DETERMINATION OF REFERENCE RANGES FOR SELECTED CLINICAL LABORATORY TESTS FOR A MEDICAL LABORATORY IN NAMIBIA USING PRE-TESTED DATA

by

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(i) Declaration

I, Cornelia de Waal-Miller, declare that the content of this thesis represents my own unaided work, and that this thesis has not previously been submitted for academic examination towards any qualification. Furthermore, this thesis represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

12th March 2015

Signed **Date**

(ii) Abstract

Aim: The aim of the study was to compile pre-tested laboratory results stored in the laboratory database of the Namibia Institute of Pathology (NIP). The study also aimed to assess the usefulness and validity of using retrospective laboratory results of different patients in varying degrees of health and which were produced using various methods in different laboratories in Namibia.

Methods: 254,271 test results (female: 134,261, male = 117,091, unknown gender= 2,919) consisting of Haemoglobin, serum Urea, serum Creatinine, plasma Glucose (fasting and random), serum Cholesterol, serum Triglycerides and serum Uric Acid was extracted from NIP Laboratory Information System over a period of four years and of the 13 different regions of Namibia were analyzed.. Each data set was sorted in ascending order and outliers were eliminated using SPSS Box plot function.

Data available for analysis were Haemoglobin: 18,999 (male = 7,716, female = 11,283, serum Urea: 8,111 (male = 3,836, female=4.275), serum Creatinine**:** 8,794 (male=4,099, female= 4,506), plasma Glucose: 78,106 (fasting=32,591, random=45,515), serum Cholesterol: 48,354 (male=24,815, female=23,539), Serum Triglycerides: 22,138 (male=9,291, female=12,847) serum Uric Acid: 37,389 (male=18,972, female=18,427). Results of tests were also analysed according to the 13 regions in Namibia. Outliers were removed using the Box plot function of SPSS and statistics were calculated for each of the parameters. Tables and histogram as well as percentile ranges (2.5^{th} -97.5^{th} and 5^{th} -95^{th}) were determined for each parameter.

Results: Non-parametric percentile ranges were as follows: Haemoglobin (2.5-97.5: M=6.64-16.9, F=7.81-15.2 and 5-95: M=7.39-16.3, F=8.48-14.7) g/L, Urea (2.5-97.5: 1.3-9.1, 5-95:1.6-8.4) mmol/L, Creatinine (2.5-97.5: M=37-141, F=33-103 and 5-95: M=43-133, F=39-117) µmol/L, Glucose (2.5-97.5: fasting=3.4-9.5, random=3.7-7.1 and 5-95: fasting=3.9-9.1, random 4-6.9) mmol/L, Cholesterol (2.5-97.5: M=2.6-6.9, F=2.8-7.0 and 5-95: M=2.9-6.1, F=3.1-6.2) mmol/L, Triglyceride (2.5-97.5: 0.39-2.72 and 5-95: 0.46-2.5) mmol/L and Uric Acid (2.5-97.5: M=0.21-0.62, F=0.17-0.51 and 5-95: M=0.24-0.58, F=0.19-0.48) mmol/L.

Conclusion: A statistically significant difference between the mean values of the study and the mean values of NIP reference range was detected and differences between these values and reference values in the region were observed. More work needs to be done to improve the data extraction process, data selection criteria and improvement of statistical analysis. If these can be addressed, it can be stated that using patient laboratory data values is a relatively easy and cost effective method of establishing laboratory and population specific reference values if skewness and kurtosis of the distribution are not too large.

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(iv) Dedication

To my children, Maryke Jebavy, Hannes de Waal, Erik de Waal and Elize Heyns who often had to make sacrifices to allow their mother to continue with her studies.

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(v) Glossary

Definitions

Definitions are based loosely on literature from Solberg (2006); Geffre *et al.,* (2009); Ceriotti *et al.*, (2008) and Horn (2010).

Reference individual	This a person selected for testing on the basis of well-defined criteria. They are usually assumed to be "healthy".
Reference population	This is a group of persons who meet the defined criteria for a reference individual.
Reference sample	An adequate number of reference individuals meeting the selection criteria to be included into the sampling group and who represent the reference population.
Reference value	This is the value, test result obtained by measurement or observation of a specific quality or quantity of any reference individual.
Reference interval (1)	"A reference interval is the interval between, and including, two reference limits, which are values derived from the distribution of results obtained from a sample of a reference population" (Ceriotti, *et al.,* 2008).
Reference interval (2)	It is the interval between the upper limit and lower limit of the reference distribution and will include two reference limits. It mostly comprises the central 95% of the measured values, (i.e. between 0.025 and 0.975 fractiles or 2.5th and 97.5th percentiles as defined by IFCC).
Reference distribution	A reference distribution is the distribution of reference values.
Reference limits	Define the value of the upper limits and the lower limits of the reference distribution, and are estimates of true limits.
Reference range	A reference range is usually defined as the set of values within which 95 percent of the normal population fall.
Observed value	The patient laboratory test result to be compared with the reference value.
Decision limit	Indicates the cut-off point or thresholds between health and disease used by clinicians to make diagnostic decision and medical action.
Parameter	A quantity that defines certain features of a population (mean, SD, CV, mode, average).
Variable	Quantity that varies within or between individuals, not be confused with parameter.
Confidence interval (CI)	A value that, within a given probability, will contain the value of the unknown population parameter and indicates the imprecision of that estimate.

Inclusion and exclusion criteria	Determining factor to determine eligibility of a person to be included or excluded from the population selected for reference study.
Tolerance interval	Interval within which, with predictable confidence, a specific proportion of a population falls, and is based on the confidence limits.
Type of quantity	A particular type or quantity in laboratory medicine is a test result, component, analyte of which the concentration has been measured.
Outlier	Observation in population that is different from the reference population. It can be an erroneous result or observation that does not conform to attributes of the reference range.
Gaussian	Symmetrical distribution also called "normal distribution"
Normal	In the context of health and reference values, it means persons without any disease indications, i.e. healthy.
Abnormal	Test results outside the reference limits, results from people that are not in good health.
Partitioning of reference values	Process of separating reference intervals based on criteria like gender, age, race, and even statistical analysis which show significant differences between populations (Horn, 2010).
Healthy	Absence of disease.
Standard deviation (SD)	Measure of variability.
Mean	Average obtained from a group of observations.

Abbreviations

NCCLS	National Committee for Clinical Laboratory Standards.
WHO	World Health Organization.
IFCC	International Federation of Clinical Chemistry.
CLSI	Clinical Laboratory Standards Institute.
SEMDSA	Society for Endocrinology, Metabolism and Diabetes of South Africa.
SI units	International System of Units (abbreviated SI from French: Système international d'unités.
SD	Standard Deviation
SQL	Structured Query Language.

Chapter 1. Background and Literature Review

1.1 Background

Apart from a few HIV and tuberculosis related studies (Hamers *et al.,* 2008; de Beer *et al.,* 2009) and un-published reports of the Ministry of Health and Social Services, no further studies, to the author's knowledge, have been carried out using data generated in a diagnostic medical laboratory in Namibia. Documented information on health trends based on laboratory data does not exist and population-based reference ranges of any test and analyte values for the Namibian populations have never been determined.

The population of Namibia is approximately 2 million, widely spread across a vast country and is ethnically diverse. It would, therefore, be helpful to the medical fraternity to have appropriate regional reference values available. A generic set of values determined could be used across the population as a whole, but results based on local groupings would be best for clinical usage.

The principal aim of this study was to utilize the pre-tested or historical laboratory data that exists in the Laboratory Information System (LIS) of the Namibia Institute of Pathology (NIP) to determine population-based laboratory reference ranges for Namibia. Reference values used in all disciplines in the medical laboratories in Namibia have been established elsewhere, either in South Africa or values are used as supplied by the reagent manufacturers. It is, therefore, assumed that the inaccurate and inappropriate reference ranges used currently may impact negatively on the treatment and monitoring of various disease conditions (Solberg, 2006; Horn, 2010). It may even be that some conditions are not treated where necessary and others are treated inappropriately.

In lieu of this, it is envisaged that the huge amount of data available in the laboratory information system (LIS) database of laboratories in the country is sufficient to determine reference ranges using statistical analysis. With an appropriate software interface selected parameters could be extracted. This information can then be analyzed according to pre-determined criteria.

Since Namibia covers a vast geographic area with a small population comprising of many ethnic groups, it is assumed that data generated in the laboratory might vary according to regions and population groups, and interesting patterns and trends might emerge if the data is collected and analyzed.

The objective of this study, therefore, was to analyze the existing clinical laboratory data in the LIS database of the Namibia Institute of Pathology (NIP) and assess the suitability of the data for the determination of population-based reference ranges for general laboratory tests for the clinical laboratory by age, gender and location of origin. This information could then be utilized to develop and define national population-based clinical laboratory reference ranges for Namibia.

1.1.1 Clarification of terms

Many studies propose the use of the term “reference interval” (Friedrichs *et al.,* 2011) and discourage the use of the term “reference range”, whereas others use the terms “reference range”, “reference interval”, “reference values” and even “normal values” interrelated. For the purpose of this study, the term “reference range” will be used because it is felt that the word “interval” suggests an upper and lower boundary, which is in fact the case. Yet, the author of this dissertation asserts that the word “range” better describes the fluidity of biological values, and the term would therefore convey a movement across values which are then hypothetically capped by an upper and a lower limit. This may also have a confidence interval of 95%, i.e. most of healthy subjects would fall within these reference limits, but a few may be healthy or not healthy, although still be outside these limits.

1.2 Research questions

1.2.1 Research problem

No locally developed reference ranges for the clinical laboratory exist in Namibia. Reference ranges used have been adopted from various sources. Since a huge amount of pretested laboratory results exists in the laboratory database of the NIP, the suitability of this data to develop local reference ranges was investigated in this study.

1.2.2 Hypotheses

1.2.2.1 Hypothesis One

It was hypothesized that the vast amount of historical or pre-tested data which is housed in the laboratory information system of the Namibia Institute of Pathology (NIP) would provide valuable health information once extracted and analyzed. The laboratory test result values in the existing LIS data base of the NIP laboratory information system from general patients, both healthy and diseased, is sufficient to generate values that are statistically valid.

1.2.2.2 Hypothesis Two

It was hypothesised that the results derived from this study will create a basis for defining reliable clinical laboratory reference ranges for the Namibian population.

1.2.2.3 Hypothesis Three

It was hypothesised that the results derived from this study are comparable to the reference range in use in Namibia Institute of Pathology (NIP).

1.3 Research aims

The main objectives of this study were to:

1. Extract existing and pre-tested clinical laboratory data from the LIS of the Namibia Institute of Pathology (NIP) into a database in a format which is suitable for statistical analysis.
2. Define reference ranges for each test and determine suitability of this indirect method for the determination of population based reference ranges for selected routine tests for a clinical laboratory.
3. To discuss possible reasons for differences between reference ranges obtained to those in use in NIP.

1.4 Literature review

1.4.1 Theory and development of reference ranges

Patient results produced in the laboratory are used by clinicians to make medical diagnosis and assess the patient's physiologic functions, thus enabling treatment, monitoring and managing therapy. During interpretation of laboratory data, measured test values are compared to reference ranges, considered to be the range in which "normal" values fall (Solberg, 2006; Rohan *et al.,* 2007; McCudden *et al.,* 2010). Researchers (Murphy, 1966; Neumann, 1968; Gräsbeck, 1990; Gräsbeck, 2004; Petitclerc, 2004; Ceriotti and Henny, 2008) have battled with the concept "what is normal, and what is not normal". As stated by Schneider (1960), the first function of a clinician is to decide if the patient belongs to a group of individuals defined as "healthy" or to the group of individuals who do not belong to the group defined as "healthy". It is, therefore, important to identify the state of health or disease. As Schneider (1960) states, "healthy persons are defined as those who have values of specific and selected attributes not characteristic of those defined states which seem important for the immediate purposes of the physician making the classification". Gräsbeck (1990) extensively discussed the concept health and disease where he stated that "health is relative, and the same individual may be regarded as both healthy and ill, depending on the situation". This was re-iterated by Geffre and co-workers (2009) who stated that "health is relative and lacks a precise and quantifiable definition".

The concept of reference intervals was first introduced by Gräsbeck and Saris in 1969 (Gräsbeck, 1990). He stated that before 1969, hardly any information existed regarding the theory and importance of reference ranges. This was partly due to the limited number of laboratory tests offered as well as the lack of quality indicators, which resulted in large deviations from values usually found in the general population. This concept first started out as

a philosophy but has since become a major discussion point in laboratory medicine and one of the most powerful tools to assist in the clinical decision making process (Geffre e*t al.,* 2009).

Barth (2009) stated that "reference ranges provide critical information to guide clinicians in their decision making". He also stated that discussion on development and use of reference ranges should consider how clinicians utilize this information in medical decision making. The most common type of clinical data is *health-associated* and is derived from a sample of a healthy reference population and another type is used for clinical decision making, termed *decision-based* and defines specific medical decision limits that clinicians use to diagnose or manage patients (Friedberg *et al.,* 2007). Often these values are used interrelatedly and determine the clinical and medical decision limits that define values that require prompt attention by the clinician (Geffre *et al.,* 2009; Horn, 2010).

The importance of correctly determined reference values was emphasized by Friedberg and co-workers, who stated that "test results are 'framed' by reference intervals, and the use of aberrant frames can bias decision-making" (Friedberg *et al.,* 2007).

It is apparent from many sources, that the importance of reference values are not so much to define "normal" and "abnormal" or health or disease, but assist clinicians to make appropriate diagnosis and monitor treatment of patients. Clinicians require decision-making values, rather than "normal values" (Barth, 2009). According to Bock and co-workers (2003) have shown that 60% to 70% of all critical decisions in medicine are made based on information provided through laboratory results. It is also stated that although medical decision making is increasingly based on laboratory results, thus emphasizing the importance of laboratory reference values, the approach to the generation of these values has remained unchanged for the past 20 years (Bock *et al.,* 2003).

1.4.2 Principles for determining reference ranges

The three principal methods to determine reference ranges are: 1) conventional method or *priori* method which conducts a comprehensive reference range determination study using the International Federation for Clinical Chemistry (IFCC) recommendations, 2) the *posteriori* method where data is analysed that has been pretested and where sufficient information to determine selection criteria is available, and 3) indirect determination analysing large amounts of data from laboratory databases and applying statistical calculation to the data set (Westgard, 2004; Arzdeh, 2008; Ceriotti *et al.,* 2009). While option 1 is probably the best method, conventional reference studies are normally done when sufficient resources (money, time and human capacity) are available. However, for various reasons alluded to in this discussion, it is not possible to use this method most of the time.

Laboratories generally use various techniques to determine health associated reference ranges. These include transference and validation of reference values from published data or from other laboratories and adopting standards recommended by regulatory agencies or basing the laboratories reference values on statistical analysis of the laboratories own assayed specimen results (Boyd, 2008; Geffre *et al.,* 2009). Many laboratories also adopt reference values from manufacturers, often without on-site correlation with healthy persons, however it is stated that reference values that are not re-tested and compared with healthy individuals are not statistically much different from those that have been correlated with the laboratories' healthy patient population (Friedberg *et al.,* 2007).

The classical method, as presented in paragraph 1.4.2.1 to determine reference ranges, has been discussed by many authors (Hyltoft Petersen, 2004; Solberg, 2006; Rohan *et al.,* 2007; Horowitz, 2008; Barth, 2009; Horn, 2010 and McCudden *et al*., 2010). Aytekin and Ermerk (2008) even demonstrated this process using a flow diagram (**Figure 1.1**) to illustrate this traditional process.

