COMPARATIVE ANALYSIS OF RANDOM BLOOD GLUCOSE LEVELS IN SERUM, PLASMA AND WHOLE BLOOD USING GLUCOSE OXIDASE AND HEXOKINASE METHODS UNDER SPECTROPHOTOMETRIC AND ELECTROCHEMICAL PLATFORMS

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Comparative analysis of random blood glucose levels in serum, plasma and whole blood using glucose oxidase and hexokinase methods under spectrophotometric and electrochemical techniques

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DECLARATION

I declare that this is my original work and has not been presented in any other institution for the award of a degree.

Gachoki Joyce Muthoni

Signature…….. Date……

This thesis has been submitted for examination with my approval as University Supervisors.

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Signature…….. Date……

DEDICATION

I wish to dedicate my work to my dear husband Mr. Robert K. Njoroge, my children Melanie Kairungi and Manuel Kairungi for their endless support both financially and morally. I also wish to extend this to the people suffering from diabetes and other non-communicable diseases.

ACKNOWLEDGEMENT

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ABBREVIATIONS AND ACRONYMS

2hrPPGT	Post prandial glucose test
ADA	American Diabetes Association
ADP	Adenosine Diphosphate
ATP	Adenosine triphosphate
β	Beta
CBGM	Continuous blood glucose monitoring
CO_2	Carbon dioxide
EDTA	Ethylenediaminetetraacetic acid
FBS	Fasting blood sugar
G6PDH	Glucose-6 Phosphate Dehydrogenase
GOD	Glucose oxidase
GDH	Glucose Dehydrogenase
H_2O_2	Hydrogen peroxide
HB	Haemoglobin
HbA1C	Glycated Haemoglobin
HK	Hexokinase
KNH	Kenyatta National Hospital
NAD	Nicotinamide Adenine Dinucleotide
NADH	Nicotinamide Adenine Dinucloetide Hydrogen
NCD	Non Communicable Diseases
NPV	Negative predictive value
O_2	Oxygen
OGTT	Oral glucose tolerance test
POD	Peroxidase

PPV	Positive predictive value
RBS	Random blood sugar
RPM	Revolution per Minute
SBGM	Self Blood Glucose Monitoring
CBGM	Continuous blood glucose monitoring
SPSS	Statistical Package for social science
WHO	World Health Organisation

ABSTRACT

Diabetes is, a condition associated with the impairment of the body's ability to produce or utilise the hormone insulin. This leads to abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. This serious non-communicable disease has been on the rise in Kenya partly because many people are not aware that they are diabetic since there are no serious early symptoms associated with the disease. It is estimated that the prevalence of diabetes in Kenya ranges from 2.7% (rural settings) and 10% (urban areas) affecting both the affluent and non-affluent population. It is expected that the actual numbers could be higher since many cases go unreported for lack of regular screening in the general population. In Kenya there is no study that has been conducted to compare the performance of current blood glucose testing methods being used. The suitability of serum or plasma as alternatives to whole blood has also not been well researched. The sample of choice for glucose testing has always been whole blood, either in fluoride or from a finger prick. The aim of the study was to compare the results obtained from these three sample types (whole blood, serum and plasma) using two methods: glucose oxidase and hexokinase methods. The investigation was carried out using 299 study subjects that included 149 diabetic patients attending diabetic clinic either for management, diagnosis or monitoring of blood sugar and 150 healthy individuals in the blood donation centre at Kenyatta National Hospital Nairobi County. The analytical instruments used were glucometers and a spectrophotometer to compare blood glucose levels in serum, plasma and whole blood using electrochemical and spectrophotometric platforms. While comparing the serum and plasma glucose concentration using hexokinase method under electrochemical and spectrophotochemical platforms, the mean difference for the two protocols was 0.145 which was found to be statistically insignificant ($p=0.342$) using paired T-test. Similarly while comparing the same using glucose oxidase method under the electrochemical and spectrophotochemical platform, the mean difference for the two protocols was 0.012 which was found to be statistically insignificant ($p=0.135$) using paired T-test. The results revealed that the two methods used in glucose concentration analysis were similar irrespective of the method or sample used. There was no significant difference in their means. Either of the two methods can be used interchangeably in the analysis of glucose and either serum or plasma in fluoride can be used as a specimen of choice.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Glucose is a simple sugar (Molecular weight $C_6H_{12}O_6$) which circulates in blood as blood sugar and is converted for storage in the body as glycogen. Glucose is the primary metabolic fuel for tissues in the body and its sources include: dietary carbohydrates, gluconeogenesis and hepatic glycogenolysis. ((Greenhaff, Hultman, & Harris, 2003).

Glucose concentrations in the blood are maintained within a narrow range by the action of the hormones insulin (hypoglycaemic action) and glucagon (hyperglycaemic activity) which regulate glucose availability, usage and storage. The hormones are produced by the pancreas and released in the circulation when required. Dysfunction in normal physiological regulation of glucose levels in blood is the essence of the diabetes. Glucose analysis is therefore an important test for screening of individuals to identify those who may be prediabetic or diabetic, as well as for the care management of diabetics under treatment.

Diabetes (commonly called diabetes mellitus) is a metabolic condition (non-communicable disease/ lifestyle disease) in which the body's ability to produce or respond to the hormone insulin is impaired. This results in abnormal metabolism of carbohydrates leading to elevated glucose levels in blood (hyperglycaemia) which marks the diagnosis of the disease. Diabetes affects a large population in the world many of whom are not aware of their condition with diagnosis being done either too late, done through medical outreach camps or on arrival to the hospital with related complications with hyperglycaemia being the hallmark of the

disease (Guariguata et al., 2014)

It was estimated that 415 million people lived with diabetes worldwide as of 2015 with diabetes Type 2 estimated to have 90% of the cases. This represented 8.3% of the total adult population for both male and female (Roglic, 2015). This number (415 million) has quadrupled since 1980 thus calling for health systems to be able to diagnose, treat and care for diabetic patients. There is an estimated 40 million living with Type 1 diabetes (WHO, 2016). The disease doubles ones risk of early death with an approximate 1.5 to 5.0 million deaths in every year from 2012 to 2015 resulting from the disease. Globally in 2014 it was estimated that the economic cost for

diabetes was US$612 billion, out of this 80% was found in the developing world (Roglic, 2015). This figure is expected to rise to around 18.6 million by 2030 if no interventions are done to try and curb the disease. The economic burden results from medical costs and this can impose burden not only to the people living with the disease and their families but also on the health-care system and the national economy (*WHO* 2016). In Kenya about 1% of the deaths in 2012 were directly attributed to diabetes (WHO, 2012) though this could be an underestimate since patients with diabetes do not die directly with the disease but from complications (Roglic, 2015).

