochemical parameters in adult Rwandan population to serve as standards for the interpretation of laboratory results during screening and follow-ups in clinical trials and routine healthcare.

This study provides the established clinical chemistry reference ranges for adults 19-58 years for both males and females in Rwanda derived from healthy individuals. Out of 467 participants recruited, the number of males (333) was quite high comparing to that of females (134); each group exceeded the minimum of 120 participants for nonparametric estimates required for 95% reference interval determination as recommended by National Committee for Clinical Laboratory Standard (NCCLS, 2000). Emphasis was laid on external and internal quality control methods which ensured accuracy and precision in addition to following all the set standard operating procedure of Kigali University Teaching Hospital.

The results of this study shown that measured biochemical parameters Albumin, Alkaline Phosphatase, Sodium, Potassium, Magnesium, Phosphate serum concentrations are comparing with the ranges established by consensus by Roche Diagnostics Company, Germany.

Bilirubin Direct, Bilirubin Total, Aspartate Aminotransferase and Creatinine are slightly higher than the limits established by consensus by the Roche Diagnostics. Alanine Aminotransferase, Urea and Chloride are below the established limits by consensus by the manufacturer.

The results of this study confirmed the results reported in a study conducted in Northern Province (Gahutu, 2013), and shown the similar results as the previous study (Gahutu & Wane, 2006) in Rwandan students in Northern Province.

The significantly higher values of the reference values for Creatinine and Chloride in male compared to female, and the higher values of the reference values for Sodium in female compared to male indicates sex differences in these clinical chemistry parameters. Sex differences in Creatinine have been known to exist due to differences in muscle mass. Similar findings have been reported in adult black populations of Kampala, Uganda; Kericho, Kenya; Mbeya, Tanzania; Huye, Rwanda; Kintampo, Ghana and adult white USA populations (Eller et al. 2008; Gahutu & Wane 2006; Kibaya et al. 2008; Roche Diagnostics, 2015; Saathoff *et al*. 2008; Dosoo *et al*. 2012).

The slight differences due to gender (sex) in the reference range for total protein and albumin could be attributed to the size; however, this difference may not have any clinical significance. The sex difference in the reference range values for serum total protein observed in this study are in contrast to that reported for the American population (Roche Diagnostics, 2015) where males and females have common reference range values but agrees with the findings of a Rwandan study (J. Gahutu & Wane, 2006).

Serum concentration of Bilirubin Total and Bilirubin Direct, Total proteins and Albumin show high values in age of 30 years and below comparing to that of above 30 years individuals in accordance with Gahutu 2013 and Wane 1985.

Sex differences in the total bilirubin and direct bilirubin values could be partly due to influence of sex hormones. These findings are in agreement with those of similar studies done in Uganda (Eller et al. 2008) for adults.

Differences in values of liver enzymes higher compared to our study for both males and females have been reported in Kenya(Kibaya *et al*., 2008). Bilirubins, Glucose, electrolytes values compare well

with the reported values in our study, except low values in serum concentration of Chloride in our study.

This study shows low levels of urea in females compared to that in males, and shows a slight decrease in age, this confirming a study done in Congo by Wane 1985. Serum concentration of urea in this study is low compared to the results published in other studies. This may due to a diet low in animal proteins common in Rwanda.

Overseas similar results have been reported with differences in higher serum concentrations of Bilirubin Total, Creatinine, Urea, Chloride and Alkaline Phosphatase for both males and females(Sluss et al., 2008).

Variations have been observed in other studies in the region (Karita et al., 2009; Palacpac et al., 2014; Saathoff et al., 2008) which may result in differences in age and living environment.

In conclusion, the results of our study on Clinical Chemistry parameters are similar to those published in other African countries, with variations due to the diet and geographical location.

This study has shown that a strict adherence to reference ranges developed from industrialized countries could qualify many healthy Rwandans as pathological cases, and also exclude them from participating in clinical trials.

Compared to other reference ranges established, reference values in our study presented remarkably low levels of urea which may be due to the diet low in proteins generally in Rwandan population.

Our study not only defined the reference ranges of healthy Rwandans attending NCBT in CHUK, but also enhanced laboratory capacity of Clinical Chemistry service as it was conducted under the guidelines of Good Clinical and Laboratory Practice, thus preparing the site to Clinical trials as a center of research.