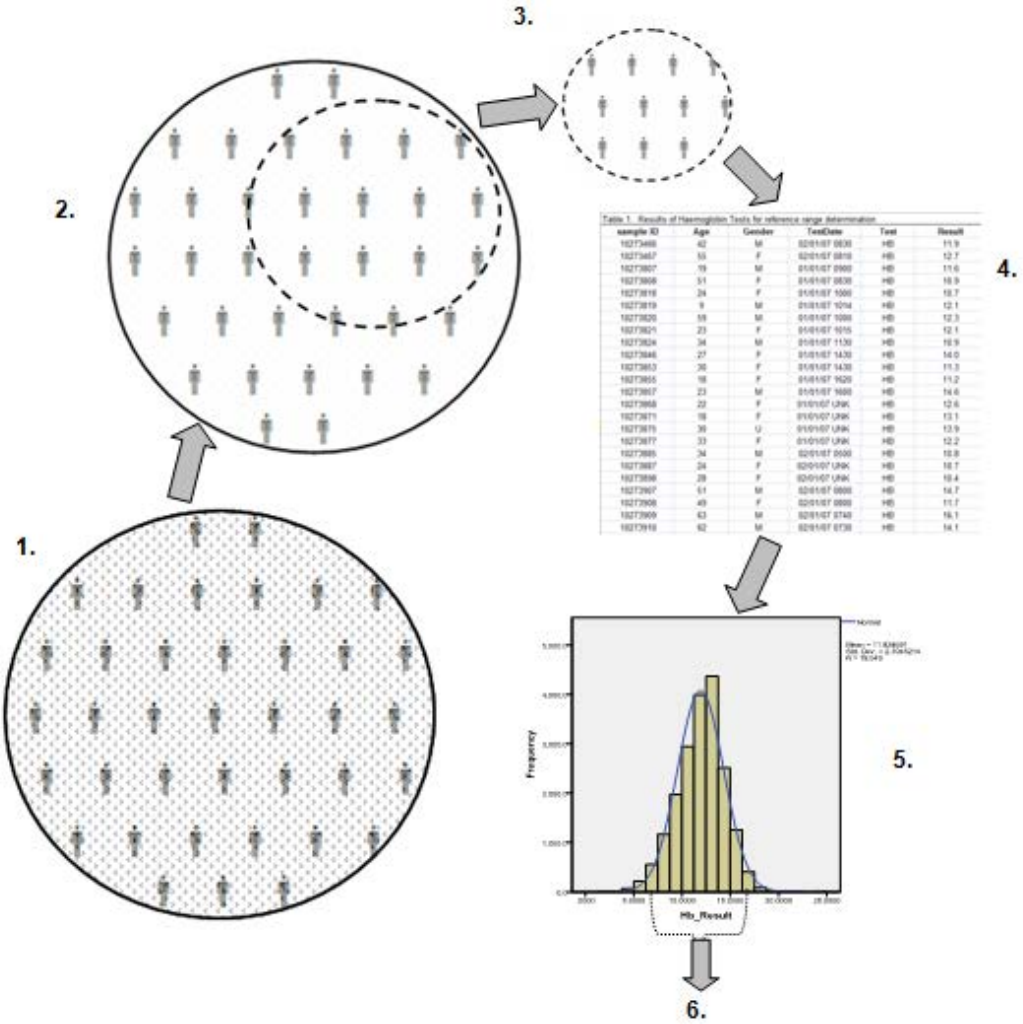
The protocols described are based on recommendations laid out in the International Federation of Clinical Chemistry (IFCC) in the Clinical Laboratory Standards Institute (CLSI) C278-P3 guidelines (IFCC, 2008). This conventional method to determine reference ranges describes how a reference study should be conducted as an independent investigation, using a comprehensive sampling plan, inclusion / exclusion criteria and carefully designed questionnaires.

1.4.3 Steps for reference interval determination

The conventional protocol for establishing reference ranges is proposed in IFCC/CLSI document C28-P3 (IFCC, 2008), and is loosely adapted as follows:

1. Consideration of biological variables and analytical interferences.
2. Identification and control of pre-analytical and analytical variables.
3. Selection of reference individuals carefully considering exclusion and partitioning criteria. Health status of individuals to be documented. Selection of one particular group, e.g. young adults should be avoided. Laboratory test results should also not be used, according to IFCC recommendations.
4. Reference individual questionnaire prepared.
5. Potential reference individuals to be categorized by using health investigations and questionnaire responses for selection of test population.
6. Inappropriate individuals that do not meet the stipulated criteria must be excluded.

7. Specimens are then collected from the selected group of reference individuals. Collection procedure should be consistent with procedures used to collect patient samples.
8. Specimens are then analyzed in a controlled environment with approved and validated technology.
9. After data is collected, data is analyzed and a frequency distribution histogram prepared. This should be examined visually to detect outliers. Statistical tests should then be applied to determine outliers.
10. IFCC strongly recommends the nonparametric method whereby 120 samples are to be collected and analyzed. If that is not possible the robust method is also recommended for use.
11. Reference interval is determined by the values above the lower 2.5% and below the 97.5 % interval.



1. General Population
2. Reference Population
3. Reference sample group
4. Table of Test results of sample group
5. Reference distribution graph
6. Reference interval (approximately 90% of inner values of distribution)

- *Persons who do not match selection criteria*

Persons who match selection criteria / reference individual

Figure 1.1 Process of Selection of reference individuals and determination of reference interval (adapted from Aytekin and Ermerk, 2008)

1.4.4 Challenges when establishing reference ranges

1.4.4.1 Concept of health and disease in selecting reference individuals

What is health? This question has been debated by many people since antiquity. The World Health Organization (WHO) defines health as “a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity” (WHO, 1948).

Since then many authors have discussed the philosophy of health relating to laboratory test results, and the subsequent results related to “healthy” or normal persons. The WHO definition appears to be very rigid and is often not met by what are considered “normal” healthy people. The decision concerning who is healthy and who is not is an important aspect for consideration when selecting an appropriate population on which to base reference range studies. According to the ICFF protocol, firm criteria have to be defined for the selection of reference population and for carrying out the study.

One can therefore infer that many challenges exist in determining reference ranges using the IFCC protocol. Henny (2009) discussed the various steps and challenges in each when conducting a reference range study. He alluded to selection of reference individuals, where definition of “good health” was extremely important, selecting individuals with no underlying disease conditions as well as dividing the selected population into well-defined inclusion criteria. He also referred to the importance of pre-analytical and analytical factors, as well as determination of the minimum number of individuals to include (Henny, 2009). It is therefore obvious, as observed by Petitclerc (2004), that it is nearly impossible to select an appropriate number of reference individuals who are representative of the biological diversity and complexity in the health environment. It is asserted that the health definition of the WHO can be criticized as being too rigid which is reiterated by several authors. Murphy (1966) stated: “try as we may, we cannot come up with anything like an absolute definition of the normal from a scientific viewpoint” and that “normalcy is a vestigial concept left in medicine from its unscientific era”. Gräsbeck (1972) asserted that "Absolute health does not exist. Some degree of pathology is present in every individual, like entropy in a chemical system." Gräsbeck (1990) gave the following definition for health: "Health is characterized by a minimum of subjective feelings and objective signs of disease, assessed in relation to the social situation of the subject and the purpose of the medical activity, and is in the absolute sense an unattainable ideal state". It can be seen that it is virtually impossible to select a suitable population for reference value studies that are absolutely healthy as per the WHO definition.

From this discussion it follows that the use of reference ranges is more about detecting abnormal results than to determine the values for normal healthy persons (Ritchi and Palomaki, 2004).

In determining reference ranges, even using the recommended methods, there will always be a level of uncertainty regarding the health status of the subject, since some may have various subclinical conditions, which may not have been detected in the selection process (Katayev *et al.,* 2010). Baadenhuijsen and Smit (1985) conclude that the use of the traditional method for determining reference values would not always provide the information needed for proper clinical decision making, because "what is gained in sensitivity is lost in specificity". Since 80% of clinical decisions are based on information provided by laboratory reports, clinicians rely heavily on laboratory results. Therefore, the test result is of little value unless it is supported by comparative information in the form of reliable reference values (Katayev *et al.,* 2010).

1.4.4.2 Variations in results of biological parameters

Difficulties relating to reference value determination are true standardization, true local population differences, agreement on format of results and reference intervals across laboratories (Jones *et al*., 2004). They reported that "the current paradigm for generation and maintenance of laboratory reference intervals is difficult and expensive to implement correctly, in general it is poorly performed, and does not meet the needs of patients and doctors."

Adeli (2008) asserted that healthy populations to be sampled for reference studies should span a range of anthropometric parameters including ethnicity, gender and age since many parameters display interdependencies which could affect the outcome of the results. This position is confirmed by Henny (2009) who reported that the metabolic and physiologic processes may differ between individual's age, gender, different ethnic and genetic backgrounds, lifestyle, diet and many others. As such, it is nearly impossible to find a sufficient number of individuals who meet all the criteria, especially taking into account the ageing populations in many countries (Petitclerc, 2004). Critical gaps in reliable reference values can potentially contribute towards incorrect disease diagnosis and inappropriate treatment (Adeli, 2008). It is critically important that more reliable and comprehensive reference ranges be established for specific populations.

Taking into account the variables that affect laboratory analyte concentration such as age, environment, lifestyle, nutrition, ethnicity, gender, age and demographics, it becomes challenging to select a sample group that complies to all the criteria, especially considering that a hospitalized patient often has different variables in terms of diet, activity, diurnal rhythm and environment (Harris, 1974; Ceriotti and Pesce, 2003; Ritchi and Palomaki, 2004; Arzdeh, 2008; Ceriotti *et al.,* 2009). Another challenge is the influence and effect of biological variation and

inter-individual variability on reference ranges, especially for a hospitalized patient or one who is not completely “healthy” (Fraser, 2004; Henny, 2004; Petitclerc, 2004; Queralto, 2004; Ricos *et al.,* 2007; Liu, 2009; Ceriotti and Henny, 2008; Houilier *et al.,* 2010). Different analytes have different ranges of biological variation (Houilier *et al.,* 2010), and seasonal variations may also influence the baseline range of reference values (Janecki, 2009). Ceriotti and co-workers reported further that the width of the reference interval is influenced by three sources of variability: the intra- and inter-individual biological variability of the selected reference individuals and analytical variability of the measurement system (Ceriotti *et al*., 2009).

Therefore the identification and selection of a meaningful reference population for testing that meets all the inclusion criteria identified, but which is also representative of the population, is extremely challenging, logistically difficult and prohibitive due to the high costs in time and money (Solberg, 1994; Ilcol and Aslan, 2006; Inal *et al.,* 2010).

1.4.4.3 Globalization and population shifting

Another important aspect becoming more pertinent is the global diversity appearing as a result of populations mixing.

Due to this global phenomenon, previously homogenous populations are increasingly becoming heterogeneous. As a result, the reference ranges of several analytes might change. The effects on reference values of this heterogeneity is an important aspect mentioned in several publications (Punyadeera *et al.,* 2001; Horn and Pesce, 2002; Johnson, *et al*., 2004; Ritchi and Palomaki, 2004; Hyltoft Petersen, 2005). In the past, ethnicity was perceived to be an important criterion in determination of reference ranges. However, this issue is losing its distinction due to the development of multicultural societies. Most populations in recent times are becoming more mixed, causing disease patterns and prevalence that do not necessarily reflect the original endogenous pattern (Aytekin and Emerk, 2008; Barth, 2009).

As the diversity of the global population increases, a serious need exists to develop meaningful and reliable reference values that can be used across regions and population groups (Adeli, 2008). One question that arose is: what are the risks of using outdated, inaccurate, and inappropriate reference values? Critical gaps in reliable reference values can potentially contribute towards incorrect disease diagnosis and lead to inappropriate treatment. It is critically important that more reliable and comprehensive reference ranges be established for specific populations (Adeli, 2008). Due to the population diversity, it becomes difficult to identify race and ethnicity and also most laboratories do not record the race and / or ethnicity of a patient sample. This applies specifically to laboratories in Namibia. This would mean that reference ranges must be determined specifically for the population that a laboratory serves.

1.4.4.4 Regionalization and patient movement

In the past patients stayed in one hospital for the majority of their treatments. With more options and better transportations options, patients often move between hospitals and different areas, and are treated by several doctors and tests are done in various laboratories. Comparability of results and standardized reference ranges is therefore important for optimal patient care. Incorrect interpretation of results may be due to inconsistency in test results and inconsistencies in appropriate reference ranges (Jones *et al.,* 2004).

Horn (2010) stated, "it is sometimes acceptable to transfer a previously established reference interval that is based on a valid reference value study from a donor laboratory or manufacturer to receiving laboratory without performing a new, full-scale study". However, such a transfer would only be acceptable if the test population, the methodology and the environment is the same or reasonably comparable to the testing laboratory that wishes to adopt these reference values. Although transference of reference values are permitted from one population to the next provided that certain criteria are met, there is an increased risk that these transferred values may not be representative of the geographic variations in patient demographics and disease prevalence (Bock *et al*., 2003).

Although it is allowed to "transfer" reference values from one population to the next if certain criteria are met, it still increases the risk that the set of reference values so obtained does not represent the unique variations in patient demographics and disease prevalence, as is the case in Namibia.

1.4.4.5 Technological advances

Another important challenge is the introduction of more and more sophisticated and advanced technologies into medical laboratory science such as molecular diagnostics, point of care testing, proteomics and genomics. Together with the rapid globalisation of human beings, these new technologies create enormous challenges for those participating in reference range studies or establishing reference intervals for laboratory tests (Petitclerc, 2004; Aytekin and Emerk, 2008).

Previously text books published detailed lists of reference ranges for most of the conventional analytes tested in the laboratory (Tietz (ed), 1986; Vaughn, 1999; Bishop (ed), 2005;, Wu, 2006; Chernecky and Berger, 2008). However, textbooks no longer publish reference ranges because of the issues discussed above.

Internet websites from various laboratory organisations now publish reference ranges for some clinical chemistry tests (Duh and Cook, 2005; CML HealthCare, 2012; Oak and Platter, 2012). For many developing countries the reference ranges used are developed in other countries.

Namibia uses reference ranges which have been established elsewhere, some have been adopted from Tietz (1986) or from other laboratories like the National Health Laboratory Services (NHLS) in South Africa. In many instances, where instruments are used that were manufactured in either Europe or the United States of America, reference values have been determined by the manufacturers in those countries. These countries also mostly reflect values of a homogenous Western population which might not be transferable to a more heterogenous population such as is the case in Namibia.

1.4.4.6 How reference ranges are used currently

For most developing countries, the determination of reference values using the traditional recommended method is not feasable due to excessive cost, time constraints and the challenges of identifying an appropriate reference population in a multicultural environment (Shine, 2008). Added to these challenges is the difficulty in identifying enough "healthy" subjects due to underlying non-recognisable sub-clinical processes (Dimouro, 2008).

Regardless of the various recommendations and guidelines for establishment of acceptable reference ranges, Friedberg and co-workers (2007) asserted that "little is known about how laboratories establish health associated reference intervals in practice. During 2004, 1.3% of laboratories enrolled in the College of American Pathologists (CAP) Laboratory Accreditation Program were cited by inspectors for failing to validate reference intervals properly. The specific types of omissions were not documented and it is not clear whether inspectors approached this problem in a consistent manner" (Friedberg *et al.,* 2007).

Although IFCC recommendations are widely adopted as the "gold standard" for reference range determination, Hyltoft-Petersen (2004) stated that more discussion on reference range determination is needed, since the "concepts of reference values and reference intervals are not static, and they are still changing" although the fundamental ideas still remain the basis for reference study discussions.

1.4.5 Recent and current international initiatives to determine reference ranges

The applicability of reference ranges in specific settings and for heterogeneous populations is currently undergoing serious reconsideration. It is being realized more and more that reference ranges need serious reconsideration, for their applicability in the specific setting and for the population that is being served.

1.4.5.1 Lack of uniform and harmonized units of measure

One important factor affecting clinical decision making is the lack of uniform and harmonized units of measure (Berg and Lane 2011). This is fortunately not the case in Namibia since SI

units are used in all laboratories. Yet many textbooks, especially those published in the United States, still use imperial or traditional units of measure. Berg and co-worker (2011) reported that harmonisation of laboratory reference values are extremely important in clinical decision making. In their investigations it was identified that current reference values are based on historical ranges which were developed by manufacturers of instrumentation or were provided by reagent manufacturers, or were modified locally and adopted from other sources published in standard textbooks such as Tietz (ed) (1986) and Bishop (ed) (2005).

It is, therefore, recognised by all authors consulted that the concept of reference ranges and their importance in medical decision making needs to be revisited and reference ranges across populations need to be standardized to eliminate disjointed information and to allow movement of patients from one hospital to the next without compromising their care based on laboratory results.

1.4.5.2 Current standardization projects

Several projects are currently being carried out to standardize and harmonize reference values in different parts of the world for example, NORIP in Nordic countries, REALAB in Italy, TURA in Turkey, and CALIPER in Canada (Aytekin and Emerk, 2008).

The Nordic Reference Interval Project 2000 (NORIP) was carried out in 102 laboratories in the five Nordic countries, Norway, Sweden, Denmark, Finland and Iceland for 25 analytes frequently measured in the laboratory according to the IFCC protocol. The protocol for selection of individuals and testing was tightly controlled, as well as the testing methodologies where controls and calibrators were provided. The RefVal 4.0 software program was used to analyze the results. This program (RefVal or NORIP) implemented the IFCC recommendations for calculation of reference intervals (Solberg, 1995; Rustad, 2004; Rustad *et al.*, 2004; Solberg, 2004). These NORIP reference values were then used to validate a 70-year-old population (Carlsson *et al.,* 2010).

Another project is the 'Pathology Harmony Initiative' conducted in the United Kingdom. The initiative was started because studies discovered that many different reference ranges were used across laboratories in Britain, but were not based on scientific reasoning and needed to be standardized (Berg and Lane, 2011; Berg, 2012).

1.4.5.3 International reference range determination studies

Many individual initiatives to determine reference ranges are currently occurring across the globe. These studies have all been conducted using the recommended IFCC protocol.