There are various methods used in the laboratory for analysing glucose levels in blood these include glucose oxidase, glucose dehydrogenase and hexokinase methods. In the hexokinase method, nicotinamide adenine dinucleotide reduced form (NADH) is measured and it is directly proportional to the glucose concentration in the sample. When using glucose oxidase the glucose concentration is measured indirectly by detecting the levels of quinoneimine. According to Freckmann blood glucose levels vary among the GOD oxygen-sensitive system. For proper diagnosis and management of diabetes, there is need to compare screening methods performance and also selection of sample. Different researchers have conducted studies on effect of oxygen on glucose using different methods (Freckmann *et al*., 2012) but none has compared the performance of the methods which is the purpose of our study.

1.2 Statement of the problem

Diabetes is a serious non-communicable disease that is on the rise (WHO,2009) in Kenya. Many people are not aware of their status in the absence of serious symptoms associated with early stages of the disease. There are several methods currently used in the screening, diagnosis and management of the disease. No published study has been carried out in Kenya to compare the methods and test their performance characteristics. The sample of choice also has not been well researched on though the choice has always been whole blood, either in fluoride or from a finger prick. There may be time saving benefits to the use of serum and plasma in glucose analysis since in laboratory setup they are the most widely used specimen in clinical chemistry investigations and their suitability may avoid the necessity to obtain a separate blood sample for testing.

1.3Justification

Currently the two methods in the proposed study are available in reagents used for qualitative and quantitative techniques for glucose analysis. GOD and HK analytical methods are available in form of spectrophotometric and electrochemical platforms. The glucose levels produced by the two methods have not been compared in any Kenyan study. Clear information on the utilisation of these two methods in blood glucose analysis can be achieved through the comparison of the results generated using the three specimens of choice and this forms the basis of the proposed study.

1.4 Research Questions

Is there a difference in blood glucose levels of serum, plasma or whole blood when using glucose oxidase and hexokinase methods under spectrophotometric and electrochemical platforms?

1.4 Hypothesis

Null hypothesis

There is no difference in glucose concentrations in serum, plasma and whole blood specimens when using glucose oxidase and hexokinase methods under spectrophotometric and electrochemical platforms.

1.5 Objectives

1.5.1 General Objective

To compare blood glucose levels in serum, plasma and whole blood using glucose oxidase and Hexokinase analytical methods when using spectrophotometric and electrochemical platforms.

1.5.2 Specific Objectives

1. To determine glucose concentration in whole blood, serum and plasma using glucose oxidase and hexokinase methods under electrochemical platform from diabetic and non-diabetic individuals.

2. To determine glucose concentration in serum and plasma using glucose oxidase and hexokinase methods under spectrophotometric platform from diabetic and non-diabetic individuals.
3. To compare glucose concentration in serum and plasma using glucose oxidase and hexokinase methods under spectrophotometric and electrochemical platforms from diabetic and non-diabetic individuals.
4. To compare the performance **(**sensitivity, specificity, positive predictive value and negative predictive value**)** of glucose oxidase and hexokinase methods in determination of glucose levels in serum, plasma and whole blood under spectrophotometric and electrochemical platforms from diabetic and non-diabetic individuals.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Glucose which is the end product of carbohydrate metabolism is form of a simple sugar which provides energy to all human body cells some of which like brain cells and red blood cells rely solely on glucose for fuel(Steffes, 2005). Glucose is the main fuel for some tissues (e.g. erythrocyte) in the body and a preferred fuel for some (e.g. nervous system). Sources in the body include dietary carbohydrate, gluconeogenesis and hepatic glycogenolysis. A constant concentration of glucose supply to the cells is maintained at a constant rate to ensure that the cells do not starve and die in between meals and overnight. The body does this by help of glucagon and insulin hormones produced by the pancreas that act antagonistically (Dunning, *et al*., 2006). When glucose is in excess in the circulation the body responds by converting it into glycogen and then storing it in the liver and body muscles(Kori, Schmid-Priscoveanu, & Straumann, 2001). When glucose is in short supply or when the levels go below the normal ranges in the circulation, the body mobilizes glucose from stored glycogen and this is the key to maintain a constant blood-glucose level.The principal form of storage is glycogen which is a glucose polymer and is stored in the skeletal muscle. Glucose is maintained within a narrow range (children 60-100mg/dl, Adults 74-100mg/dl) by the action of insulin (hypoglycaemic action) and glucagon (hyperglycaemic action). The two hormones are secreted by islets of Langerhans cells in the pancreas and thus referred to as pancreatic endocrine hormones.

2.2 The aetiology and types of diabetes

Diabetes mellitus as currently understood, is almost certainly more than a single disorder of particular importance, yet poorly understood, as the propensity for persons with Diabetes mellitus to develop specific complications e.g. retinopathy with blindness, kidney failure with uremia, nerve damage and circulatory problems that predispose to tissue damage and may lead to amputations, heart disease and stroke. Diabetes which is non- communicable disease is a

threat to national development due to the complications resulting from the conditions which are costly to treat (Ministry of Health, *2015)*. Diabetes is an increasing burden in the Kenyan population and has become a threat to the economy (Jones, 2013). Diabetes mellitus is a common disorder affecting many people half of whom are not aware that they have the disease. Hyperglycemia is the hallmark of diabetes mellitus though there are some other factors like pancreatitis pituitary/thyroid dysfunction, renal failure and liver disease that can also cause hyperglycemia. Hypoglycemia which is less frequently observed can be found in conditions like insulinoma, hypopituitarism, neoplasm or insulin-induced hypoglycemia. The common underlying effect that can result to development of hyperglycemia is deficiency in secretion or action of the insulin hormone. This may be either an absolute or relative deficiency of insulin hormone production i.e. absolute deficiency (insulin dependent diabetes mellitus (IDDM) or Type 1) or deficiency (Non-insulin dependent diabetes mellitus (NIDDM) or Type II) (Greenhaff et al., 2003). Diabetes either develops at adulthood (Type II) or one can also be born with a defective pancreas that is not able to produce insulin and therefore developing diabetes at birth which is Type I (Juvenile diabetes).

If the disease is not diagnosed early and well managed it can be fatal. The condition can progressively drain the family resources making them unproductive, poor which eventually retards the economy eventually leading to poverty. Through collaboration with NGO, regional and international diabetes support bodies the Kenyan Government has come up with Kenya National Diabetes strategy 2010-2015 Diabetes policies and programmes in the view of reducing disease impact to diabetic people, people at risk and also to the country's economy (ministry of Public Health and Sanitation, 2010). This is also to help the government achieve her objective of non-communicable diseases reduction.