Table 3
Comparison of established reference ranges to those in literature

Analytes (unit)	Gender	Established reference ranges		Northern province-Rwanda		Central Province-Rwanda		Central part of Ghana		Ugandan adult blood bankdonors		Kericho, Kenya		Case records of Massachusetts General Hospital		Mbeya, Tanzania	
		Lower	Upper	Lower	Upper	lower	upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
BIL-D (µmol/L)	Male	2.0	6.9	2	7	0.4	8.8	0.9	4.1	1.71	8.5	1.3	9	1.7	5.1	0.93	8.43
	Female	2.6	6.5	-	-	-	-	0.8	4	0	6.8	0.8	6.7	-	-	0.70	5.83
BIL-T (µmol/L)	Male	4.8	21.6	3	29	2.9	37	3.8	32	6.8	44.4	5.6	41.9	5.1	17	6.0	42.0
	Female	5.9	17.3	-	-	-	-	2.7	26.6	5.1	32.5	4.4	26.8	-	-	4.5	31.3
TP (g/L)	Male	68.2	87.6	65	85	58	88	46.7	86.4	65	89	-	-	55	80	67.2	85.2
	Female	66.6	85.7	-	-	-	-	55.2	86.9	68	90	-	-	-	-	65.8	85.5
ALB (g/L)	Male	39.7	55.5	34	54	35	52	32.7	49.8	39	55	36.9	48.5	35	55	37.06	50.72
	Female	40.0	54.5	-	-	-	-	33.5	50.4	37	52	34.4	47.5	-	-	35.57	49.27
ALP (U/L)	Male	43.8	145.7	27	122	48	164	101	353	42	199	-	-	-	-	45.4	170.4
	Female	50.3	138.4	-	-	-	-	82	293	47	160	-	-	-	-	45.3	155.0
ALT (U/L)	Male	7.2	36.2	12	43	8	61	8	54	7.2	43.3	10.8	53.9	0	35	9.1	55.3
	Female	7.3	33.9	-	-	-	-	7	51	5.3	39.9	8.6	47	-	-	6.7	44.9
AST (U/L)	Male	16.1	49.2	16	47	14	60	17	60	13.2	35.9	14.9	45.3	0	35	15.2	53.4
	Female	16.8	45.1	-	-	-	-	13	48	11.4	28.8	13.1	38.1	-	-	13.5	35.2
GGT (U/L)	Male	8.0	75.6	9	77	-	-	9	71	8.7	70.7	-	-	-	-	9.3	120.8
	Female	7.1	63.3	-	-	-	-	6	53	8	41.3	-	-	-	-	7.3	51.8
CREA (µmol/L)	Male	65.2	107.1	44	97	47	109	56	119	53.1	106.2	62	106	0	133	48	96
	Female	62.5	98.6	-	-	-	-	47	110	44.2	79.6	51	91	-	-	40	81
UREA (mmol/L)	Male	1.3	5.8	-	-	-	-	0.9	6.2	-	-	-	-	3.6	7.1	-	-

	Female	1.4	5.2	-	-	-	-	0.9	5.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Na+ (mmol/L)	Male	134.5	145.5	137	147	-	-	135	151	136	148	141.8	152.1	136	145	-	-	-	-	-	-	133.6	143.1	
	Female	134.5	146.5	-	-	-	-	135	150	135	146	140.3	155.3	-	-	-	-	-	-	-	-	133.4	142.3	
K+ (mmol/L)	Male	3.7	5.0	3.3	5	-	-	3.6	5.2	3.4	4.8	3.9	5.8	3.5	5	-	-	-	-	-	-	3.8	55	
	Female	3.5	5.0	-	-	-	-	3.4	5.1	3.4	4.8	3.8	5.8	-	-	-	-	-	-	-	-	-	-	
Cl- (mmol/L)	Male	89.9	104.2	100	112	-	-	101	115	96	104.6	100.4	110.8	98	106	-	-	-	-	-	-	97.9	108.3	
	Female	90.6	103.1	-	-	-	-	103	113	97.4	104.5	101.1	113.4	-	-	-	-	-	-	-	-	97.8	107	
Mg+ (mmol/L)	Male	0.7	1.0	-,	-	-	-	-	-	0.4	1	-	-	0.8	1.2	-	-	-	-	-	-	0.67	0.97	
	Female	0.7	1.0	-	-	-	-	-	-	0.4	1	-	-	-	-	-	-	-	-	-	-	0.71	0.95	
P3+ (mmol/L)	Male	0.8	1.5	-	-	-	-	0.742	2.032	-	-	-	-	-	-	-	-	-	-	-	-	0.7	1.48	
	Female	0.7	1.6	-	-	-	-	0.876	1.806	-	-	-	-	-	-	-	-	-	-	-	-	0.72	1.52	
GLU (mmol/L)	Male	3.2	7.7	-	-	-	-	-	-	3	5.6	4.2	6.4	4.2								2.88	5.3	
	Female	3.1	6.7	-	-	-	-	-	-	3.2	5.7	-	-	-	-	-	-	-	-	-	-	3.3	5.06	

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