These international reference range projects conducted in recent years are "Reference Ranges for Serum Creatinine and Urea in Elderly Coastal Melanesians" (Erasmus *et al.*, 1997),

"Diagnostic and epidemiological implications of regional differences in Serum Concentrations of Proteins Observed in Six Asian Cities" (Ichihara *et al.,* 2004), "Reference Intervals for Serum Proteins: Similarities and Differences Between Adult Caucasian and Asian Indian Males in Yorkshire, UK" (Johnson *et al.*, 2004), "Reference Intervals of Common Clinical Chemistry Analytes for Adults in Hong Kong" (Lo and Armbuster, 2012), "Reference Ranges of 17 Serum Biochemical Constituents in a Singapore Population" (Chua *et al.,* 1978), and "Age-specific PSA Reference Ranges in Chinese Men without Prostate Cancer" (Liu *et al.,* 2009).

1.4.5.4 Reference ranges in Africa

The traditional reference ranges that are currently used in many laboratories in Africa, notably in Namibia, were established by instrument manufacturers or using text book values, mostly from Tietz (ed), (1986). For most times they were not transformed to the specific laboratory as was proposed by Henny (2009), i.e. transference tests were not carried out.

In recent years several studies have been carried out in Africa using the recommended IFCC guidelines. The main purpose of many of these studies was to prepare the laboratory environment for drug trials. While several reference determination studies have been carried out in other parts of Africa (listed below), none have been carried out in Namibia.

Some examples of the studies carried out in Africa:

1. Plasma Lipids and Fatty Acids in urbanized Bushmen, Hereros and Kavangos of Southern Africa (Namibia) (Tichelaar *et al.,* 1992),
2. Lipoprotein(a) determination and risk of cardiovascular disease in South African patients with familial hyperCholesterolaemia (Scholtz *et al.*, 2000),
3. Reference Range of Serum Haptoglobin and Haptoglobin Phenotype-Dependent in Blacks (Kasvosve *et al.*, 2000),
4. Ethnic Differences in Lipid Metabolism in Two Groups of Obese South African Women (Punyadeera *et al.*, 2001),
5. Haematology and Biochemistry Reference Intervals in Eastern and Southern Africa (Karita *et al.*, 2004),
6. Haematologic and Immunologic Reference Values for a Healthy Ugandan Population (Lugada *et al.*, 2004),
7. Reference Intervals Studies Carried Out in Uganda On Blood Donors (Eller *et al.,* 2008),
8. Reference Ranges for the Clinical Laboratory Derived from a Rural Population in Kericho, Kenya (Kibaya *et al.,* 2008),
9. Establishment of Reference Values of CD4 and CD8 Lymphocyte Subsets in Healthy Nigerians (Oladepo *et al.,* 2009),

10. Reference Ranges for Some Biochemical Parameters in Adult Kenyans (Waitaka *et al.*, 2009),
11. Reference Intervals for Serum Total Cholesterol, HDL Cholesterol and non-HDL Cholesterol in Batswana Adults (Maphephu and Kasvosve, 2011),
12. Biochemistry, Immunologic and Haematological Reference Values for Adolescents and Young Adults in a Rural Population in Western Kenya (Zeh *et al.*, 2011),
13. Haematological and Biochemical Reference Values in the Middle Belt of Ghana (Dosoo *et al.,* 2012),
14. Reference Intervals for Biochemical Analytes in the Greater Accra Region in Ghana (Asare *et al.,* 2012).

To date, no study has determined specific reference ranges for Namibia, taking into account the diversity of the population, its diverse ethnicity and demographics. All the reference ranges currently used in Namibian laboratories were developed somewhere else.

It therefore important to take a new and innovative approach to development of population based reference ranges. As Theodorsen (2008) asserted, “The time seems ripe for a fresh look at techniques used for sampling, measuring and calculating reference intervals in order to take new bold steps in this field, the results of which are so useful and widely used in patient care”.

1.5 Indirect methods and alternative methods, pros and cons

1.5.1 Indirect method developments

In recent years many authors have investigated the use of pre-tested data produced in the laboratory to determine reference ranges for individual analytes. This is a very attractive alternative to the elaborate method of selecting healthy reference individuals and conducting the reference study according to the conventional recommended protocol. Since most laboratories have electronic records, huge data sets exist that could be utilized if an acceptable method was found which could produce reliable reference values especially in a resource constrained environment (Arzideh, 2008). Killeen (2009) stated that patient data can be used to determine reference ranges, although they may be generated from people presumed to be ill or not healthy. A variation in patient results due to pre-analytical factors will also be more comparable to the “real life” situation. It is stated that in a huge data set, the “abnormal” results will become statistically negligable (Oosterhuis *et al.,* 1990; Killeen, 2009).

This ”indirect” method has been discussed by many (Neumann, 1968; Amador and Hsi, 1969; Cook *et al*., 1970; O’Holloran *et al.,* 1970; Glick, 1972; White, 1978; Tsay, 1979; Naus *et al.,* 1980; Baadenhuijsen and Smit, 1985; Hemel *et al.,* 1985; Tango, 1986; Kouri *et al.,* 1994; Solberg, 1994; Kairisto *et al.,* 1994; Kroll and Saxtrup, 1998; Ferre-Masferrer *et al.,* 1999; Bock

et al., 2003; Grossi *et al.,* 2005; Giavarina *et al.,* 2006; Ilcol and Aslan, 2006; Dimouri *et al.,* 2007; Shine, 2008; Barth, 2009; Geffre *et al*., 2009; Inal *et al*., 2010; Katayev *et al.,* 2010; Dorizzo *et al.,* 2011). Several publications have discussed the advantages and disadvantages of using this indirect method (Solberg, 1994; Ilcol and Aslan, 2006; Katayev *et al.,* 2010). Although Arzdeh (2008) has proposed a sophisticated statistical method for use in separating two populations, he stated that "the main criticism of the indirect approach is about the assumption involved. It is asserted that in the presence of pathological values, it is impossible to apply some statistical tests to the estimated distribution for non-pathological values" (Arzdeh, 2008).

Hoffman (1963) stated that "normal values" are hidden in data sets of the total distribution of clinical laboratory results , and if an appropriate statistical tool could be employed, the "normal" population could be separated from the "non-healthy" population. This was also reported by others (Murphy and Abbey, 1967; Neumann, 1968).

1.5.2 Techniques for analysis of data obtained through indirect method

Techniques for statistical analysis to identify and separate "normal" from "non-normal" patient populations were developed by Hoffmann (1963) and Bhattacharya (1967). These techniques for statistical analysis were adopted and adapted by other studies (Neumann, 1968; Amador and Hsi, 1969; Reed *et al.,* 1971; White, 1978; Naus *et al.,* 1980; Baadenhuijsen and Smit, 1985; Hemel *et al.,* 1985; Ferre-Masferrer *et al.,* 1999; Bock *et al.,* 2003; Ilcol and Aslan, 2006; Katayev *et al.,* 2010). These statistical methods were used to attempt to separate the "healthy" from the "non-healthy" individuals. Other authors reported using different techniques for statistical analysis (Reed *et al.,* 1971; Tsay *et al.,* 1979; Tango, 1986; Kouri *et al.,* 1994; Kroll and Saxtrup, 1998; Grossi *et al.,* 2005; Inal *et al.,* 2010). Some authors have discussed the pros and cons using this technique (Oosterhuis, 1990; Solberg, 1994; Ilcol and Aslan, 2006; Katayev *et al.,* 2010), concluding that the "indirect" method has useful applications when considering the advantages that this entails.

1.5.3 Advantages of using the indirect method

By using the bulk of the hospitalized and laboratory patient population, a one-on-one relationship is automatically obtained between the selected population group and the group of people for whom the reference intervals are intended, i.e. the group under investigation and presumed "non-healthy" group. Glick (1972) asserted that, by using patient data to evaluate normal ranges, specific factors that may influence the reference range may be identified. This then presents information concerning how many subjects or which criteria are relevant when the conventional reference study is done on that specific population. With regard to assessment of quality control in the laboratory, it would also provide an opportunity to use the patient data to

evaluate all the areas including pre-analytical, analytical and post-analytical areas in the laboratory's operations. This was also reported by White (1978), who stated that values obtained from patient data using statistical analysis, would be useful to incorporate into laboratory standardization systems. Determining reference ranges from patient data can be done when taking into consideration the direct relationship that exists between the population from which the reference ranges were determined and those for whom it will be used, i.e. the non-healthy patient population (Gräsbeck, 2004).

Various sources propose that data in the laboratory's information or computer system (LIS) is a "rich" source of laboratory results that are a combination of healthy people that come for routine analysis as well as diseased individuals (Grossi *et al.,* 2005; Inal *et al.,* 2010). Therefore, the data required for determination of reference ranges can be readily obtained from data warehouses and pre-tested or pre-analyzed data from laboratory information systems (Kouri *et al.,* 1994; Barth, 2009). Two main advantages using this approach are: (a) a significant amount of time and money can be saved by using historical or pre-tested data in a laboratory data base, and (b) reference values obtained will match new clinical results considering patient demographics, site of origin, and ethnicity (Solberg, 1994). Another advantage is that huge data sets are available which cover a wide range of age, demographics and other relevant criteria. Also, invasive collection techniques are omitted, especially for pediatric and geriatric patients, where it is often difficult to obtain samples due to physiologic factors like frail veins. In these cases, results can be used from samples that had already been collected and laboratory test results already generated for other purposes (Barth, 2009). Finally, another advantage is that the bias due to patient consent would be eliminated since samples do not receive any treatment that is different from the normal routine sample handling and results can be anonymized.

Putting all these advantages together, if a method can be developed to use laboratory generated data to determine reference ranges in a resource constrained country, which also meets stringent scientific criteria, it could be useful in developing and implementing important values for clinical decision making. Many studies have demonstrated that appropriate collection of samples for testing of reference values can be overcome using the data from a large number of patients like those stored in the laboratory computer system (Kouri *et al.,* 1994; Solberg, 1994; Grossi, *et al.,* 2005; Katayev *et al.,* 2010).

In this discussion of using laboratory data sets, one should differentiate between two methods which are often confused and used interrelatedly: the *posteriori* and the indirect method. The *posteriori* method selects reference individuals that have already been tested using specific selection criteria such as information obtained from medical records (Kouri *et al.,* 1994; Arzdeh, 2008; Ceriotti *et al.,* 2009). In this method, patients are screened retrospectively for suitability for inclusion into the reference individual sampling group. Criteria such as medical record, reflecting

the disease state, as well as elimination of patient results from obviously sick persons who visit the laboratory more that once in a short period of time, or results from patients in wards like oncology, intensive care unit, cardiac and kidney units. The other method, the indirect method, was proposed many years ago but lost credibility due to the difficulties involved in defining a strategy for statistical analysis. This happened even though Hoffmann (1963) proposed a method for statistical analysis which could use patient data.

Since the mid-1990, several studies have used the indirect method (Oosterhuis, 1990; Kairisto, *et al.,* 1994; Kouri *et al.*, 1994; Kroll and Saxtrup, 1998; Bock *et al.*, 2003; Dimouri *et al.,* 2007; Grossi *et al.*, 2005; Dorrizzi *et al.*, 2011; Giavarina *et al.*, 2006; Ilcol and Aslan, 2006; Shine, 2008; Inal *et al.,* 2010; Katayev *et al.,* 2010). This method is becoming more attractive and is used more often, especially since computerisation of virtually all laboratories, and the accumulation of massive amounts of data waiting to be “mined” (Arzdeh, 2008). In addition more and more technologically advanced computer analytical power and software become available.

In a study conducted by Ilcol and Aslan (2006) in Turkey, data from reference intervals obtained using the traditional IFCC method was compared with reference interval data obtained from data stored in the laboratory information system from the hospital population. It was found that for many of the reference intervals determined, no significant difference was observed between the values obtained from the direct and from the indirect method.

It can be observed, that no significant differences were reported between the reference interval (RI) values obtained from the indirect versus the direct method. It can even be noted that the established values differed from those recommended by the manufacturers. This suggests that establishing reference values using the indirect method and patient data is worth evaluating.

Bock and co-workers (2003) proposed a rigorous screening of patient population in the laboratory data base to maximize the number of healthy individuals and therefore the likelihood of obtaining a true normal range. Yet, this is not always possible unless patients are screened and this information entered into the data base which also requires additional manpower, time and money. Also reference values obtained from a population outside the hospital and laboratory patient population are often not appropriate for the patients tested (Gräsbeck, 2004; Grossi *et al.,* 2005).

The following table **(Table 1.1)** is an extract of the data presented in that study.

Table 1.1: Extract of RI values for specific tests determined by the indirect method compared to results determined by the IFCC method presented by Ilcol and Aslan (2006);870-873.

	Results	Results	
Analyte	**Indirect method - RI**	**Direct Method - RI**	**MS**
Total Cholesterol, mmol/L			
Male	2.12-7.17		
Female	2.92-6.63		
Male & Female	3.13-6.96	2.49-6.70	<5.18
Triglyceride, mmol/L			
Male	0.42-2.24	0.39-3.37	
Female	0.38-2.21	0.27-2.48	
Male & Female			<1.69
Uric Acid, mmol/L			
Male	0.16-0.45	0.16-0.35	0.20-0.42
Female	0.11-0.33	0.06-0.24	0.15-0.35
Glucose, mmol/L			
Male	4.16-6.10		
Female	3.88-5.93		
Male & Female	4.05-6.10	3.55-5.6	3.88-5.82

RI, reference interval; MS, manufacturer suggestion

When considering all the advantages versus the disadvantages of the indirect method to establish reference range, it can be asserted that using the large amount of patient data in a laboratory information system (LIS) for this purpose, could be worthwhile to investigate, since this would save much time and other scares resources.

1.6 Analysis of the data

As discussed, many studies have detailed methods of using laboratory generated data to develop population specific reference ranges. However, the big challenge is to identify the healthy and diseased population and remove the “diseased” portion from the total data. While several methods have been reported, the most widely referenced method was proposed by Bhattacharya (1967). More research was conducted using the “Bhattacharya” method to analyze the patient data (Neumann, 1968; Amador and Hsi, 1969; Reed *et al.,* 1971; White, 1978; Naus *et al.,* 1980; Baadenhuijsen and Smit, 1985; Hemel *et al.,* 1985; Ferre-Masferrer *et al.,* 1999; Bock *et al.,* 2003; Ilcol and Aslan, 2006; Arzdeh, 2008; Katayev *et al.,* 2010). This method allowed refinement of “raw” laboratory data and defined reference ranges that corresponded to the study population.

When patient data is used, it must be remembered that the data is obtained from a population that included “healthy” and “non-healthy” individuals. Therefore, the population distribution would be overlapping. It can be assumed that the majority of the patient data base contains data from “normal” or healthy individuals (Baadenhuijsen and Smit, 1985). As already discussed, several authors proposed using existing data from patients to develop reference ranges and most proposed statistical analysis detecting and eliminating outliers, thereby transforming the data to meet requirements for a Gaussian distribution.

A *posteriori* selection method of selecting reference individuals has been used in some studies in which reference individuals were selected after testing in the laboratory based on clinical criteria. The patient data was "cleaned" by removing data from patients that have been tested repeatedly, including all data from patients from hospital wards that house seriously ill patients like intensive and acute care and oncology wards. Outliers were then detected and eliminated (Kouri *et al.,* 1994; Grossi *et al.,* 2005).

Discussing the use of pros and cons in using data from hospitalised patients, the main advantages of using the available patient data is that a great amount of work and money can be saved, and also that the clinical data will correspond more closely to the patient data (Solberg, 1994).

1.6.1 Statistical methods

The major challenge is to find appropriate statistical methods and tools to analyze the large data set available and interpret the data in a meaningful way.

Arzdeh (2008) asserted that it is possible to establish reference ranges using patient data if the appropriate statistical tests are carried out (e.g. truncation of Gaussian distribution), although Reed and co-workers stated that most biological data cannot adequately be described by Gaussian or log-Gaussian curves (Reed *et al.,* 1971). Many distributions of biological data tend to be unimodal and positively skewed, which will influence the determination of the reference interval. The preferred method for statistical analysis of huge numbers of data points is the parametric method, but in cases of distributions that do not display Gaussian characteristics, the non-parametric method would be preferable. The estimated “normal” range or reference range is dependent on the distribution selected, and therefore the statistical method selected (Reed *et al*., 1971).

As the methods proposed are mathematically complex and are not easy to reproduce, they have not been accepted and implemented for use. During the last 20 years, more sophisticated laboratory information systems (LIS) have been developed, thus allowing huge sets of laboratory data to be stored and analyzed. More sophisticated statistical software packages have also been developed such as SPSS (IBM). This allows the opportunity for resource

constrained countries such as Namibia to “mine” the data and transform it into useful information such as national laboratory reference ranges.

Bhattacharya **(**1967) developed a statistical method to divide the distribution into the fraction considered from “healthy” individuals from the one considered to be from “non-healthy” individuals. He stated that the distribution is a mixture of components corresponding to different populations. He reported that the relative frequency and frequency distribution has to be found, which is usually assumed to be normal. Then, for the resolution of Gaussian distribution, a statistical approach is used to resolve overlapping Gaussian distributions.

According to Baadenhuijsen and Smit (1985), a prerequisite for the effective application of the *Bhattacharya* technique is the availability of a large amount of data which would allow for absence of large statistical fluctuations, and allow for the recognition of the linear part of the distribution. The authors stated that it is necessary to collect more than 1500 values for each analyte. The *Bhattacharya* algorithm assumes that the major part of a total population of unselected samples can be considered to be “normal” and that the overlap between the “healthy” part and the abnormal (either high or low) part is only partial. Hoffmann (1963) proposed a similar technique to be used to separate different populations.