2.3 Risk factors

There are several factors which may be associated with development of diabetes; they can be either physiological or environmental. Environmental factors e.g diet and weight play a large part in contributing to the development of type 2 diabetes this is in addition to any genetic component present. Physiological factors such as stress, trauma or pregnancy may also increase the chances of developing diabetes. Glucose levels may be increased when one is stressed e.g in trauma, general anaesthesia, infection, burns, and Myocardial infarction, caffeine, drugs e.g antidepressants diuretics, estrogen can also increase the levels. Some pregnant women may

also experience glucose intolerance and a time the levels may raise to significant levels leading to gestational diabetes (Stanifer et al., 2016). Some drugs e.g alcohol, anabolic steroids, insulin acetaminophen may decrease glucose levels**.** There are some lifestyle factors also associated with diabetes e.g obesity which is a major contributing factor to the development of type 2 diabetes leading to increased insulin resistance. This mostly results from the effects of the adipose tissues (especially that in the abdomen around internal organs) which are a source of several chemicals signals e.g hormones and cytokines to other tissues within the body.

2.4 The role of genetics in diabetes

People with first-degree relatives suffering from type 2 diabetes have a much higher risk of developing the condition and these increases with the number of the relatives living with the condition. Among the monozygotic twins it is close to 100%, and about 25% of those with the disease have a family history of diabetes. According to Eberhart *et al*., 2004, among the people diagnosed with Type 2 diabetes 55% of these patients were obese at diagnosis.

2.5 Diagnosis of diabetes

Diagnosis of diabetes is key because with time hyperglycaemia erodes ability of the pancreas cells to make insulin leading to overcompensation and increase in insulin levels damaging the pancreas permanently over time. To test for the concentration of blood glucose in the circulation, there are several analytical tests that can be used either for screening, diagnosis or management of diabetes; fasting blood sugar (FBS), random blood sugar (RBS), oral glucose tolerant test (OGTT), 2 hour post prandial glucose test (2hrPPGT) and glycosylated haemoglobin (HbA1C) (Waithaka et al., 2010). Hyperglycaemia can also cause changes that lead to atherosclerosis which can lead to kidney failure, stroke, heart attack, erectile dysfunction, nerve damage, poor blood circulation to legs and feet and weakened immune system. There are various analytical methods used in diagnosis of and they rely on either glucose oxidase principle, glucose dehydrogenase principle or hexokinase principle. This depends on the type of enzyme involved in the reaction. The reaction is either coupled to chromophore or involves electric current generation. Assays that generate electric current are suitable for point of care instruments.

2.5.1 Glucose oxidase method

The technique involves a timed end point reaction in which glucose reagent is used to measure the concentration of glucose present. In the reaction, glucose oxidase (GOD) catalyzes the oxidation of glucose to gluconic acid with the formation of hydrogen peroxide (H_2O_2). The H_2O_2 formed reacts with phenol and 4-aminophenazone under the catalysis of peroxidase (POD) to form a red-violet quinoneimine which is directly proportional to the concentration of glucose in the sample. Quinonimine dye can also be quantified spectophotometrically at 505nm.

Glucose $+O_2 + H_2O$ –------ ^{GOD} --------> Gluconic acid + H_2O_2

$2H_2O_2$ + 4-aminophenazone +phenol ----------^{POD}--------->quinoneimine + $4H_2O$

2.5.2 Hexokinase method

This technique involves a timed end point reaction in which a glucose reagent is used to measure the glucose levels. Hexokinase catalyzes the transfer of phosphate group from adenosine triphosphate (ATP) in glucose to form adenosine diphosphate (ADP) and glucose-6-phosphate. The glucose-6-phosphate formed is then oxidized to 6-phosphogluconate with the concomitant reduction of NADH by the catalytic action of glucose-6-phosphate dehydrogenase (G6PDH). The reduced NADH is quantified spectrophotometrically at a wavelength of 340 nm.

Glucose + ATP ^{HK} --------->glucose-6-phosphate +ADP

Glucose-6-phosphate $+NAD^{+G6PD}$ -----> 6-phosphagluconate +NADH + H^+

2.6 Diabetes management

If not managed well diabetes can cause serious problems that include, retinopathy, kidney failure and nerve damage, stroke, foot ulcers or even heart disease (SEMDSA, 2017). The Kenyan Government National Medium Term Plan (2014-2018) and National health strategic plan (2014-2018) has identified and prioritised diabetes diagnosis and screening in a plan to achieve her millennium goals by the year 2030. The national Diabetes day is observed annually on the 14 November every year to serve as a reminder to the government on its commitment on the developed national policies for diabetes prevention, treatment and care (WHO 2014)According to Polsup *et al.,* 2010 in their study blood glucose monitoring can be used in

adjusting treatment regimens to improve glycemic control in patients with Type 2 not treated with insulin, this was demonstrated in their study by reduction of glycated haemoglobin (HBA1c) levels of the participants. Self and continuous blood glucose monitoring can provide the cornerstone and comprehensive assessment of glycemic management, (Ajjan, 2017). The issue of urgency and the seriousness of diabetes complications have brought about the use of self-blood glucose monitoring (SBGM) and continous blood glucose monitoring (CBGM) using point of care techniques, in which their accuracy and sensitivity need to be determined. Despite wide use of glucose monitoring using glucometers, its optimal use has not been determined,(Coster, Gulliford, Seed, Powrie, & Swaminathan, 2000). The performances of these methods is key in the diagnosis of diabetes and in management of diabetic patients. Blood glucose has been shown to be affected by partial oxygen (pO_2) particularly when pO_2 is decreased and when using the GOD method (Schmid *et al*., 2014) though no study has been done to compare the analytical methods in Kenya. According to Frank *et al.,* 2012, in their study on blood glucose detection in serum and plasma, when serum was used the glucose concentration was 1.15% lower than that of fluoride plasma. This difference was statistically significant though may not be physiologically relevant and therefore they recommend that when using serum an error of 1.15% applies. Intensive glucose control and management reduces the risk of developing hyperglycemic related conditions e.g. cardiovascular diseases, (Frank *et al.,* 2012). The need to identify the best real time method is therefore very crucial since most diabetic patients rely on self-monitoring systems which are based on electrochemical technique.

In the study blood glucose levels analysis was done using glucose oxidase and hexokinase methods both electrochemically and spectrophotomertically to find their performance and precision (repeatability and reproducibility) when using serum, plasma and whole blood.

2.7 Reference ranges for glucose levels

Lower reference limits for fasting specimens is <2.2 mmol/L (venous plasma), this is the value when the hypoglycemic symptoms are apparent though the value is highly variable between individuals.(Waithaka *et al*., 2010)

Upper reference limits for fasting and post standardized glucose intake specimens according to American Diabetes Association (ADA) and world health organization (WHO) are 5.6mmol/l

and 6.1mmol/l respectively. These reference limits do not differ across both genders and apply across the entire age-range (Waithaka *et al*., 2010) in the Kenyan population.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Introduction

Out of the 300 recruited study subjects one declined to be drawn blood sample and therefore we worked with 299 participants. All samples collected were analysed for glucose levels using GOD and HK methods under electrochemical and spectrophotochemical platforms. During the study total confidentiality was observed and no significant harm was reported during the whole study time.

3.2 Study area

The study was carried out at Kenyatta National Hospital (KNH) at the Medical outpatient clinic (clinic No. 17) and the KNH blood donation centre. KNH is the largest and oldest Hospital in the country which is a public, tertiary, teaching and referral hospital for the Ministry of Health. It is also a teaching hospital for the University Of Nairobi College Of Health Sciences. It has a capacity of over 1800 beds for public section and 209 for private wing. The clinic has a patient number totalling to 100 per clinic day that operates from Monday to Thursday for minor clinics, but there is a major clinic every Friday that has a total of 200 patients per clinic day. There are around 12 members of staff working in the clinic that holds at least 1000 patients weekly.

3.3 Study Design

This study was cross-sectional which included recruitment of diabetic and non-diabetic adult participants.

3.4 Study Population

The study recruited adults confirmed to be diabetic and those who sought screening for diabetes in the diabetic clinic (test). Non-diabetic blood donors in the KNH blood donation unit were recruited and considered as control. They signed a consent form, filled in a questionnaire and provided blood sample for glucose level measurement.

3.4.1 Inclusion Criteria

All adults who were screened and confirmed as diabetic who consented to take part were included in the study. A control group from the healthy population (blood donors) who consented was also included in the study.

3.4.2 Exclusion Criteria

Those participants who were below 18yrs, who were suffering from chronic diseases e.g. Cancer as well as pregnant women were excluded from the study. Those who declined to sign the consent form were also excluded from the study.

3.5 Sample Size Determination

General formula of sample size calculation for comparing two independent proportions used when comparing sensitivity and specificity of two tests or accuracy of two diagnostic tests was used. Where P_1 and P_2 denotes the expected proportion (sensitivity (Se) or specificity (Sp)) of two diagnostic tests respectively. For testing hypothesis H_0; $P_1=P_2$ versus H_1: $P_1 \neq P_2$, the required sample size with equal size based on normal approximation of binomial data with 1-α confidence level and 1-β power (Hajian-Tilaki, 2014)

$$n = \frac{\left[Z_{\frac{\alpha}{2}}\sqrt{P_0(1-P_0)} + Z_{\beta}\sqrt{P_1(1-P_1)}\right]^2}{(P_1 - P_0)^2}$$

Where $Z_{\frac{\propto}{2}}$ and Z_{β} denotes the upper $\frac{\propto}{2}$ and β percentiles of standard normal distribution and P_1 and P_2 are expected proportion for sensitivity and specificity respectively. For α=0.05 and β=0.02 (α=probability of type I and β= probability for type II errors), $Z_{\frac{\propto}{2}}$ =1.96 and Z_{β} =0.84 was inserted. The study wished to compare the Se of two alternative diagnostic tasks H0: P_1 = P_2 versus H1: $P_1 - P_2$. The sample size needed to have 95% confidence and 80% power to detect a difference of 10% from a Se of 70% (i.e. P_1 = 0.70, P_2 = 80%).

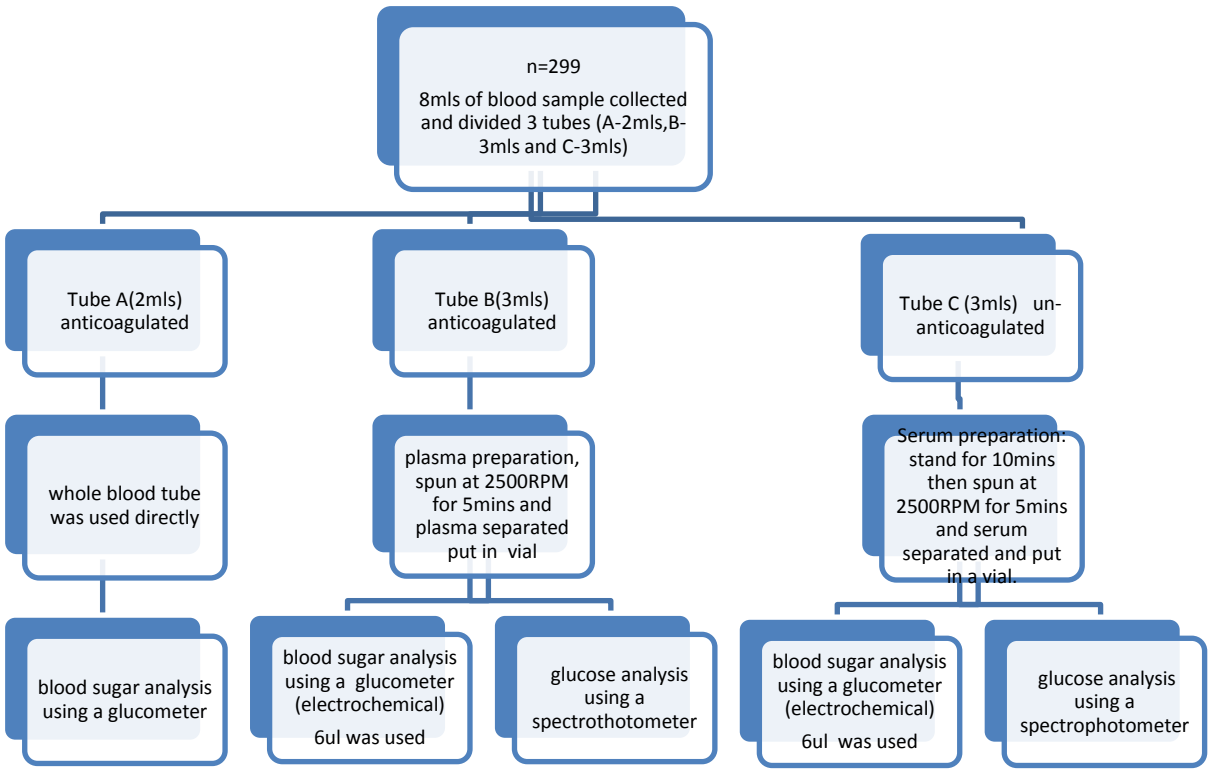
$$n = \frac{\left(1.96 \times \sqrt{0.70 \times 0.30} + 0.84 \times \sqrt{0.80 \times 0.20}\right)^2}{(0.10)^2} = 153$$

NB/ An average total of 300 participants was used with an equal number for both diabetic and non-diabetic group.

3.6 Sampling Method

Convenient sampling technique was used where the subjects were randomly selected from the diabetic clinic and blood donation centre. The subjects were provided with a consent form and a questionnaire that they filled and signed. They further provided a blood sample for blood glucose level analysis.

3.7 Flowchart of analytical procedures



The study was carried out in Kenyatta National Referral hospital in Nairobi county Kenya. Adult patients visiting the diabetic clinic for diagnosis, screening or management and healthy volunteers were randomly selected from the blood donation centre, given a questionnaire and a consent form which they read through filled and signed thereafter a blood sample was

collected for glucose level analysis. For relative suitability of serum, plasma and whole blood for blood glucose determination, fluoride plasma, whole blood and serum samples were compared for the same patient. The analysis of these samples was carried out within the first 30 minutes of drawing the blood.