One aspect addressed concerns the transformation of the data. Baadenhuijsen and Smit (1985) evaluated the efficiency of the transformation algorithm. They explored the possibility of transforming data even in cases where the underlying subpopulation appeared to be Gaussian, as detected from the first derivative function. They hypothesized that a more consistent application of this technique to all the data would yield a more comparable evaluation of the data.

Amador (1969) stated that several assumptions are usually made when examining the data obtained by indirect methods. The first and main assumption is that results fit a Gaussian distribution and are equal to the reference range of the healthy population. Yet, even when mean and standard deviation (SD) of the patient population is compared with the reference range in use, they often differ from each other. The second assumption is that the proportion of individuals affected by the disease will remain stable from one group of patient results to the next. This is also not the case since different groups may display variations in the disease composition and pattern.

1.6.2 Steps for analysis, transformation and removal of outliers

Shine (2008) proposed to normalize transformation using a three step approach for data analysis: 1) identify and remove outliers, 2) define underlying distribution of the remaining data points, 3) stratify data and model the effects of gender and age. Another approach reported was to transform data to log-normal, identify and remove outliers, and back transform (Tango, 1986).

He also stated this definition for normal range was between 2.5th and 97.5th percentiles, which are also the standards used in the medical laboratory, defining the 95% reference interval (Wright and Royston, 1999). Positive skewness is common in the distribution of reference individuals and a logarithmic transformation is effective in considerably reducing or removing it (Wright and Royston, 1999).

Some authors have also mentioned the problem of outliers, since outliers can significantly change the defined reference limits. They proposed methods to detect and eliminate these outliers (Reed *et al*., 1971; Chua *et al.,* 1978; Horn *et al.,* 2001; Ilcol and Aslan, 2006; Inal *et al.,* 2010). Ceriotti and co-workers*,* (2009) used a method proposed by Dixon (1953), (Ceriotti *et al.* 2009), while Horn and co-workers proposed the use of a Box-Cox transformation (Horn *et al*., 2001). Inal and co-workers have used modern statistical software, such as the SPSS statistics program (IBM SPSS Inc., Chicago, IL., USA) to identify and delete outliers using the stem-and-leaf and box-plots functions of the software (Inal *et al.,* 2010).

Most of the authors have reported a four step process to analyze the data using the parametric method:

1. Analyze the raw data and determine if it meets the “normal” distribution criteria.
2. Treat by log-transformation of the original values if needed
3. Identify and remove outliers
4. Define reference intervals

A further approach uses the non-parametric analysis approach and defines the inner 95% values or confidence interval which would demarcate the upper and lower limits of the specific analyte (Reed *et al.,* 1971). Ilcol and Aslan (2006) reported having used the inner 90% of the values that they have obtained.

1.6.3 Summary of steps to analyse data

The data analysis for defining reference ranges commonly uses the IFCC guidelines. The following is a sequence of analysis that is proposed in several of the studies discussed.

1. Calculate mean, median and mode of all raw data of all tests.
2. Construct frequency table and histogram.
3. Inspect visually to identify type of distribution. The distribution should be Gaussian, and if not it should be mathematically transformable to fit the Gaussian distribution (Chua *et al.,* 1978).
4. Transform to log normal if not Gaussian.

5. Identify outliers and remove using methods described. It is important to exclude outliers since they will distort the range of values. Chua *et al.,* (1978) stated that in the study conducted by them it was found that if 5 % of values fall outside three standard deviations from the mean, they should be treated as outliers and rejected.
6. Calculate statistics and determine distribution characteristics.
7. Use parametric or nonparametric method based on the number of data available. The robust method is strongly recommended when less than 120 values have been obtained. (Aytekin and Emerk, 2008)
8. Partition into subclasses and determine differences if valid by comparing the mean value of two partitioned populations, e.g. male and female (Harris and Boyd, 1990).
9. Compare to health related distribution curve of laboratory population (Ilcol and Aslan, 2006). Many include diseased individuals but extremes can be identified and eliminated. Ilcol and Aslan (2006) found that the reference values established using conventional method differed very little from those calculated from hospital patients.

As can be seen from the discussions it could be possible to develop an algorithm to use patient data to determine reference ranges which would be reliable and appropriate for the population that the medical laboratory services caters for.

1.7 Benefits for the country

The main aim for this study is to investigate the possibility to utilize the vast amount of pretested laboratory data that exists in the LIS of NIP for the determination of population based laboratory reference ranges.

As explained previously it is crucial for a mixed population like in Namibia, to have specific population based laboratory reference ranges, since inaccurate and inappropriate reference ranges may impact negatively on the treatment and monitoring of various disease conditions (Solberg 2006; Horn, 2010).

Chapter 2. Materials and Methods

2.1 Ethical Issues and Approval

Ethical Approval was obtained from the Faculty of Health and Wellness Sciences Research and Ethics Committee of Cape Peninsula University of Technology, Reference Number: CPUT/HW-REC 2011/HW-REC 2011/H08 (Appendix A).

Permission was also granted from the Namibia Institute of Pathology (NIP) to use the laboratory data (Appendix B). Permission was also granted from the Ministry of Health and Social Service to obtain and use the laboratory data for the purpose of obtaining the Master in Technology qualification (Appendix C).

2.2 Research Design

This was a retrospective, cross sectional quantitative study aimed at determining a new reference range using pretested data for the following laboratory tests:

- Haemoglobin
- Serum Urea
- Serum Creatinine
- Plasma Glucose (fasting and random)
- Serum Cholesterol
- Serum Triglycerides
- Serum Uric Acid

This study design was selected since all the data used is historical information of pre-tested laboratory results over a period of four years from January 2007 to December 2010. All data was extracted from the laboratory information system, *Meditech* of NIP.

2.3 Research Setting

Namibia is a country in Sub-Saharan Africa, where the bulk of medical laboratory services are offered by a government-owned institution, the Namibia Institute of Pathology (NIP), and rendering services mainly to the government of Namibia. In addition to NIP some private laboratory organizations service the private sector, i.e. private patients and medical aid companies. The NIP services the public health system, which varies from the most basic primary health care to the specialized hospital care. Since NIP consists of 36 laboratories all over the country in 13 regions and in many remote locations, NIP renders laboratory services to all public health facilities, as well as to some private hospitals and physicians.

2.4 Inclusion Criteria

This study extracted the available laboratory data from the existing laboratory information system database of the NIP and analysed only the data obtained for the tests listed.

The assumption was that the data in the laboratory computer system is comprehensive enough to provide all the required information as to gender and location of origin of samples.

2.5 Exclusion Criteria

Data for all the 13 regions in Namibia for all the seven tests could not be obtained, due to deficiencies in the data extraction program (**Table 2.2).** Only data from five tests from all 13 regions was extracted. Data used was identified only by the unique laboratory numbers as well as data clearly marked M (male) and F (female) of patients were used for this study. All data, where gender was identified as U (unknown gender), was deleted. Results of a patient occurring more than once in two months was deleted. Unique laboratory numbers were retained which were required to determine the origin of the data.

2.6 Process for data extraction (steps followed)

2.6.1 Step 1

A “custom report” was developed by the *Meditech* programmer which was then used to extract the following data from each individual patient on whom tests were performed and which results were stored in the *Meditech* LIS data base at NIP. The data of a patient (laboratory identification number, age, date of birth, gender, region and location of origin of sample, test date and time, test name and test result) was extracted from every one patient on whom one or a combination of the tests listed in 2.2 was performed. Although the age of patients was available in the data set, this was not considered and data was not striated according to age groups.

This information was captured when patient’s information from laboratory specimens was logged onto the LIS. The custom report’s data was presented in a text file format or “flat” file for each patient result, and could not be imported directly into a spread sheet file format without conversion into an appropriate format.

2.6.2 Step 2

An information technology expert was appointed to write an interface program for this purpose. Information that was in unstructured text files was converted into a format that could be imported into "MS Office Excel".

Data was validated by verifying that the data conforms to ranges as per number of reports in the "flat" files, exceptions where raised on discrepancies and manually investigated. Whereupon the ranges where extended, or the data was rejected as invalid. Data Integrity was ensured by defining unique identification keys which will hold true across all data sets. The extraction was then done into a database with primary key constraint, (e.g. "more than one test per two months"). Exceptions were reported and investigated and/ or discarded. Data Completeness was ensured by back referencing and checking each data set to the source, and finally numerous spot checks where performed.

After validation of data, it was exported into Microsoft Excel. Data of the different tests were separated into different Excel sheets and were sorted to be ready for statistical analysis.

2.7 Raw data available for analysis

Data of 254,271 records relating to 254,271 individual's tests results stored in the laboratory information system (LIS) of NIP over a 4-year period (Jan 2007 - Dec 2010) were retrieved. This constitutes the original database for this study. This sample group included 134,261 females and 117,091 males, as well as 2,919 results from unknown gender. Unknown gender is recorded when no gender is indicated on the patient request form.

After removal of records of "unknown" gender, 251,352 records remained, consisting of 7 different tests from 13 regions of Namibia. Three tests could only be obtained from the Khomas region due to constraints in the data collection process for these tests. These tests were; Haemoglobin (N=19,307), serum Urea (N=10,056), serum Creatinine (N=10,768). All the other test results were from all the13 regions of Namibia and consisted of fasting plasma Glucose (N=43,222), random plasma Glucose (N=55,620), serum Cholesterol (N=49,448), serum Uric Acid (N=38,464) and serum Triglycerides (N=24,467). These are presented in **Table 2.1.**

Of this total data base, 29,696 (11.9%) outliers (Haemoglobin=1.6%, Urea=18.8%, Creatinine=18.3%, Fasting Glucose=24.6%, Random Glucose=18.2%, Cholesterol=2.2%, Triglycerides=9.7%, Uric Acid=2.8%), were removed with SPSS Box Plot function and results partitioned into male and female before analysis **(Table 2.2).**

All the records were further partitioned into the 13 regions of Namibia; Caprivi (N=7,016), Erongo (N=11,593), Hardap (N=11,476) Karas (N=7,000), Kavango (N=3,972), Khomas (N=115,440), Kunene (N=3,040), Ohangwena (N=7,232) Omaheke (N=3,782), Omusati (N=5,637), Oshana (N=36,373), Oshikoto (N=30,101) and Otjozondjupa (N=8,690). The number of tests for each region available for analysis is displayed in **Table 2.2**.

Table 2.1: Number of tests for each region (before removal of outliers)

REGIONS	HB	UREA	CREAT	GLUC	CHOL	TRIG	UA	total tests
Caprivi				2 860	2 241	801	1 114	**7 016**
Erongo				6 928	1 845	1 059	1 761	**11 593**
Hardap				4 260	3 147	1 996	2 073	**11 476**
Karas				1 892	1 910	1 305	1 893	**7 000**
Kavango				2 093	840	320	719	**3 972**
Khomas	19 307	10 056	10 768	28 487	20 373	11 592	14 857	**115 440**
Kunene				1 756	622	467	195	**3 040**
Ohangwena				3 681	1 130	675	1 746	**7 232**
Omaheke				1 275	1 069	334	1 104	**3 782**
Omusati				3 002	1 024	443	1 168	**5 637**
Oshana				19 991	8 152	2 601	5 629	**36 373**
Oshikoto				18 978	4 801	2 578	3 744	**30 101**
Otjozondjupa				3 639	2 294	296	2 461	**8 690**
TOTAL	**19 307**	**10 056**	**10 768**	**98 842**	**49 448**	**24 467**	**38 464**	**251 352**

HB = Haemoglobin, Urea = Serum Urea, Creat = Serum Creatinine, Gluc = Glucose, Chol = Total Cholesterol, Trig = Total Triglycerides, UA = Uric Acid

Table 2.2 Test records of the total data after removal of outliers and partitioning into male and female results

TESTS	N before outlier removal	N after outlier removal	Outliers removed	% outliers removed	N=M	% M	N=F	% F
Haemoglobin	19 307	18 999	308	1.6	7 716	45	11 283	66
Urea	10 056	8 111	1 889	18.8	3 836	47	4 275	52
Creatinine	10 768	8 794	1 974	18.3	4 099	47	4 506	51
Plasma Glucose (fasting)	43 222	32 591	10 631	24.6	13 317	41	19 274	59
Plasma Glucose (random)	55 620	45 515	10 105	18.2	21 370	47	24 145	53
Cholesterol	49 448	48 354	1 094	2.2	24 815	52	23 539	49
Triglyceride	24 467	22 138	2 379	9.7	9 291	42	12 847	58
Uric Acid	38 464	37 389	1 066	2.8	18 972	51	18 426	50
	251 352	**221 891**	**29 446**	**11.7**	**103 416**		**118 295**	

M=Male, F=Female

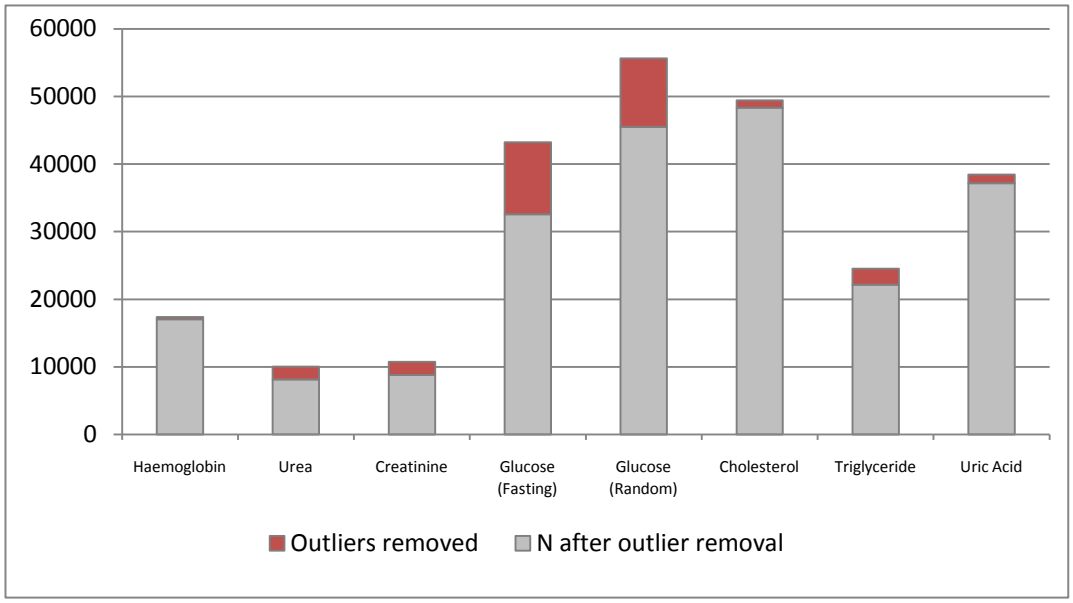


Figure 2.1: Graph to display the number of outliers removed from the total data set

Table 2.1 represents the total number of data after removal of outliers and partitioning into male and female results. **Figure 2.1** displays the total number of outliers removed and **Figure 2.2** displays the number of male and female patients in the total data set for each test.

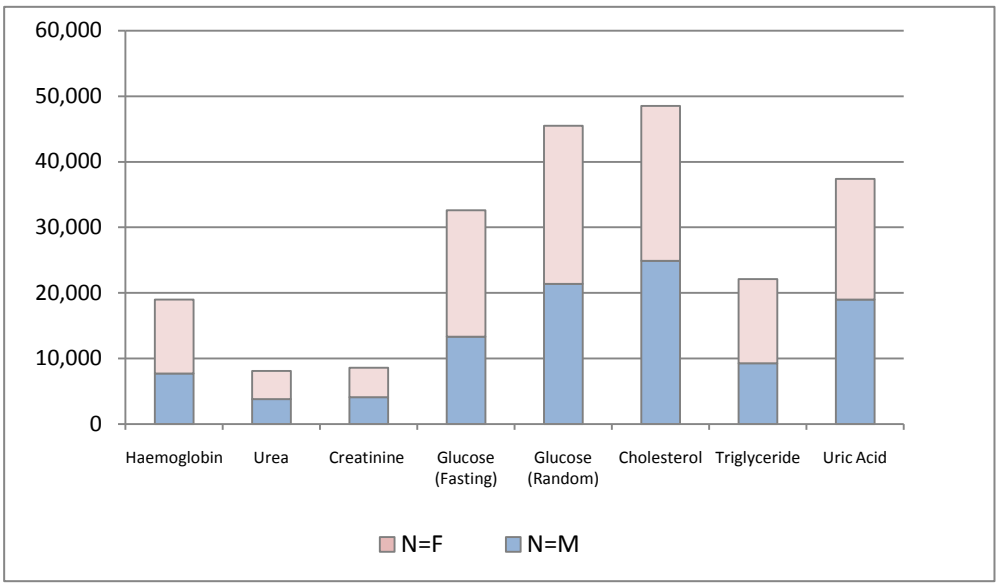


Figure 2.2: Graph to display the number of males and female results available for analysis

2.8 Data cleaning and statistical analysis

For the purposes of this study, the statistical program SPSS version 21 (IBM) was used and the following steps for analysis were proposed by many authors. (Reed et al., 1971; Chua et al., 1978; Horn et al., 2001; Ilcol and Aslan, 2006; Ceriotti et al., 2009), Inal et al., 2010).

1. Analyse the raw data and determine if it meets the "normal" distribution criteria.
2. Identify and remove outliers using the box and leaf plot in SPSS.
3. Treat by log-transformation of the original values if needed.
4. Identify and remove outliers further.
5. Transform log transformed data back.
6. Determine mean standard deviation and percentiles.
7. Determine normality of each data set using the Kolmogorov-Smirnhov test.

2.8.1 Statistical analysis

Microsoft Office Excel and a statistical program, SPSS version 21, were used to analyse the data.

For each test the following procedure was followed.

1. Different spread sheets for each of the different tests were created in MS Excel.
2. The results of each test were then sorted in MS Excel in ascending order.
3. Outliers were removed using the Box-plot function on SPSS. Box-plots were visually inspected and any outliers were removed. Outliers are assumed to lie beyond the lower quartile (Q1 = 25^{th} percentile) and the higher quartile (Q3 = 75^{th} percentile) of the data, i.e. below 25 % and above 75 % of the data. Since a huge number of data points were available this method was used to cluster the results to be analysed within the 75% range. As stated by Walfish (2006), the box-plot function can be used to remove outliers even if data are not normally distributed
4. The data was log transformed where distribution of data appeared to be excessively skewed upon visual inspection.
5. Again outlier detection and removal was performed. This was done several times until no further outliers were identified. The data was then back transformed.

6. Normality was tested using the Kolmogorov Smirnoff test using SPSS, and using significance value of $p<0.05$.
7. Frequency tables and histograms were created using the SPSS program. Statistics like mean, mode, standard deviation (SD) and percentiles (2.5th, 5th, 95th, and 97.5th) were calculated and displayed in the SPSS tables.
8. The data was further striated according to gender and region and further analysis was then done.
9. ANOVA test was performed to compare mean values of results between the 13 regions. A p value of <0.05 was considered to be statistically significant.
10. The mean value of reference ranges used in NIP was calculated assuming that the range lies between ± 2SD.
11. The mean value of results of the study was compared to the mean value of NIP reference range using a T-test, with $p<0.05$ indicating statistical significant difference.

2.8.2 Outliers and log transformation

Outliers were identified in each of the test results. (**Table 2.1**) and these were removed. For all results log transformation was carried out but it was found that it did not make any difference in the outliers identified and also not in the distribution of the results. Only for Triglycerides a marked difference was found.

Chapter 3. Results

3.1 Characteristics of the data used to produce the results

After outlier removal and cleaning of data, 221,891 result variables were available for statistical analysis and were as follows:

Haemoglobin (N=18,999; M=7,716, F=11,283),

Serum Urea (N=8,1111; M=3,836, F=4,275),

Serum Creatinine (N=8,794; M=4,099, F=4,506),

Fasting plasma Glucose (N=32,591; M=13,317, F=19,274,

Random plasma Glucose (N=45,515; M=21,370, F=24,145),

Serum Cholesterol (N=48,354; M=24,815, F=23,539),)

Serum Triglycerides (N=22,138; M=9,291, F=12,847) and

Serum Uric Acid (N=37,389; M=18,972, F=18,426)

These are presented in T**able 2.2.** .

3.2 Results of statistical analysis of each test

3.2 (A) Results of statistical analysis of tests from the Khomas region only

Results for Haemoglobin, Serum Urea and Creatinine could only be obtained from the Khomas region due to constraints in the data extraction process as explained in paragraph.

3.2.1. Results for Haemoglobin

The statistics of the Haemoglobin results of males and females are displayed in **Table 3.1**.

Table 3.1: Table of statistics for Haemoglobin results for male and female

		MALE	FEMALE
N		**7 716**	**11 283**
Mean		12.165	11.9
Std. Error of Mean		0.031	0.018
Median		12.4	12.1
Std. Deviation		2.759	1.863
Variance		7.613	3.47
Skewness		-0.256	-0.333
Std. Error of Skewness		0.028	0.023
Kurtosis		-0.557	-0.202
Std. Error of Kurtosis		0.056	0.046
Percentiles	2.5	6.64	7.81
	5	7.39	8.48
	95	16.3	14.7
	97.5	16.9	15.2

A histogram for male and female Haemoglobin result distribution is displayed in **Figure 3.**1.

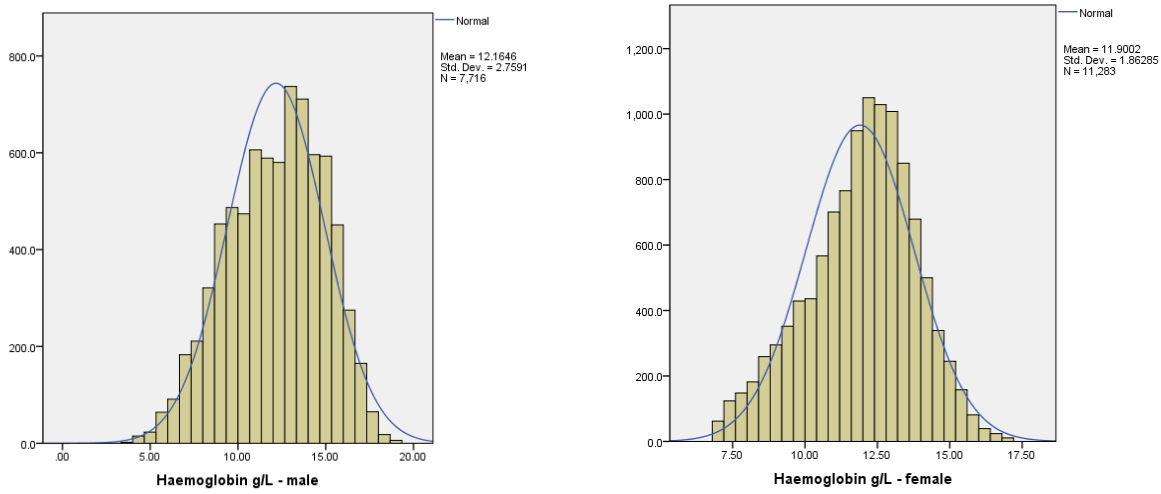


Figure 3.1: Histogram of male and female frequency distribution of Haemoglobin results

A statistically significant skewness was detected because the skewness value of -0.256 for males and 0.333 for females was more than ± 2X standard error of skewness, as indicated from the Kolmogorov-Smirnov test **(Table 3.9)** where the value of the statistic was 0.047 for males and 0.055 for females.

The percentile range from 2.5th to 97.5th was 6.64-16.9 g/L for males and 7.81-15.2 g/L for females (**Table 3.8**).

3.2.2 Results for Urea

8,111 Urea results were analysed after outliers were removed, and statistics were determined (**Table 3.2).**

Table 3.2: Statistics of Urea results before and after outlier removal

UREA		After outlier removal
N		8111
Mean		4.441
Median		4.1
Std. Deviation		2.0466
Skewness		0.605
Std. Error of Skewness		0.027
Kurtosis		-0.242
Std. Error of Kurtosis		0.054
Percentiles	2.5	1.3
	5	1.6
	95	8.4
	97.5	9.1

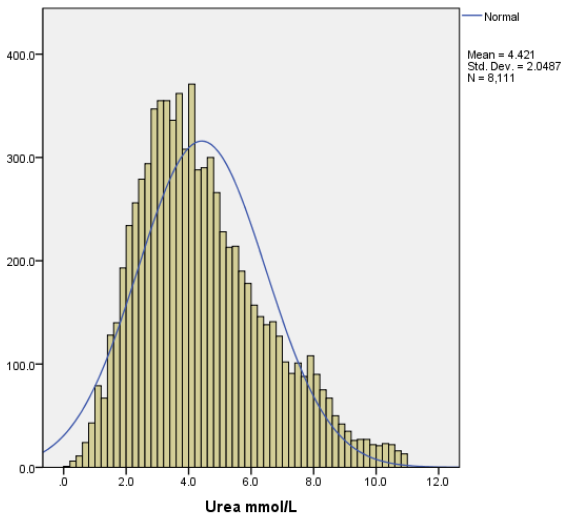


Figure 3.2: Histogram of Urea results

A histogram of the distribution of Urea results is shown in **Figure 3.2.**

A slight skewness to the right is detected by visual inspection of the histogram as well as by Kolmogorov Smirnov test for normality. The Kolmogorov-Smirnov test indicated that significant skewness existed ($p<0.05$) because the skewness was more than 2 times the standard error of skewness as indicated in the statistic of 0.079 **(Table 3.9).**

Although the t-test **(Table 3.12)** for comparison between the mean obtained in the study to the mean of NIP reference range was statistically significant ($p>0.05$), the mean, standard deviation and percentiles (**Table 3.13)** of the study population was comparable, yet slightly wider than the reference range of 2.1 – 7.1 mmol/L used by NIP **(Table 3.13).**

3.2.3 Results for Creatinine

After outlier removal, a total of 8,946 Creatinine results were analysed. The statistics of the male and female Creatinine results are presented in **Table 3.3.**

Table 3.3: Statistics of all Creatinine results of the results for male and female

		MALE	FEMALE
N		4109	4847
Mean		86.662	70.493
Median		86	67.000
Std. Deviation		25.7262	22.7354
Skewness		0.099	0.950
Std. Error of Skewness		0.038	0.035
Kurtosis		-0.086	1.363
Std. Error of Kurtosis		0.076	0.070
Percentiles	2.5	36	33
	5.0	43	39
	90	121	100
	95	133	117
	97.5	141	131

Although the histograms **(Figure 3.3)** appeared to display a normal distribution of results from both the male and female population, the Kolmogorov-Smirnov test **(Table 3.9)** for normality indicated that the distribution of the test results was not normally distributed ($p<0.05$).

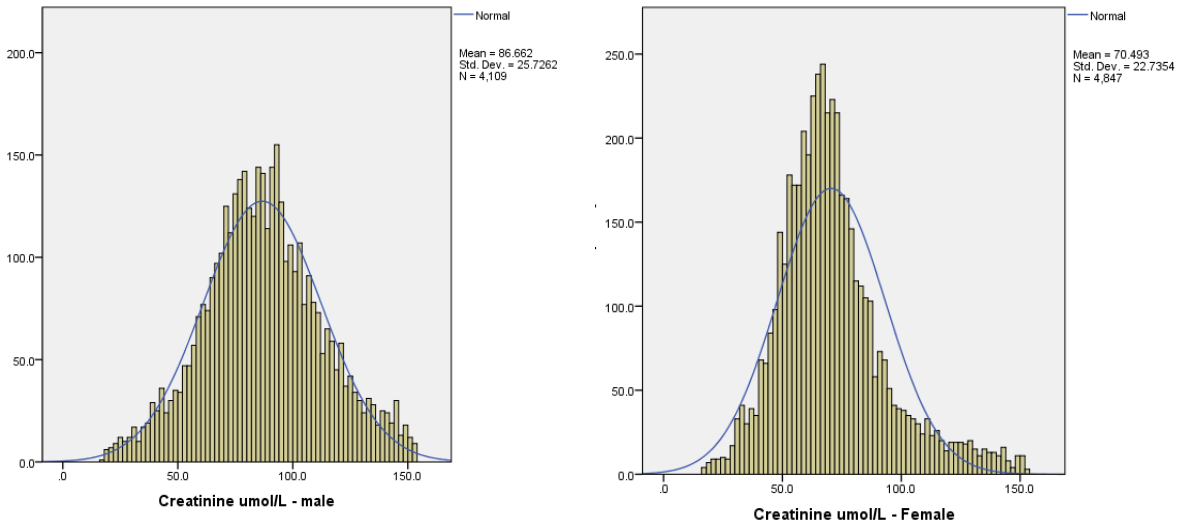


Figure 3.3: Histogram of Creatinine results for male and female

The mean, standard deviation and percentiles of the data set is displayed in **Table 3.8**, which was comparable, yet slightly higher, than the reference range (M=62-106, F=26.5-88.4 µmol/L) used by NIP, although a statistically significant difference ($p<0.05$) exists when comparing with the mean value of the NIP reference range **(Table 3.12).**

3.2 (B) Results of statistical analysis of 5 tests from all 13 regions of Namibia including the Khomas region

3.2.4 Results for Plasma Glucose

After outlier removal the fasting plasma Glucose results (N=32,591) and random plasma Glucose results (N=45,515) were analysed and statistics determined **(Table 3.4).**

Table 3.4: Statistics of Plasma Glucose (fasting and random) results

		Results of Fasting Glucose	Results of Random Glucose
N		32,591	45,515
Mean		5.945	5.268
Median		5.6	5.2
Std. Deviation		1.5618	0.8425
Skewness		0.618	0.318
Std. Error of Skewness		0.014	0.011
Kurtosis		-0.093	-0.15
Std. Error of Kurtosis		0.027	0.023
Percentiles	2.5	3.4	3.7
	5	3.9	4
	95	9.1	6.9
	97.5	9.5	7.1

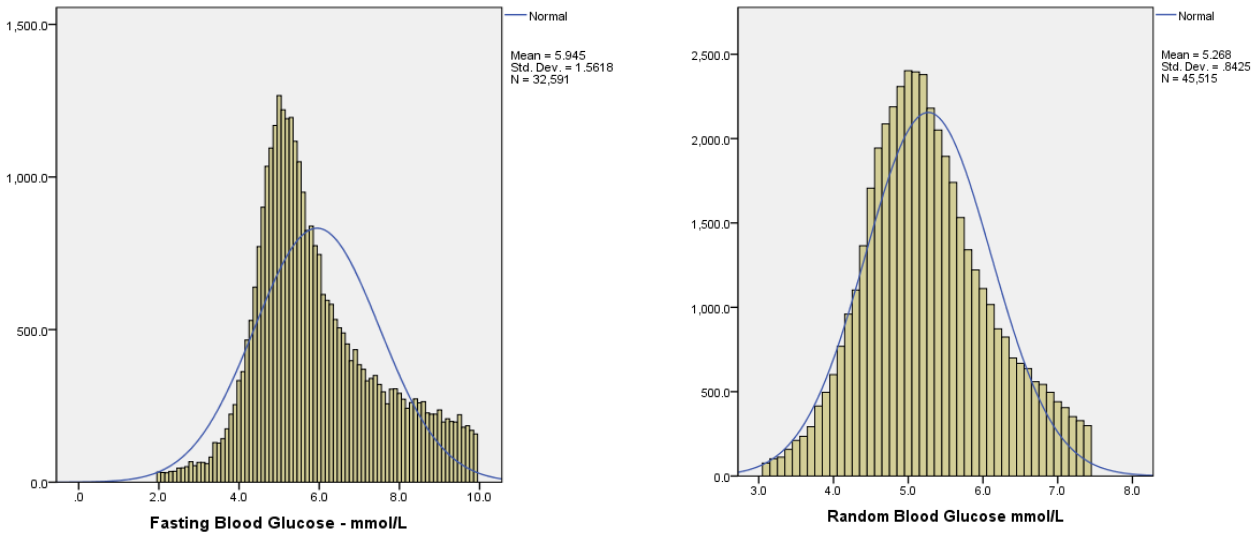


Figure 3.4 Histogram of plasma Glucose (fasting and random) results

Although more than 18 % of outliers were removed, the distributions for the plasma Glucose (fasting and random) were still not normal as can be seen from the statistics **(Table 3.4**), the Kolmogorov-Smirnov test ($p<0.05$) **(Table 3.9)** and the histogram **(Figure 3.4)** which indicated significant skewing. The distribution of results was skewed to the right with many high results on the right side of the distribution.

The mean and standard deviation results of fasting and random blood Glucose for each of the 13 regions was presented in **Table 3.10.**

ANOVA analysis **(Table 3.11)** between the various regions indicated a significant difference ($p<0.05$), but visual inspection showed that Erongo, Oshana and Oshikoto regions have a higher mean value that the other regions.