3.8 Glucose testing on whole blood, serum and plasma samples

3.8.1 Collection of samples for glucose testing

8ml of whole blood was drawn from each of the participants through venipunture. This blood was then separated into three different tubes which were labelled with letters and serial numbers specific to each participant as follows: A contained 2ml, B,3ml and C,3ml of the aliquoted blood sample Tubes A and B contained heparin as an anticoagulant and were for the unprocessed whole blood and serum preparation, respectively. The aliquots in tube C which was a plain tube (no anticoagulant) were for plasma preparation.

3.8.2 Whole blood sample handling and storage

One tube (2ml of heparinised) was used as whole blood sample and the other (3ml of heparinised) was spun at 2500RPM for 5 minutes, plasma separated from cells and put in a sample vial and stored at room temperature.

3.8.3 Preparation of serum samples

The tube containing 3ml of sample (un-anticoagulated) was let to stand for 10 minutes and spun at 2500RPM for 5 minutes; serum separated and put in a sample vial and stored at room temperature.

3.8.4 Preparation of plasma samples

The tube containing 3ml of sample (heparinised) was let to stand for 10mins and spun at 2500RPM for 5 minutes; plasma separated and put in a sample vial and stored at room temperature.

3.9 Internal Quality Control

Analytical quality control is a procedure recommended in diabetes management to provide an assessment of meter and machine performance. Quality control was done by use of the manufacturers' (humatrol® from Human manufacturer) quality control material/solution

mimicking fresh human blood. These controls (for both dry and wet methods) were run in all the 20 sessions before running the test samples which all passed (was within the given control range) and this allowed for the running of the test.

3.10 Determination of glucose levels

3.10.1 Determination of glucose levels using the Glucose oxidase method under electrochemical platform;

The test electrode from the GOD kit (Roche'®) was removed from the vial and the vial recapped, with the arrow side facing up and in the direction of the arrow and the electrode was inserted into the test port of the meter firmly. The meter automatically turned on and the code number that matched the one on the vial displayed. When the blood sample blood-shape symbol blinked, at least 1ul of the sample was applied by touching the edge of the electrode to the blood sample until it filled. The blood sugar levels then displayed within 5 seconds on the LCD window in mmol/l, and automatically stored in the meter memory.

3.10.2 Determination of glucose levels using the Glucose oxidase method under spectrophotometric platform

Separated serum/plasma samples were aliquoted in a labelled sample vial. Machine (Cobas mira chemistry systems analyser®) was switched on and let to warm up, ensuring the sample and reagent probes are straight and continuous with the syringes free of air bubbles. Glucose reagents were placed into appropriate position on the reagent rack and sample cuvette on the sample rack. Using the PROG section the glucose test profile was checked to ensure that it was programmed. The control pools (normal and elevated) and cups were well positioned. The sample work list under routine menu was programmed by entering the first and the last numbers of the samples to be analysed then pressing test GLUC. A work list appeared on the screen and start button was pressed. When analysis was complete the results were printed out automatically.

3.10.3 Determination of glucose levels using the Hexokinase method under electrochemical platform;

The test electrode from the hexokinase electrode (Roche'®) was removed from the vial **and** the vial recapped, with the arrow side facing up and in the direction of the arrow the electrode was inserted into the test port of the meter firmly. The meter automatically turned on and the

code number that matched the one on the vial displayed on the LCD window. When the blood sample blood-shape symbol blinked, at least 1ul of the sample was applied by touching the edge of the electrode to the blood sample until it filled. The blood sugar levels then displayed within 5 seconds on the LCD window in mmol/l, and automatically stored in the meter memory.

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3.11 Limitations of the Study

Hexokinase method: hemolysis (Hb >0.5 g/dL) causes negative interference in bilirubin and triglycerides (> 55 mmol/L) has a positive interference.

Glucose oxidase method: peroxidase is inhibited by various substances including bilirubin and hemoglobin, leading to low results.

3.11 .1 Delimitation

To avoid hemolysis great caution was taken while drawing the samples and low speed was used when separating the samples. Incorporation of positive and negative control during each run also ensured that the values were within the range.

3.12 Statistical Analysis

The glucose levels data was collected following analysis of the samples then analysed using statistical package for social science software programme (SPSS). Where paired t-test was used to compare the means of the two methods, Kappa statistics for testing level of agreement of the two methods and to analyse the variation of the tests one way analysis of variance ((ANOVA) were used. Descriptive statistics was used where appropriate where *p*-value of less than or equal to 0.05 was considered statistically significant and data presented inform of tables.

3.13 Ethical Approval

The Ethical approval was acquired from the institutional research and ethics committee of Kenyatta National Hospital in collaboration the University of Nairobi (KNH/UON ERC) (**P360/07/2017).** Inclusion in the study and collection of biological specimens was carried out only after obtaining a written informed consent from each participant. Participation was on voluntary basis with no payment or incentives were offered to the study participants. Interviews were conducted ensuring confidentially to all participants. Confidentiality of the results was assured to the participants and were only be revealed to the participant through the clinician. To ensure data safety the results obtained was recorded and only the principal investigator was previa to the information.

CHAPTER FOUR

RESULTS

4.1 Introduction

During the study a total of 299 adult participants both male and female aged 18 years and above were recruited. From the 299 participants, 149 (49.8%) were diabetic whereas 150 (50.2%) were non-diabetic randomly selected from diabetic clinic and blood donation centre respectively. The total number of participant's distribution according to gender was 49.7% for males and 50.3% for females, with a percentage of 40.7% males and 59.3% females for the diabetic and 59.3% male and 40.7% female for the non-diabetics.

4.2 Assessment of the study subject results.

Internal Quality Control sera was analysed prior to the analysis of the study subjects specimens in order to ascertain that the study results were precise and accurate. The study performed 20 sessions of internal quality control for glucose during the analytical period. The internal quality control report for glucose were found to be within the assigned quality control sera range as shown in tables below.

Table 4.1: Quality assessment report for glucose under electrochemical platform.

Method	***IQC manufacturers kit Assigned report**			****Current study IQC report**		
	Sessions	IQC Mean	Range	Mean	Range	**Sd**
GOD	20	14.4mmol/l	2.6-22.2mmol/l	14.0mmol/l	13-15mmol/l	**0.5**
HK	20	13.7mmol/l	2.8-23mmol/l	13.5mmol/l	12.7-15mmol/l	**0.62**

*Sysmex Educational Enhancement and Development | December 2017, **Current study

Table 4.1: Quality assessment report for glucose under spectrophotometric platform.

Method	*IQC manufacturers kit Assigned report			**Current study IQC report		
	Sessions	IQC Mean	Range	Mean	Range	**Sd**
GOD	20	4.9mmol/l	4.3-6.3mmol/l	5.3mmol/l	4.0-6.5mmol/l	**0.62**
HK	20	5.3 mmol/l	4.0-6.6mmol/l	5.2mmol/l	4.2-6.4mmol/l	**0.55**

*Sysmex Educational Enhancement and Development | December 2017, **Current study

4.3 Glucose levels using GOD and HK methods in whole blood, serum and plasma under electrochemical platform form.

Out of the 299 subjects that were involved in the study, their glucose concentration was determined using whole blood, serum and plasma. The mean glucose concentrations for the diabetic on using whole blood, serum and plasma in the GOD method under electrochemical platform were 10.60mmol/l, 12.16 mmol/l and 11.98 mmol/l respectively. While for the non-diabetics mean glucose concentrations when using whole blood, serum and plasma were 7.74mmol/l, 8.11mmol/l and 7.89 respectively with glucose oxidase method. Consequently the mean glucose concentrations for the diabetic on using whole blood, serum and plasma in the hexokinase method under electrochemical platform were 9.94 mmol/l, 11.93 mmol/l and 11.94 mmol/l respectively. While for the non-diabetics mean glucose concentrations when using whole blood, serum and plasma were 7.74mmol/l, 7.94mmol/l and 7.01mmol/l respectively with Hexokinase method as shown in table below.

Table 4.2: Means for glucose oxidase and hexokinase methods under electrochemical platform.

	GOD		**Hexokinase**	
	Diabetic	Non-diabetics	Diabetic	**Non-diabetic**
Whole blood	10.60 mmol/l	7.75 mmol/l	9.93 mmol/l	**7.74 mmol/l**
Serum	12.16mmol/l	8.12 mmol/l	11.94mmol/l	**7.94 mmol/l**
Plasma	11.98 mmol/l	7.89 mmol/l	11.94mmol/l	**7.0 mmol/l**

4.3 Glucose levels in GOD and HK methods in serum and plasma under spectrophotometric platform.

The glucose concentration was determined using serum and plasma only (whole blood cannot be run spectrophotometrically) and the mean glucose concentrations for the diabetic subjects were 8.69mmol/l and 9.74mmol/l for serum and plasma respectively. While the mean glucose concentrations for the non-diabetics when using serum and plasma were 7.3mmol/l and 6.38mmol/l respectively with glucose oxidase method under spectrophotometric platform. The mean glucose concentrations for the diabetic on using serum and plasma in the hexokinase method under spectrophotometric platform were 8.93mmol/l and 9.93mmol/l respectively. While for the non-diabetics mean glucose concentrations when using serum and plasma were 7.00mmol/l and 6.94mmol/l respectively with hexokinase method under spectrophotometric platform as shown in the table 4 below.

Table 4.3: Means for glucose oxidase and hexokinase methods under spectrophotometric platform.

	Glucose oxidase means		**Hexokinase means**	
	Diabetics	Non-diabetics	Diabetics	**Non-diabetics**
Serum	8.69 mmol/l	7.35 mmol/l	8.94 mmol/l	**7.00 mmol/l**
Plasma	9.74 mmol/l	6.38 mmol/l	9.93 mmol/l	**6.94 mmol/l**

4.4 Comparison of glucose levels in serum and plasma (mmol/l) using GOD and HK methods under spectrophotometric and electrochemical platforms.

Two hundred and ninety-nine serum specimens were used to determine the glucose concentration using spectrophotometric and electrochemical techniques under hexokinase method. The mean serum glucose concentrations for spectrophotometric and electrochemical techniques were 7.46mmol/l and 7.45mmol/l respectively. The means difference for the two protocols was 0.017 which was found to be statistically insignificant (p=0.193) using paired T-test. Similarly, the same number of plasma specimens was used to determine glucose concentration using spectrophotometric and electrochemical techniques under hexokinase method. The mean plasma glucose concentrations for spectrophotometric and electrochemical platforms were 7.445mmol/l and 7.590mmol/l respectively. The means difference for the two protocols was 0.145 which was found to be statistically insignificant (p=0.342) using paired T-test. The above results are as shown in table 5 below

Table 4.4: Comparison of serum and plasma glucose concentration using hexokinase analytical method under electrochemical and spectrophotometric platform.

Sample type	Platform	N	Mean	SD	SEM	95% CI		MD	t-value	Sig. (2tailed)
						Lower	Upper			
Serum	electrochemical	299	7.452	4.311	0.249	0.085	0.281	0.017	1.980	**0.193**
	spectrophotometric	299	7.469	4.194	0.243					
Plasma	electrochemical	299	7.590	4.303	0.249	0.024	0.344	0.145	1.685	**0.342**
	spectrophotometric	299	7.445	4.216	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference

Serum specimens were used to determine the glucose concentration using spectrophotometric and electrochemical techniques under glucose oxidase method. The mean serum glucose concentrations for spectrophotometric and electrochemical techniques were 7.445mmol/l and 7.561mmol/lrespectively. The means difference for the two protocols was 0.084 which was

found to be statistically insignificant (p=0.185) using paired T-test. Similarly, the same numbers of plasma specimens were used to determine glucose concentration using spectrophotometric and electrochemical techniques under glucose oxidase method. The mean plasma glucose concentrations for spectrophotometric and electrochemical platforms were 7.325mmol/l and 7.337mmol/l respectively. The mean difference for the two protocols was 0.012 which was found to be statistically insignificant (p=0.135) using paired T-test. The above results are as shown in table 6 below

Table 4.5: Comparison of serum and plasma glucose concentration using glucose oxidase analytical method under electrochemical and spectrophotometric platform.

Sample type	platform	N	Mean	SD	SEM	95% CI of the difference		MD	t-Value	Sig. (2tailed)
						Lower	Upper			
Serum	electrochemical	299	7.561	4.244	0.361	3.6889	4.3442	0.084	1.126	**0.185**
	spectrophotometric	299	7.445	4.216	0.244					
Plasma	electrochemical	299	7.337	4.057	0.350	3.5724	4.2098	0.012	1.028	**0.135**
	spectrophotometric	299	7.325	4.116	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference

4.4.1 Difference between GOD and HK analytical methods using serum and plasma under spectrophotometric and electrochemical platforms.

Serum and plasma specimens from two hundred and ninety-nine study subjects were used to determine whether the hexokinase and glucose oxidase analytical methods for glucose estimation produce similar or different results when used to analyze same specimen in the same conditions. The mean serum glucose concentrations for electrochemical technique using glucose oxidase and hexokinase analytical methods were 7.561mmol/l and 7.452mmol/l respectively. The means difference for the two protocols was 0.115 which was found to be statistically insignificant (p=0.431) using paired T-test. The mean serum glucose concentration for spectrophotometric technique using glucose oxidase and hexokinase analytical methods

were 7.445mmol/l and 7.469mmol/l respectively. The means difference for the two protocols was 0.12 which was found to be statistically insignificant (p=0.532) using paired T-test.