The percentile 2.5th, 5th, 95th and 97.5th of fasting and random Glucose results for the total data set is displayed in **Table 3.8.**

3.2.5 Results for Cholesterol

After removal of outliers 48,354 Cholesterol results (M=24,815, F=23,539) were analysed. Statistics for total results, as well as for male and female Cholesterol results were displayed in **Table 3.5.**

Table 3.5: Statistics of Cholesterol results

		Results after outlier removal	
		MALE	FEMALE
	N	24 815	23 539
Mean		4.625	4.771
Median		4.600	4.700
Std. Deviation		1.1108	1.0823
Skewness		0.203	0.196
Std. Error of Skewness		0.016	0.016
Kurtosis		-0.317	-0.245
Std. Error of Kurtosis		0.031	0.032
Percentiles	2.5	2.6	2.8
	5	2.9	3.1
	95	6.6	6.2
	98	6.9	7.0

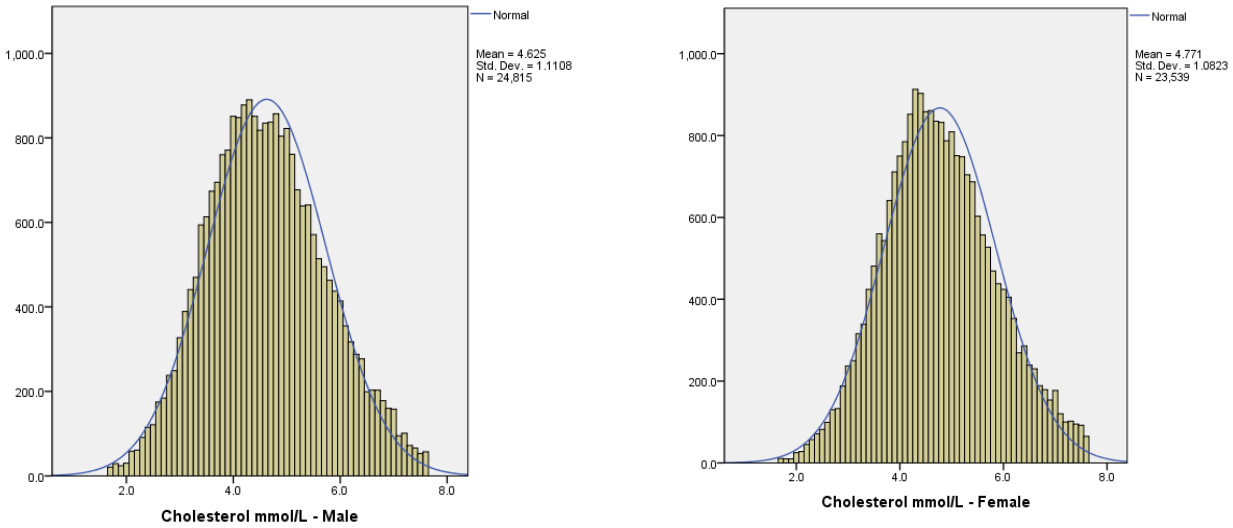


Figure 3.5: Histogram of Cholesterol results for male and female

As can be seen from the histograms (**Figure 3.5**), the distribution of results of male and female, upon visual inspection appeared to be normally distributed, but the Kolmogorov-Smirnov test for normality indicated differently and presented a significant skewness ($p<0.05$) **(Table 3.9).**

The percentiles 2.5^{th}, 5^{th}, 95^{th} and 97.5^{th} of Cholesterol results for male and female are presented in **Table 3.8**.

Mean value and standard deviation Cholesterol results of the 13 regions is displayed in **Table 3.10**. ANOVA analysis presented in **Table 3.11** displayed a significant difference ($p<0.05$) between the mean values of results of the various regions.

3.2.6 Results for Triglycerides

A total of 22,138 Triglyceride results were analyzed. The statistics of the Triglyceride results are shown in **Table 3.6**. The distribution is still slightly skewed to the right (skewness=0.714), but overall the results compare favourably with the ranges used in NIP.

Table 3.6: Statistics of Triglyceride results before and after log transformation and outlier removal

		Original Results before outlier removal	Results after log transformation and back transformation	Results after final outlier removal
N		24 467	22 834	22 138
Mean		1.5893	1.3262	1.2680
Median		1.23	1.17	1.1500
Std. Deviation		1.73039	0.69419	0.62088
Skewness		20.862	0.886	0.714
Std. Error of Skewness		0.016	0.016	0.016
Kurtosis		970.871	0.18	-0.217
Std. Error of Kurtosis		0.031	0.032	0.033
Percentiles	2.5			0.390
	5			0.46
	95			2.50
	97.5			2.72

A histogram of Triglyceride values is displayed in **Figure 3.6.**

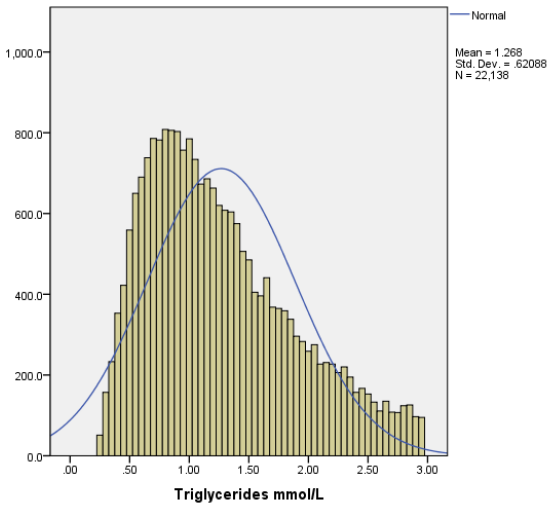


Figure 3.6: Histogram of Triglyceride results after back transformation from log transformation.

Although the results were log transformed and back transformed after outlier removal when an initial skewness of more than 20 was detected, the distribution was still severely skewed as can

be seen from the histogram **(Figure 3.6)** as well as the statistic of 0.714 skewness and the result of 0.082 obtained in the Kolmogorov-Smirnov test **(Table 3.9).**

The mean, standard deviation, 2.5th, 5th, 95th and 97.5th percentile of the Triglyceride results are displayed in **Table 3.8.**

Table 3.10 displays the mean value and standard deviation of Triglyceride results of the 13 regions of Namibia, and **Table 3.11** displays the ANOVA analysis to compare results between the 13 regions. It can be seen from this table that a statistically significant difference ($p<0.05$) was found between the mean values of the regions.

3.2.7 Results for Uric Acid

After partitioning the total data set that was analysed was 37,398 (M=18,972; F=18,426).**Table 3.7** displays the statistics for the analysis of the total Uric Acid data set, as well as for results of male and female.

Table 3.7: Statistics of Uric Acid results before and after outlier removal

		Results after outlier removal	Male	Female
	N	37 155	18 972	18 426
Mean			0.3942	0.3113
Median			0.38	0.3
Std. Deviation			0.10181	0.087721
Skewness			0.35700	0.47812
Std. Error of Skewness			0.01778	0.01804
Kurtosis			-0.13723	-0.19531
Std. Error of Kurtosis			0.03556	0.03609
Percentiles	2.5		0.21	0.17
	5		0.24	0.19
	95		0.58	0.48
	97.5		0.62	0.51

Figures 3.7 display the histogram of the frequency distribution of male and female Uric Acid results. This distribution appeared to be slightly skewed, but not excessively so, but the Kolmogorov-Smirnov test **(Table 3.9**) for skewness indicated a significant skewness ($p<0.05$)**.**

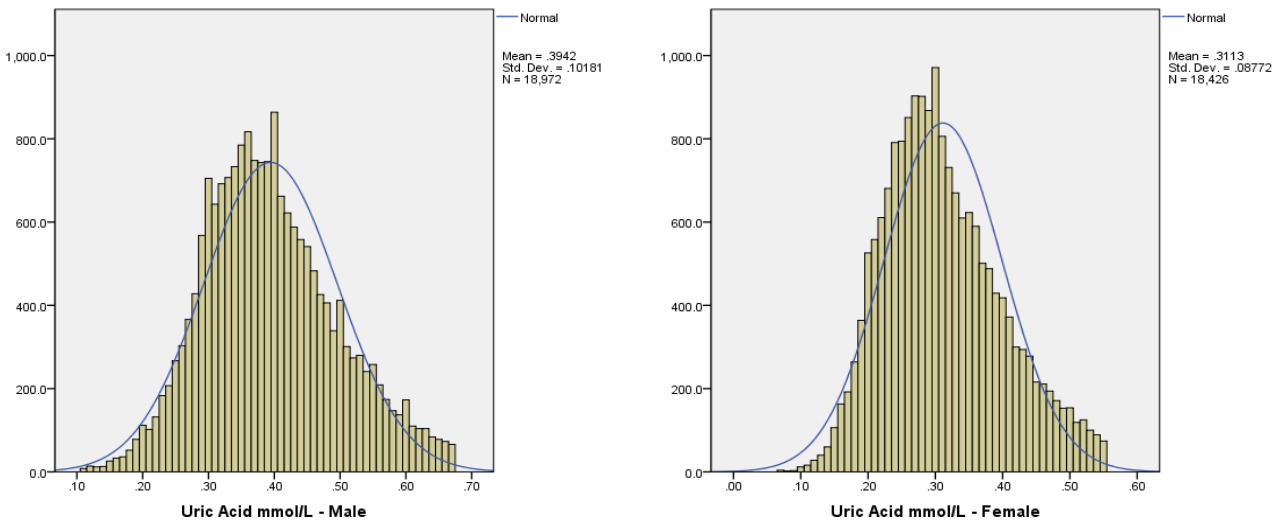


Figure 3.7: Histogram to display the frequency distribution of Uric Acid results of males and females

The mean, standard deviation, 2.5th, 5th, 95th and 97.5th percentile of the total Uric Acid data distribution as well as from male and female is displayed in **Table 3.8.**

Table 3.10 displays the mean value and standard deviation of the 13 regions, and **Table 3.11** displays an ANOVA analysis to indicate the significance or not of the differences between the mean values of the total data set and the mean value of the individual region.

3.3 Summary of results

Table 3.8 is a summary of all the results for the seven test parameters investigated and indicates the total number of data, the mean and standard deviation of results as well as the percentiles (2.5^{th} 5^{th}, 95^{th} and 97.5^{th})

Table 3.8: Mean, standard deviation (SD) and percentile range of results

		N	Mean	SD	Percentiles				Unit
					2.5	5	95	97.5	
Haemoglobin	Male	7,716	11.28	2.76	6.64	7.39	16.30	16.90	g/dL
	Female	11,283	11.90	1.86	7.81	8.48	14.70	15.20	g/dL
Urea	Total	8,111	4.44	2.05	1.30	1.60	8.40	9.10	mmol/L
Creatinine	Male	4,099	86.83	25.54	36.00	43.00	133.00	141.00	µmol/L
	Female	4,506	66.64	16.89	33.00	39.00	117.00	131.00	µmol/L
Glucose	Fasting	32,591	5.95	1.56	3.40	3.90	9.10	9.50	mmol/L
	Random	45,515	5.27	0.84	3.70	4.00	6.90	7.10	mmol/L
Cholesterol	Male	24,815	4.63	1.11	2.60	2.90	6.10	6.90	mmol/L
	Female	23,539	4.71	1.08	2.80	3.10	6.20	7.00	mmol/L
Triglyceride	(male and female)	22,138	1.27	0.62	0.39	0.46	2.50	2.72	mmol/L
Uric Acid	Male	18,972	0.39	0.10	0.21	0.24	0.58	0.62	mmol/L
	Female	18,426	0.31	0.09	0.17	0.19	0.48	0.51	mmol/L

Table 3.9 represents statistics of the Kolmogorov-Smirnov test for normality of data distributions of each test.

Table 3.9: Table representing results of Kolmogorov-Smirnov test for normality for all results

TEST		Kolmogorov-Smirnov		
		Statistic	**df**	**p value**
Haemoglobin	Male	0.047	7716	0.0
	Female	0.055	11283	0.0
Urea	Result	0.0605	8111	0.0
Creatinine	Male	0.033	4109	0.0
	Female	0.099	4847	0.0
Glucose	Fasting	0.111	32591	0.0
	Random	0.067	45515	0.0
Cholesterol	Male	0.042	24815	0.0
	Female	0.043	23539	0.0
Triglyceride	Result	0.082	22138	0.0
Uric Acid	Males	0.063	18972	0.0
	Females	0.078	18426	0.0

Table 3.10 represents the mean and standard deviation of five test parameter for each of the 13 regions of Namibia.

Table 3.10: Table to display the mean and standard deviation of results of the 13 regions of Namibia

	FASTING GLUCOSE			RANDOM GLUCOSE			CHOLESTEROL			TRIGLYCERIDES			URIC ACID		
Region	Mean	N	SD	Mean	N	SD	Mean	N	SD	Mean	N	SD	Mean	N	SD
Caprivi	5.572	844	1.4678	5.281	1397	0.8789	4.5	2204	1.1272	1.2579	698	0.54632	0.3577	1073	0.09559
Erongo	6.351	3638	1.6021	5.232	1319	0.8494	4.919	1777	1.0806	1.2665	939	0.55633	0.3415	1700	0.10212
Hardap	5.676	2015	1.3232	5.289	1299	0.835	4.985	3043	1.0938	1.3415	1760	0.56618	0.3494	1967	0.10707
Karas	5.766	588	1.5195	5.125	919	0.8751	4.816	1875	1.1238	1.3233	1097	0.57709	0.3542	1834	0.1013
Kavango	5.993	619	1.7011	5.16	941	0.8526	4.341	824	1.1313	1.251	298	0.55647	0.3597	704	0.09257
Khomas	5.597	6070	1.245	5.257	17953	0.8163	4.755	19863	1.0878	1.1759	10450	0.57257	0.3531	14336	0.10084
Kunene	5.958	581	1.5242	5.041	829	0.8557	3.85	613	1.1022	1.2391	446	0.5371	0.3477	190	0.09421
Ohangwena	5.949	1708	1.625	5.089	1179	0.8867	4.418	1120	1.0415	1.174	619	0.56473	0.3514	1659	0.10528
Omaheke	5.556	328	1.2069	5.115	744	0.8882	4.857	1052	1.0596	1.3258	298	0.56846	0.3578	1071	0.09849
Omusati	5.619	1230	1.389	5.205	1239	0.9154	4.471	1010	1.0577	1.2798	394	0.60177	0.3722	1125	0.10913
Oshana	6.202	5489	1.6315	5.357	10175	0.8452	4.571	8021	1.0845	1.2228	2355	0.60204	0.3534	5477	0.09568
Oshikoto	6.066	8559	1.7057	5.286	5511	0.8494	4.739	4695	1.0607	1.2323	2492	0.55331	0.359	3646	0.09984
Otjozondjupa	5.558	922	1.333	5.281	2010	0.8364	4.576	2257	1.0796	1.1996	292	0.56149	0.3508	2373	0.10236
Total	5.945	32591	1.5618	5.268	45515	0.8425	4.696	48354	1.0994	1.2201	22138	0.57373	0.3538	37155	0.10069

An ANOVA test was carried out to determine if any significant difference exists between the means of each parameter across the 13 regions of Namibia. Results of this are presented in **Table 3.11**

Table 3.11: ANOVA table to determine statistical difference between mean values of the different results of the 13 regions of Namibia

		Sum of Squares	df	Mean Square	F	p value
Glucose (Fasting)	Between Groups (Combined)	2423.925	12	201.994	85.379	0.001
	Within Groups	77074.59	32578	2.366		
	Total	79498.52	32590			
Glucose (Random)	Between Groups (Combined)	220.221	12	18.352	26.025	0.001
	Within Groups	32086.47	45502	0.705		
	Total	32306.69	45514			
Cholesterol results for male and female	Between Groups (Combined)	1397.92	12	116.493	98.717	0.001
	Within Groups	57045.69	48341	1.18		
	Total	58443.6	48353			
Triglyceride results for male and female	Between Groups	66.52	12	5.543	16.99	0.001
	Within Groups	7054.368	21621	0.326		
	Total	7120.888	21633			
Uric Acid results for male and female	Between Groups (Combined)	0.878	12	0.073	7.234	0.001
	Within Groups	375.803	37142	0.01		
	Total	376.681	37154			

Table 3.12 displays the One-Sample T-Test Statistics comparing the data array for each test with the mean value of NIP reference range. The mean values compare favourably and no statistical significant difference was detected between the mean values obtained in the study with the mean values of the reference range of NIP, assuming the range in use is ± 2 SD.

Table 3.12: T-Test - One-Sample Statistics comparing the data array for each test with the mean of NIP reference range

Test	N	Mean	Std. Deviation	Std. Error Mean	**Test Value (NIP mean value)**	t	df	P value (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
										Lower	Upper
Haemoglobin (M)	7 716	12.165	2.759	0.0314	**16.40**	-134.841	7 715	0.000	-4.23541	-4.2970	-4.1738
Haemoglobin (F)	11 283	11.9002	1.86285	0.0175	**14.45**	-145.391	11 282	0.000	-2.54977	-2.5842	-2.5154
Urea	8 111	4.421	2.0487	0.0227	**4.60**	-7.861	8 110	0.000	-0.1788	-0.2234	-0.1342
Creatinine (M)	4 109	86.662	25.726	0.4013	**84.00**	6.633	4 108	0.000	2.662	1.875	3.449
Creatinine (F)	4 847	70.493	22.7354	0.3266	**57.50**	39.788	4 846	0.000	12.9931	12.353	13.633
Glucose (fasting)	32 591	5.945	1.5618	0.0087	**5.00**	109.264	32 590	0.000	0.9453	0.928	0.962
Glucose (random)	45 515	5.268	0.8425	0.0039	**7.55**	-577.832	45 514	0.000	-2.2819	-2.29	-2.274
Cholesterol (M)	24 815	4.625	1.1108	0.0071	**3.81**	116.237	24 814	0.000	0.8196	0.806	0.833
Cholesterol (F)	23 539	4.771	1.0823	0.0071	**4.28**	69.583	23 538	0.000	0.4908	0.477	0.505
Triglycerides	22 138	1.2680	.62088	.00417	**1.55**	-67.575	22 137	0.000	-0.2820	-0.2902	-0.2738
Uric Acid (M)	18 972	0.3942	0.1018	0.0007	**0.41**	-14.648	18 971	0.000	-0.01083	-0.0123	-0.0094
Uric Acid (F)	18 426	0.3113	0.0877	0.0007	**0.32**	-13.413	18 425	0.000	-0.00867	-0.0099	-0.0074

Table 3.13: Summary of mean, standard deviation, 2.5th and 97.5th percentile and confidence intervals of all the test results as well as comparison with NIP reference ranges currently in use.