Table 4.6: Comparison of Hexokinase and Glucose Oxidase analytical methods

Sample type	platform	Method	N	Mean	SD	SEM	95% CI of the difference		MD	t-value	Sig. (2tailed)
							Lower	Upper			
serum	electrochemical	GOD	299	7.561	4.244	0.361	3.256	4.111	0.115	1.282	**0.431**
		HK	299	7.452	4.311	0.249					
	spectrophotometric	GOD	299	7.445	4.216	0.244	3.421	4.251	0.12	1.244	**0.532**
		HK	299	7.469	4.194	0.243					
plasma	electrochemical	GOD	299	7.337	4.057	0.350	3.419	4.418	0.029	1.372	**0.751**
		HK	299	7.590	4.303	0.249					
	spectrophotometric	GOD	299	7.325	4.116	0.244	3.221	4.331	0.054	1.333	**0.673**
		HK	299	7.445	4.216	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference, HK= hexokinase, GOD= glucose oxidase

The mean plasma glucose concentration for electrochemical platform using glucose oxidase and hexokinase analytical methods were 7.337mmol/l and 7.59mmol/l respectively. The means difference for the two protocols was 0.029 which was found to be statistically insignificant (p=0.751) using paired T-test. The mean plasma glucose concentrations for spectrophotometric technique using glucose oxidase and hexokinase analytical methods were 7.325mmol/l and 7.445mmol/l respectively. The means difference for the two protocols was 0.054 which was found to be statistically insignificant (p=0.673) using paired T-test. The above results are as shown in table 7 below

4.4.2 Difference between glucose concentration in plasma and serum specimens used for glucose analysis

Serum and plasma specimens from two hundred and ninety-nine study subjects were used to determine whether the two types of specimens have any difference in glucose concentration. The mean plasma and serum glucose concentration using glucose oxidase method under electrochemical technique were 7.561mmol/l and 7.337mmol/l with mean difference of 0.224 and statistical t value of 1.324 which was statistically significance at p=0.013. On the other hand, the mean plasma and serum glucose concentration using hexokinase method under electrochemical technique were 7.59mmol/l and 7.452mmol/l with mean difference of 0.138 and statistical t value of 1.124 which was statistically significance at p=0.044.

The mean plasma and serum glucose concentration using glucose oxidase method under spectrophotometric technique were 7.325mmol/l and 7.445mmol/l with mean difference of 0.09 and statistical t value of 1.097 which was statistically significance at p=0.018.On the other hand, the mean plasma and serum glucose concentration using hexokinase method under spectrophotometric technique were 7.445mmol/l and 7.469mmol/l with mean difference of 0.024 and statistical t value of 1.041 which was statistically significance at p=0.039. The above results are as shown in table 8 below.

Table 4.7: Difference between glucose concentration in plasma and serum specimens used for glucose analysis

Specimen	Method	platform	N	Mean	SD	SEM	95% CI of the difference		MD	t	sig
							L	U			
P	GOD	electrochemical	299	7.561	4.244	0.361	3.111	4.212	0.224	1.324	**0.013**
S			299	7.337	4.057	0.350					
P	HK	electrochemical	299	7.59	4.311	0.249	3.108	4.123	0.138	1.124	**0.044**
S			299	7.452	4.303	0.249					
P	GOD	spectrophotometric	299	7.325	4.216	0.244	3.042	4.202	0.12	1.097	**0.018**
S			299	7.445	4.116	0.149					
P	HK	spectrophotometric	299	7.445	4.194	0.243	3.117	4.531	0.024	1.041	**0.039**
S			299	7.469	4.358	0.341					

KEY:S=serum, P=plasma, GOD=glucose oxidase, HK=hexokinase, N=number,SD=standard deviation, SEM=standard mean error, CI=confidence interval, L=lower limit, U= upper limit, MD=means difference, t=t value, sig=significance difference

4.4.3 Comparison between spectrophotometric and electrochemical platforms.

Serum and plasma specimens from two hundred and ninety-nine study subjects were used to determine whether spectrophotometric and electrochemical platforms for glucose estimation produce similar or different results when used to analyze same specimen using the same analytical method. The mean plasma glucose concentrations for glucose oxidase method under electrochemical and spectrophotometric platforms were 7.461mmol/l and 7.507 respectively. The means difference for the two platforms was 0.215 which was found to be statistically insignificant (p=0.415) using paired T-test. The mean serum glucose concentrations for glucose oxidase method under electrochemical and spectrophotometric platforms were 7.601mmol/l and 7.352mmol/l respectively. The means difference for the two platforms was 0.228 which was found to be statistically insignificant (p=0.514) using paired T-test. The above results are as shown in table 9 below.

Table 4.8: Comparison of Electrochemical and Spectrophotometric platforms

Method	**Specimen**	**platform**	**N**	**mean**	**SD**	**SEM**	**95% CI of the difference**		**MD**	**t**	**sig**
							L	U			
GOD	P	Elec	299	7.461	4.344	0.351	3.112	4.211	0.214	1.224	**0.415**
		Spec	299	7.507	4.050	0.360					
HK	S	Elec	299	7.601	4.301	0.509	3.109	4.133	0.228	1.134	**0.514**
		Spec	299	7.352	4.311	0.500					

KEY:S=serum, P=plasma, GOD=glucose oxidase, HK=hexokinase, N=number,SD=standard deviation, SEM=standard mean error, CI=confidence interval, L=lower limit, U= upper limit, MD=means difference, t=t value, sig=significance difference.

4.5 The performance of GOD and HK methods in determination of glucose levels in serum, plasma and whole blood.

Serum and plasma specimens from two hundred and ninety-nine study subjects were used to determine whether the two types of specimens have any difference in sensitivity and specificity when run using the two methods under different platforms. The sensitivity and specificity for the GOD method were 100% and 70% respectively with a PPV and NPV was 51% and 100% respectively as shown in table 10 below.

Table 4.9: Evaluating the performance characteristics of the glucose oxidase method Vs hexokinase method under electrochemical and spectrophotometric platform.

Glucose analysis	**Performance characteristics**			
Method	Sensitivity (%)	Specificity (%)	PPV (%)	**NPV (%)**
Hexokinase	100	100	100	**100**
Glucose oxidase	100	70	51	**100**

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

Results generated from the clinical laboratory are mostly utilized either to make a diagnosis or determine the prognosis of any pathological disorder. It is of paramount important therefore that these results are reliable and goes a long way in ensuring that the clients get the benefit of the services offered in the laboratory. The current study incorporated internal quality control procedure in every step of the undertaking in the generation of study results. The internal quality control results produced in this study were within the expected range according to the assigned glucose reagent kits manufacturer values. The internal quality control was found to be accurate and precise for both dry and wet chemistry glucose analytical procedures undertaken in the current study. Other qualitative and quantitative studies undertaken here in Kenya are in agreement with this good laboratory practice. (Waithaka et al., 2010) in a study on quantitative reference ranges for fasting profiles and oral glucose tolerance test for healthy adults from metropolitan region of Kenya emphasized the need to carry out internal quality control procedure prior to analyzing specimens from clients as a measure of ensuring that the results generated are accurate and precise.