Test		N	Mean	SD	Percentiles								NIP REFERENCE RANGES			p value
					2.5	CI	5	CI	95	CI	97.5	CI	Range	Mean	SD	
Haemoglobin	male	7,716	11 283	2.759	6.64	(6.57-6.71)	7.39	(7.31-7.47	16.3	(16.12-16.48)	16.9	(16.72-17.08)	14.0-18.8	16.4	1.2	<0.05
Haemoglobin	female	11,283	11.900	1.863	7.81	(7.74-7.89)	8.48	(8.39-8.57)	14.7	(14.57-14.86)	15.2	(15.06-15.36)	12.0-16.9	14.45	1.23	<0.05
Urea	male and female	8,111	4.441	2.047	1.3	(1.29-1.31)	1.6	(1.58-1.62)	8.4	(8.31-8.49)	9.1	(9.00-9.20)	2.1-7.1	4.6	1.25	<0.05
Creatinine	Male	4,109	86.827	25.539	36	(36.45-37.40)	43	(42.53-43.47)	133	(131.03-134.44)	141	(138.91-142.52)	62.0-106.0	84	11	<0.05
Creatinine	Female	4,847	66.636	16.892	33	(32.53-33.36)	39	(38.58-39.42)	117	(1295.34-1358.27)	131	(101.54-104.11)	26.5-88.4	57.5	15.5	<0.05
Glucose	Fasting	32,591	5.945	1.562	3.4	(3.38-3.44)	3.9	(3.86-3.94)	9.1	(9.05-9.20)	9.5	(9.45-9.60)	4.1-5.9	5	0.45	<0.05
Glucose	Random	45,515	5.268	0.843	3.7	(3.68-3.74)	4.00	(3.96-4.04)	6.9	(6.87-6.97)	7.1	(7.07-7.18)	4.1 - 11.0	7.55	1.73	<0.05
Cholesterol	Male	24,815	4.625	1.111	2.6	(2.58-2.63)	2.9	(2.87-2.93)	6.1	(6.06-6.17)	6.9	(6.86-6.97)	2.12-5.49	3.805	0.84	<0.05
Cholesterol	Female	23,539	4.711	1.082	2.8	(2.78-2.83)	3.1	(3.07-3.13)	6.2	(6.16-6.27)	7.0	(6.96-7.08)	2.46-6.1	4.28	0.91	<0.05
Triglyceride	male and female	22,138	1.268	0.621	0.39	(0.388-0.394)	0.46	(0.455-0.465)	2.5	(2.48-2.53)	2.720	(2.703-2.749)	0.5-2.6	1.55	0.53	<0.05
Uric Acid	Male	18,972	0.394	0.102	0.21	(0.209-0.212)	0.24	(0.237-0.243)	0.58	(0.576-0.586)	0.62	(0.616-0.627)	0.29-0.52	0.405	0.06	<0.05
Uric Acid	Female	18,426	0.311	0.088	0.17	(0.169-0.172)	0.19	(0.188-0.192)	0.48	(0.477-0.485)	0.51	(0.506-0.516)	0.16-0.48	0.32	0.08	<0.05

Table 3.13 presents the percentiles 2.5th, 5th, 95th and 97.5th, their 90% confidence intervals (CI), as well as a summary of the statistical values obtained comparing the mean values obtained to the mean values of the reference rages in use in NIP, assuming the reference range of NIP ranges over ± 2 standard deviations. It can be seen from the results that although the ranges are close, the statistical comparison when performing a T-test between the mean values of the study and NIP is statistically significantly different indicated by a p<0.05 **(Table 3.12).**

Table 3.14: Table to present 5th -95th and 2.7th -95.7th percentile obtained in the study against the reference ranges used by different laboratory organization and published in a text books (stated in SI units)

		PERCENTILES OBTAINED IN STUDY		NAMIBIA		SOUTH AFRICA		UNITED STATES		CANADA			TEXT BOOKS		
Test	Sex	5th - 95th	2.5th-97.5th	NIP reference ranges (in use)	PathCare Laboratories Namibia & SA	National Health Laboratory Services (NHLS)	LANCET Laboratories	University of Maryland School of Medicine		CML HealthCare (Canada)	BCBiomedical (British Columbia)		Tietz (Ed) 1986		Bishop (5th Ed) 2005
Hb	male	8.5-15.2	8.1-15.4	14.0-18.8	13-18	13-17	no values obtained	14-18		135-180 g/dL	135-180 g /L		13.5 - 17.5		
	female	8.8-14.6	8.3-15.0	12.0-16.9	12-16	11-15		12-16		115-165 g/dL	115-160 g/L		12.0 - 16.0		
Urea	both male & female	1.6-8.4	1.3-9.1	2.1-7.1	2.5-6.7	2.1-7.1	2.1-7.1	2.1-7.1		3.8-8.2	2.0-9.0				2.5-6.4
Creatinine	male	43-133	366-141	62-106	57-113	64-104	80-115	62-115		60-115	45-110		53 - 106		53-106
	female	39-117	33-131	35-88	39-91	49-90	53-97	53-97		60-127	45-90		44 - 97		44-97
Glucose (F)	both male & female	3.9-9.1	3.4-9.5	3.89-6.38	3.5-5.5	≤ 7.0	3.3-6.0	4.1-5.9		3.3-6.0	3.6-5.5	serum	4.44 - 6.38		3.9-6.5
Glucose (R)		4.0-6.9	3.7-7.1	critical 26.9	3.5-5.6	≤ 11.1	3.3-7.8	<6.7		3.3-7.8	3.6-8.3	serum	3.63-5.70		Not stated
Cholesterol	male	2.9-6.1	2.6-7.0	2.12-5.49	< 5.0	< 5.0	<5.00	<5.2	desirable	< 4.6 < 29 yrs	2-4.6	< 30 yrs	3.63 - 8.03	both male & female	3.6-5.2
	female	3.1-6.7	2.8-7.1	2.46-6.1	< 5.0	< 5.0	<5.00	5.2-6.2	borderline	< 5.2 > 29 yrs	2-5.2	> 30 yrs	3.63 - 5.70	desirable	3.6-5.2
Triglycerides	both male & female	0.46-2.5	0.39-2.72	0.5-2.6	< 1.7	< 1.7	<1.70	<2.83	fasting	< 1.71	≤2.2	>18 yrs	0.45 - 1.81	male	0.11-2.15
								2.83-5.67	borderline				0.40 - 1.53	female	
Uric Acid	male	0.2-0.58	0.21-0.62	0.21-0.42	0.24-0.52	0.21-0.43	0.26-0.45	0.27-0.47		180-450 umol/L	150-430 umol/L		0.27 - 0.48		2.08-4.28
	female	0.19-0.48	0.17-0.51	0.15-0.35	0.16-0.48	0.16-0.36	0.14-0.39	0.15-0.37		120-400 umol/L	140-360 umol/L		0.18 - 0.38		1.55-3.57

Table 3.14 displays many different reference ranges / reference intervals, reported in mmol/L except for Haemoglobin reported in g/dL and Creatinine reported in µmol/L are in use in Namibia and other countries. It should also be considered that some laboratories have specific reference ranges for special conditions, e.g. diabetes (SAMDSA guidelines) or for Cholesterol in cases of cardiovascular risk stratification

Chapter 4. Discussion

4.1 Pre-tested data used in the study

The aim of this study was to use pre-tested laboratory results from the laboratory information software data base (*Meditech)* in the Namibia Institute of Pathology (NIP) to determine the suitability of this huge data source to determine population based reference ranges for the Namibian population. Unfortunately, at the stage of data collection, no Sequential Query Language (SQL) data base module existed in the NIP *Meditech* programme, which presented enormous challenges, as a laboratory report programme feature had to be used to extract data separately from all the locations, wards and hospitals, resulting in possible exclusion of clinician consulting room's results where most healthy patient data would have been obtained.

It has then been found that after analysis and statistical removal of data from the assumed non-healthy patient population group, a reference range for a selected group of laboratory tests, i.e. Haemoglobin, serum Urea, serum Creatinine, plasma Glucose, serum Cholesterol, serum Triglycerides and serum Uric Acid, is comparable by visual observation to the reference ranges presently in use in NIP (**Table 3.13**). However the comparison between the mean values obtained in the study and values currently used in NIP using the t-test, showed significant differences ($p<0.05$) for some of the tests done **(Table 3.12)**, and it is asserted that a refinement of the analytical statistics method and a more stringent selection of data could provide a reliable means of reference value determination.

It is also observed that many different reference values and reference ranges **(Table 3.14)** exist internationally, probably because of different methodologies, adoption and transference of reference ranges from manufacturer, literature of other sources. It is not always clear if clinicians are made aware of these differences and it would be much simpler if reference values could be more or less standardized, and only in cases of extreme differences could be stated specifically in the laboratory reports.

4.2 General introductory comments

4.2.1 The importance of outlier removal

For a study such as this, where indirect data is used, and where it is not clear whether patients are healthy or diseased, it is important to remove outliers from the data set. Several

studies have reported on the importance of outlier removal. It is also one of the steps in the reference range determination recommended by IFCC (2008). Osborne and Overbay (2004), as well as Walfish (2008) stated that researchers should always check for outliers and endeavour to eliminate these from the data set. Many authors have reported on various methods to remove outliers from the data to be analysed. (Reed *et al*., 1971; Chua *et al.,* 1978; Horn *et al.,* 2001; Ilcol and Aslan, 2006; Inal *et al.,* 2010), while Ceriotti and co-workers*,* (2009) used a method proposed by Dixon (1953). Horn and co-workers (2001) proposed the use of a Box-Cox transformation and Inal and co-workers (2010) have used modern statistical software, such as the SPSS statistics program (IBM SPSS Inc., Chicago, IL., USA) to identify and delete outliers using the stem-and-leaf and box-plots functions of the software.

For the purpose is this study the box-plot function of the SPSS software, version 21, was used to delete outliers, i.e. only values in the inner 75 % of values remained while the lower (Q1) and upper (Q4) quartiles were removed.

As found in this study, from Urea, Creatinine and Glucose results more than 18% outliers (Urea=18.85%, Creatinine=18.33%, fasting Glucose=24.6%, random Glucose=18.17%) were removed. From the other test results less than 10% outliers had to be removed **(Table 2.3**). This is seen as an indication that in general from the huge representative data set not many results were beyond the inner 2 quartiles (inner 50%) of the distribution.

4.2.2 Observed skewness of distribution of data

It is also important to note that biological data tends to be skewed. It is stated by several authors that positive skewness is common in biological data, and therefore can be expected to be present in the distribution of reference individuals (Reed *et al*., 1971; Solberg, 1986; Wright, 1999). Schork and co-workers (1990) have stated that “skewness may be an integral part of a biological trait, and may, in fact, have a biological meaning”.

To calculate skewness the Kolmogorov-Smirnov test **(Table 3.9)** was performed in the SPSS program for every analyte and it was found that although the visual appearance was only slightly skewed to the right, as could be expected, when tested for significance of skewness with the Kolmogorov-Smirnov test, all the distributions displayed a significant skewness, which was more than ± 2 time the standard error of skewness.

A logarithmic transformation is effective in considerably reducing or removing the skewness (Wright, 1999), and it was tested for all the analyte distributions. Since it only made a real difference in the Triglyceride distribution, only that was therefore reported.

4.3 Discussion of specific test results

4.3.1 Selected results of data only obtained from one region (Khomas) in Namibia

Raw data from Haemoglobin, Creatinine and Urea results could only be obtained from the Khomas region due to data extraction constraints as was explained in Par. 2.5 and 4.3. Therefore, this data set did not represent the population of Namibia and it is also assumed to contain an excess of results from non-healthy individuals since most of the data originated from hospital wards of the two major government reference hospitals in Namibia, which could be seen from the location ID in the data set. It is therefore assumed that many of the patients tested were from these hospitals since the majority of the patients from consulting rooms and private hospitals are not tested by the NIP central laboratory. Therefore, most of the patients' results of these three tests can be assumed not to be healthy.

A) Haemoglobin

Data of Haemoglobin results was only obtained from the Khomas region and therefore cannot be seen to be representative of the Namibian population.

The percentile range from 2.5thth to 97.5thth was 6.64-16.9 g/L for males and 7.81-15.2 g/L for females (**Table 3.13**). This differs significantly ($p<0.0$) from the reference ranges used in NIP (M=14.0-18.8 g/L and F=12.0-16.9 g/L) as well as those proposed by the WHO and also from those used in other laboratories **(Table 3.14).**

The recommendation for diagnosis of anaemia of the WHO state that the reference ranges for non-pregnant women (15 years of age and above) are ≤12.0 g/L and for pregnant women it is ≤11.0 g/L, while for men it is ≤13.0 g/L. The mean value of the distribution obtained differs significantly from the reference ranges used in NIP **(Table 3.12),** and this may be because the Haemoglobin results were only from the Khomas region and originated mostly from Windhoek.

The implication of the values obtained in the study relating to the WHO recommendation indicates that most of the Haemoglobin values from the Khomas region are from patients who are anaemic. Although information on the clinical data of patients was not available, certain assumptions were made.

Many of the results of females were assumed to be from a pregnant population, since all anti-natal testing include a Haemoglobin test. This is data that should have been excluded from the study, but since no information on pregnancy or not no clinical data was available, this data could not be excluded. Therefore it is assumed that the majority of female

Haemoglobin results were lower than that of healthy normal females. Another assumption may be that since many of the results come from patients being hospitalised in Windhoek in the two major reference hospitals of the country, many results could therefore have come from individuals who might have nutritional deficiencies and possibly many may be HIV infected.

B) Urea

Data of Urea results was also only obtained from the Khomas region and therefore cannot be seen to be representative of the Namibian population.

8,111 results were analysed and a the 2.5^{th} 97.5^{th} percentile range obtained was 1.3–9.1 mmol/L and the 5^{th} – 95^{th} percentile range was 1.6-8.4 mmol/L, while the reference range used by NIP is 2.1 – 7.1 mmol/L. Reference ranges used in South Africa are also comparable to what NIP uses (2.1-7.1 mmol/L). Comparing the mean value of 4.444 obtained with the mean value of 4.60 of the reference range used in NIP resulted in $p<0.05$. Although the difference is statistically significant, the results for the 5^{th}-95^{th} percentile is higher than those used in South Africa, but are comparable to upper limit of reference range used in Canada **(Table 3.14),** which is 2.0-9.0 mmol/L. However the results obtained are not comparable to those that Ilcol and Aslan (2006) determined using the indirect method from a defined population and reported ranges of 2.83–7.34 mmol/L for males and 2.33–6.50 mmol/L for females.

The wider reference range obtained could be attributed again to the fact that data was only obtained from the Khomas region, where, as explained before, most of the data came from the two big government reference hospitals in Windhoek.

C) Creatinine

It must again be stated that the data collected in the Khomas region only was mainly from the two government hospitals, and therefore it is assumed that this population contained many non-healthy results. After these assumed non-healthy results had been removed, the 2.5^{th} and 97.5^{th} percentiles (36-141 µmol/L for males and 33-131 µmol/L for females), was obtained. The 5^{th} – 95^{th} percentile range was 43-133 µmol/L for males and 39-117 µmol/L for females, while the reference range used in NIP was found to be lower, i.e. M=62-106 and F=35-88 µmol/L, and a statistically significant difference ($p<0.00$) was detected when performing a t-test **(Table 3.13).**

Many different reference ranges used in the region (NIP: M=62-106, F=35-88; Pathcare (Namibia & SA): M=57-113, F=39-91; NHLS: M=64-104, F=49-90; Lancet (SA): M=62-115,

F=53-97) µmol/L which also differs from some ranges used internationally for example in CML (Canada): M=60-115, F=60-127 µmol/L, and United States (Maryland School of Medicine): M=62-115, F=53-97 µmol/L). **(Table 3.14).** Ilcol and Aslan (2006) reported obtaining a reference range of M=59.2–119.3 and F=52.1–91.01 µmol/L by using the indirect method.

Although these many different reference ranges are used, the upper limits are still lower than those obtained in the study. This again could be attributed to the extreme high number of hospital patients from the two government hospitals in Windhoek in the Khomas region.

As can be seen from this discussion, for Creatinine many different reference ranges are used in various laboratories, which again demonstrates the need of population based reference ranges.

4.3.2 Results of data from all thirteen regions in Namibia

For four of the seven tests investigated raw data from all the administrative regions, were extracted from NIP LIS. During 2007 to 2010 NIP operated 30 laboratories in various towns in 13 different regions of Namibia representing the total Namibian population. This resulted in a substantive amount of data and therefore contributing towards solid statistical results.

A) Plasma Glucose

43,222 fasting Plasma Glucose and 55,620 random Plasma Glucose results were analysed.

Although nearly 25 % of outliers were removed from the fasting Glucose results, the distribution was still not normal as can be seen from the statistics (skewness=0.618), the histogram and probability plot and Kolmogorov-Smirnov test **(Table 3.9)** for normality $p<0.05$. The distribution of results is skewed to the right with many increased results on the right side of the histogram. Although data from the same patient who was tested more than once every second month was removed, repeat testing of diabetic patients, and possible high incidence of diabetic patients could be the reason. No clinical data was available, and therefore this could not be verified. This could also be because of possible incorrect recording or patients that did not fast and possibly were not correctly informed. For the purpose of determining reference ranges for Glucose using patient data, this method would not be reliable, unless strict supervision in fasting and correct recording of patient data is possible.

The percentiles obtained for the fasting Glucose results are presented in **Table 3.8**, and display a range of values that is not comparable with the reference range used in NIP when

performing a t-test ($p<0.05$) and the upper limit percentile 97.5^{th} = 9.5 mmol/L, and 95^{th} = 9.1 mmol/L obtained is not comparable to 6.1 mmol/L of WHO criteria. (6.1-7.1 mmol/L which is considered a grey zone for fasting blood Glucose defined by the Society for Endocrinology, Metabolism and Diabetes of South Africa (SEMDSA), the World Health Organization **(**WHO**)** and the American Diabetes Association (ADA) criteria, above which results are diagnostic of diabetes mellitus). There is a significant difference between the expected upper limit of 6.1 mmol/L according to SEMDSA criteria and the 95^{th} percentile of 9.1 mmol/L and the 97.5^{th} percentile of 9.5 mmol/L obtained**.**

The distribution of random Glucose results presents a better picture than that of the fasting Glucose results. The percentiles 97.5^{th} = 7.1 mmol/L and 95^{th} = 6.9 mmol/L which is the upper limit of the results is comparable to the SEMDSA, ADA and WHO recommendations for random Glucose estimation which is within 6.1-7.1 mmol/L **(Table 3.8).** There is still a shift to the right and again certain assumptions can be made in terms of a population that has a high incidence of diabetes. This again is worth further investigation.

ANOVA analysis of both the fasting and the random data set between the various regions indicated a significant difference ($p<0.05$), but visual inspection shows that Erongo, Oshana and Oshikoto regions have a higher mean value that the other regions, which may be due to possible non-fasting specimens in the data set, or also due to a possible high incidence of diabetes in these regions. This aspect would also be worth investigating further.

B) Cholesterol

A total of 48,354 (M=24,815 and F=23,539) results were analysed.

Although the results have a normal distribution upon visual inspection, the Kolmogorov-Smirnov test **(Table 3.9)** indicated significant skewness of $p<0.05$ for the normality test. The 95^{th} and 97.5^{th} percentile of 6.1 and 6.9 mmol/L for males and 6.2 and 7.0 mmol/L for females are above the upper limit of the reference ranges or 5.49 mmol/L for males, and 6.1 mmol/L for females which is currently in use in NIP. If the 5^{th} and 95^{th} percentiles (1.3-9.1) are used, this comes closer to the upper range of 7.1 mmol/L used in NIP. The results are also above the upper ranges used in medical laboratories in South Africa. However as mentioned before reference ranges differ from country to country, as can be seen in **Table 3.14.** According to Adult Treatment Panel III (ATPIII) report (2002) total Cholesterol should be below 5.2 mmol/L and borderline value should not be more than 6.2 mmol/L.

Results obtained in the study were higher than the recommended upper limits. This may be due to non-recording of fasting or non-fasting of the specimens tested. Although 1,094 outliers were removed, it is also assumed that a portion of the results could still be from a

non-healthy population, but it may also be due to an inherent tendency of high Cholesterol in the Namibian population. These results of this study should not be used, unless confirmed by rigorous patient selection criteria, clinical data and confirmation using a small conventional reference range estimation study. This would be worth further investigation.

An ANOVA comparison **(Table 3.11)** between the Cholesterol result mean values of the various regions indicated a statistical significant difference of $p<0.05$. The differences may be due to inherent ethnic and dietary differences, and this is also worth further investigation.

C) Triglyceride

The percentile ranges (2.5^{th} -97.5^{th} = 0.39-2.72 mmol/L and 5^{th} -95^{th} = 0.46-2.50 mmol/L) compare favourably with the NIP reference ranges (0.5-2.6 mmol/L), although the difference from comparison between the mean values was significant ($p<0.05$). Comparing the results with other laboratories in South Africa, it is noticeable that upper limit values of <0.7 mmol/L are used when a patient has fasted. According to the APTIII guidelines (2002) a fasting value of <0.7 is recommended, with the borderline limit of <2.6 mmol/L. It is also interesting to observe how many different reference ranges are in use in various countries in the world (**Table 3.14).**

Since Triglyceride is very dependent on diet, the many high values encountered in the study population might be due to non-fasting specimens, because information was not available on whether results originated from a fasting specimen or not. It might also be due to a high incidence of results of HIV positive patients in the data set, since incidence of HIV in the Namibian population is 18.8% (Namibian Sentinel Survey for HIV Prevalence 2010). Studies have shown that HIV positive persons on ARV treatment show increases in their blood Triglyceride levels (Fauvel *et al.,* 2001; Heath *et al*., 2001; Worm *et al.,* 2010).

The ANOVA analysis which compared result mean values of the different regions showed a significant difference between mean values ($p<0.05$) **(Table 3.11).** This may be due to different ethnic groups and also different lifestyle and dietary habits. This is another aspect that warrants further research.

D) Uric Acid

A total number 37,398 results were analysed. Since NIP uses different reference ranges for male and females, results were partitioned and 18,972 results for males and 18,426 for females were analysed.

The percentiles ranges 2.5th - 97.5th = 0.21-0.62 mmol/L and 5th - 95th = 0.24-0.58 mmol/L for males and 2.5th -97.5th = 0.17-0.51mmol/L and 5th - 95.5th = 0.19-0.48 mmol/L for females was found. This is not comparable to the ranges currently used in NIP **(Table 3.13).**

The upper limits of both percentile ranges are higher than the upper limit of the reference range used in NIP. This may be because the statistical method to separate the healthy from the non-healthy population is not adequate, but may also be due to dietary and life style differences in the Namibian population who consume a high volume of red meat, compared to populations from where reference ranges were obtained. It is therefore imperative to ensure accurate reference value determination for the Namibian population. This aspect also warrants further investigation. When comparing the results obtained with reference ranges used in laboratories in other countries it could be seen that the upper limit differs from country to country **(Table 3.14).**

4.3.3 Summary comments

When considering the general and individual study results, the indirect method used in this study to establish reference ranges is not robust enough and it is considered that much more rigid data selection criteria should have been used. Statistical manipulation of data to remove possible non-healthy population should also have been more robust. Although some authors (Ilcol and Aslan, 2006; Inal *et al*., 2010; Katayev *et al*., 2010) have demonstrated that they have used the indirect method for determining the reference ranges, this was only applied to a specific population group. The question remains if this method can be applied in Namibia with its heterogeneous population.

4.4 Limitations of the study

Although the total data set was more than 250,000, it cannot be said that it was truly representative of all results that NIP had produced during the 4 year period. This is because data was extracted selectively since no SQL data base module existed in *Meditech* at the stage of collection. Subsequently, a specific report program was written in *Meditech* to extract patient results according to the location of origin or testing location which was, in most cases, from hospital wards. This resulted in results predominately originating from hospitalized patients of which the majority is presumed to be non-healthy. Since a huge number of test results were available for analysis, it was asserted that by using a method of outlier removal, it was possible to remove most of the results from non-healthy persons.

For the tests only collected from the Khomas region (Haemoglobin, Urea and Creatinine), it was assumed that these originated mostly from hospital wards, and therefore included a great proportion of non-healthy values. This would have been prevented if the collection method used would have incorporated results from the other 12 regions of Namibia.

Much more healthy patients' data could have been obtained if it would have been possible to also utilize data in the data base collected of private laboratory companies. A perceived lack of data originating from private clinicians' consulting rooms may also be considered a limiting factor since most of these results would have been obtained from healthy individuals.

Another limitation could be considered to be the non-partitioning of results into age groups. Although age data was available, this was not considered to be accurate enough to be included in the study, and therefore not age partitioning was carried out. It is suggested that that if results could have been partitioned into age categories (Harris, 1975), considerable improvement in sensitivity over non-specific ranges, even when age-sex differences are significantly different, would be achieved.

4.5 Conclusion and recommendations

4.5.1 Hypothesis One

The hypothesis stated that the vast amount of historical or pre-tested data which is housed in the laboratory information system of the Namibia Institute of Pathology (NIP) would provide valuable health information once extracted and analysed. The laboratory test result values in the existing LIS data base of the NIP laboratory information system from general patients, both healthy and diseased, is sufficient to generate values that are statistically valid.

This was found to be true since although the method of extraction was cumbersome and required improvement, 254,271 records from seven laboratory tests could be extracted from the LIS of NIP which represents huge data set.

4.5.2 Hypothesis Two

It was hypothesised that the results derived from this study will create a basis for defining reliable clinical laboratory reference ranges for the Namibian population.

Although this study has proven that this could be done, much more work needs to be done to prepare and select the data set for defining reliable clinical laboratory reference ranges for Namibia.

4.5.3 Hypothesis Three

It was hypothesised that the results derived from this study are comparable to the reference range in use in Namibia Institute of Pathology (NIP).

The study demonstrated that pre-tested laboratory data can be used to determine laboratory reference ranges provided that:

1. Data set is representative, i.e. all results from all laboratories, if possible also from private laboratory companies, are surveyed. The laboratory specimens analysed by private laboratory companies come from predominately healthy people, because many laboratory tests done by private clinicians are mostly routine. The NIP laboratory mainly performs laboratory tests for patients from government hospitals and health centres which have a high volume of non-healthy patients.
2. If possible, more care should be taken to prevent inclusion of non-healthy patients' results. It is asserted that if it would be possible to eliminate patient results from high-care wards, and also find ways to ensure recording of accurate clinical data of patients tested, more non-healthy patients' results could be removed and prevent inclusion of those into the data set. This would be possible if a SQL database system would be available in *Meditech* to selective eliminate patients from high-care wards and hospitals.
3. Fasting and non-fasting specimens are recorded for Glucose, Cholesterol and Triglyceride as well as patients appropriately informed when a fasting specimen is required.
4. More stringent statistical analysis is carried out to accurately remove non-healthy population data, and determine suitability or different statistical tests to compare results.

It was also demonstrated that many different sets of reference ranges are in use and it is asserted that often responsible laboratory managers and pathologists introduce changes to reference intervals based on instrument selection and reference range transference from literature. Often clinicians may not be informed or even if the report forms indicate abnormal values, these might not be appropriate for a specific patient or patient population.

4.6 Future research

It is suggested that the method for determination of reference ranges for Namibia be further developed and reference values for additional laboratory tests be determined. Sufficient

information exists in the data base of the Namibia Institute of Pathology (NIP) laboratory information system (*Meditech*) to carry out extensive further investigation. It is also suggested that a specific and appropriate algorithm for data extraction and statistical analysis could to be developed to reliably determine reference ranges.

The results of this study have provoked other questions, which should be investigated further, for example:

- Why were the bulk of the blood Glucose results high even after removal of outliers?
- Why are differences in results between the 13 regions significant for most of the tests? Could it be genetic, ethnic or lifestyle (diet) factors?

It would be of benefit to Namibia if a comprehensive study on the incidence of diabetes in Namibia could be carried out with related identification of population specific risk factors.

It would also be of interest to investigate further specific differences between reference ranges between the various regions, and determine the reasons for this.

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Chapter 6 Figures and Tables

6.1 Figures

6.2 Tables

Chapter 7. Appendices

Appendix A **CPUT ethics certificate**

Cape Peninsula
University of Technology

Prof A J Esterhuyse
Head: Biomedical Sciences
Faculty of Health and Wellness Sciences
Tel.: +27(0)219596562
Fax: +27(0)219596096
Email: esterhuysejs@cput.ac.za

Date: 25 May 2012

Re: Extension of ethical approval for Cornelia de Waal-Miller

Dear FRC/REC Chairperson

We hereby request the committee for extension of ethical approval for the following research project:

Title: Compilation of clinical laboratory results and determination of reference ranges for the Clinical Laboratory in Namibia

Registration number: CPUT/HW-REC 2011/H08

Principle Investigator: Mrs Cornelia de Waal-Miller

Internal Supervisor: Prof AJ Esterhuyse

External Supervisor: Dr Bruce Noden

Although it was very challenging to get the data from NIP, which delayed the research significantly, good progress has been made over the last 6 months and the project is ongoing.

Data from NIP was received in "flat" text file and has to be extracted into a format for statistical analysis. The first batch of data has been received and "cleaning up" of the data is in progress whereby fields are to be extracted from ASCII and reformatted and linked in tabular form for further analysis in a statistical data processing in three parts.

Yours sincerely

AJ Esterhuyse
Associate Professor

Cape Peninsula
University of Technology

13 April 2011
CPUT/HW-REC 2011/H08

P.O. Box 1906 • Bellville 7535 South Africa •Tel: +27 21 442 6162 • Fax +27 21 447 2963
Symphony Road Bellville 7535

OFFICE OF THE CHAIRPERSON:
HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

At the meeting of the Health & Wellness Sciences Research Ethics Committee on 21 February 2011 approval was granted to Cornelia de Waal-Miller pending amendments that have now been received and reviewed. This approval is for research activities related to an MTech: Biomedical Technology at this institution.

TITLE:

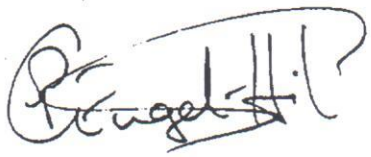
Compilation of clinical laboratory results and determination of reference ranges for the Clinical Laboratory in Namibia

INTERNAL SUPERVISOR:	**Prof J Esterhuyse**
EXTERNAL SUPERVISOR:	**Dr Bruce Noden**

Comment:

Research activities are restricted to those detailed in the proposal and application submitted in September 2010.

Approval will not extend beyond 13 April 2012. An extension must be applied for should data collection for this study continue beyond this date.



Prof PENELOPE ENGEL-HILLS
CHAIR: HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE

e-mail: *engelhillsp@cput.ac.za*

Appendix B Letter from Namibia Institute of Pathology (NIP)

NAMIBIA INSTITUTE OF PATHOLOGY (NIP) LTD.

Reg. No. 2000/431

Tel: +264-61-295 4200, Fax: +264-61-255 566, P.O. Box 277, Windhoek, Namibia

25 October 2010

Mrs. Cornelia de Waal-Miller
Department of Biomedical Science
School of Health and Applied Sciences,
Polytechnic of Namibia

Dear Madam,

Letter dated 11th October 2010 to request for permission to use data for research

This is to acknowledge receipt of you letter requesting permission to use the data in the laboratory information system of NIP for research purposes, dated 11th October 2010.

Although I, as the chairperson of the research committee of NIP, do not foresee a negative response, we have to convene a research committee meeting to table this matter for discussion and resolution. This meeting is anticipated to take place at the beginning of November after which you will be informed about the outcome.

This provisional permission should enable you to continue with the planning for this project, and I trust that we will be able to give you a favourable response before the middle of November.

NAMIBIA INSTITUTE OF PATHOLOGY (NIP) LTD.
HEAD OFFICE
20 OCT 2010
P.O. BOX 277 WINDHOEK
TEL: 264-61-2954200
FAX: 264-61-255566
REPUBLIC OF NAMIBIA

Harold T. Kaura

Harold Kaura,
Chairperson, Research Committee

General Manager: Technical Operations

Appendix C Letter from Ministry of Health and Social Services, Namibia

REPUBLIC OF NAMIBIA

Ministry of Health and Social Services

Private Bag 13198 **Ministerial Building** **Tel: (061) 2032510**
Windhoek **Harvey Street** **Fax: (061)272286**
Namibia **Windhoek** **E-mail:** eshaama@mhss.gov.na
Enquiries: Ms. Ester Shaama **Ref:** 17/3/3 **Date:** 28 February 2011

OFFICE OF THE PERMANENT SECRETARY

Ms. C de Waal-Miller
P.O. Box 31771
Windhoek

Dear Ms. de Waal-Miller

REQUEST FOR ETHICAL APPROVAL TO USE LABORATORY DATA FOR RESEARCH PURPOSES

1. The Ministry of Health and Social Services hereby acknowledge receipt of your request as stated above.

2. **Kindly be informed that permission to access the laboratory data has been granted under the following conditions;**

2.1 The data to be collected must **only** be used for your Master's degree in Biomedical Technology,

2.2 No other data should be collected other than the data stated in the requesting letter submitted to the Ministry.

Yours sincerely,

MR. K. KAHUURE
PERMANENT SECRETARY