The current study was able to make use of whole blood, plasma and serum as the specimens of choice since these are the type of specimens used for analysis of glucose concentration in a routine clinical laboratory. Other studies carried out elsewhere have widely used the three specimens in undertaking similar studies on glucose concentration (Carstensen, *et al.*,2008). The choice of the two categories of study population that is the diabetic and non-diabetic study subjects was made to ensure that the analysis is not biased. Normal study subjects enabled the study to use glucose concentration that is within the normal reference ranges using the studied techniques that is wet and dry chemistry. The anticipation of the current study was to reveal whether the glucose concentration in the three types of specimen is affected by the type of technique applied in a clinical laboratory. Studies carried out in other parts of the world are in agreement with the current study as far as the use of normal subjects referred in this study as non-diabetic study subjects. On the other hand, the current study had to use diabetic patients as study subjects, so as to determine whether the application of electrochemical or

spectrophotometric platforms in the analysis of high concentration of glucose is affected by the type of specimen used from a single subject. The utilization of diabetic patients as study subjects in an effort to establish the effect on the techniques applied on the three specimens under study is in agreement with a study carried out on a similar subject by (Jorgetto & Franco, 2018)**.**

It is very clear from the current study findings that the two methods applied in glucose analysis, are able to pick high glucose concentration in diabetic study subjects as well as picking normal glucose concentration in non- diabetic study subjects. This picture of glucose concentration is irrespective of the type of glucose analytical method applied. The current study has established that the glucose concentration in whole blood is lower than the glucose concentration in both serum and plasma of the study subjects. This trend is expressed in the two methods used that is glucose oxidase and hexokinase methods. The reason attributed to this low glucose concentration in whole blood is that glycolysis taking place in the blood cells utilizes glucose therefore lower the glucose concentration in the whole blood specimen. The current study findings are similar to other studies carried out elsewhere in the world. (Turchiano *et al.*, 2013) researching on the impact of blood sample collection and processing methods on glucose levels in community outreach studies established similar findings of low glucose levels due to glycolysis taking place in whole blood specimen. Serum and plasma specimens when analysed for glucose concentration produce similar results irrespective of the type of method used for analysis. This similarity in glucose concentration in both serum and plasma is expressed in glucose concentration for both diabetics and non-diabetics study subjects. The current study findings on the similarities of glucose concentration in serum and plasma concur with a similar study by Hye Soon Kim (2016) which aimed at establishing whether serum and plasma from the same individual using same or different glucose conventional methods generates similar or different results.

The study compared the means of the two methods and we found that there was no significant difference between the means in GOD in relation to HK method. This finding was similar to other studies conducted by Ayyaanar *et al., (*2018) in their study that showed that there was good correlation between GOD and HK methods.

Due to liberation of reagent market in our country today, clinical laboratories are at liberty to choose which reagent kit to use for analysis of glucose. The current study considered that these reagents kits are representation of the conventional methods widely used in our clinical

chemistry laboratories today. The current study also considered the possibilities of health workers making request for glucose analysis using either plasma or serum as the specimen of choice. It is evident from the current study that when plasma specimen is used for the analysis of glucose concentration using either the hexokinase or glucose oxidase analytical methods, the results generated are similar. Likewise, serum specimen generates similar results when the two analytical methods are used for analysis of glucose concentration. The current study finding is in agreement with a study carried out by Jia and Zhang, (2010), which evaluated serum and plasma glucose concentration results generated by routine glucose analytical methods.

In this study no significant difference was found between GOD method vis a vie Hexokinase while using plasma sample. But when using serum sample there was a significant difference (p=0.093) when using serum sample and this could be attributed to the fact that glycolysis could have taken place prior to analysis. This is in agreement with other studies carried out carried out by Eldin *et al.*,2010.

The current study has also established that there is no significant difference between the two platforms while using the same sample type under the same analytical method and this agrees to a study done by Manuela link *et al.,* 2013**.**

It is desirable to have tests that are both highly sensitive and highly specific to the analyte being analysed, though at times this is not usually possible. When choosing the cut-off point there is need to trade-off between sensitivity and specificity since when one is increased the other reduces. When sensitivity increases there is little decrease in specificity up until when high levels of sensitivity is reached. Screening methods should be valid, reliable and reproducible in the population where screening is taking place (WHO, 2003). To ensure this, uniform procedures and methods, standardized techniques, proper functioning equipment and quality assurance were used in this study. For any particular test reliability and validity must be tested. Validly is testing how accurate the results from a particular test are compared to another method. The current study tested the validity of GOD comparison to HK method in the determination of glucose levels. Measure of validity was done by testing the tests sensitivity, specificity, positive predictive value and negative predictive values in relation to the spectrophotometric (gold standard) when using serum and plasma samples. The sensitivity and specificity of the GOD method was 100% and 70% respectively while for hexokinase the sensitivity and specificity was 100% and 100% respectively. Predictive values of a test are

determined by sensitivity, specificity of the test and the prevalence of the disorder in the population being screened. In Kenya the prevalence of diabetes was estimated to be 3.3% (*WHO 2016)* and was projected to increase to 4.5% by 2025 though most of the cases undiagnosed. It is evident from our current study that the two methods are highly sensitive and specific on glucose. This evident on the fact that they were able to pick both high and low glucose levels on the study subjects used during the study. This was in comparison with hexokinase which is considered as the gold standard.

The level of agreement between GOD and hexokinase was determined using kappa statistics. A 2X2 contingency table was used to tabulate the results. The Kappa value was 0.52 and this therefore depicts that the level of agreement between the two techniques is substantial. The two methods are able to pick hyper and hypoglycemic status of individuals since they have shown to be having high sensitivity but hexokinase based have been found to be more specific than glucose oxidase based. Though either of the two methods can be used for diagnosis, screening and management of diabetes, hexokinase based method is more superior.

Hexokinase which was used as the gold standard in glucose screening diagnosis and management reported 68 true positive and 231 true negatives 0 false negatives and 1 false positive. . These were found to be in line with other studies conducted by (Ayyanar *et al.*, 2018)**,** in their study that showed that there was good correlation of both GOD and HK methods. This also correlates with the WHO 2003 guidelines on diabetes type 2 screening methods for venous blood sugar.

5.1 Conclusion

1. Glucose analysis in our clinical chemistry laboratories can be achieved by the use electrochemical and spectrophotometric analytical techniques with equal chance of generating similar results. This is an assurance that there is no bias in the results generated. Neither of the two techniques has an advantage over the other in relation to the techniques application. Clinical chemistry laboratories can interchangeably use the two techniques in glucose analysis.
2. Sodium fluoride plasma and serum can be used as specimens of choice for the analysis of glucose concentration for the purpose of diagnosing and monitoring glucose concentration of an individual. The current study has established that these two specimens generate different results. If plasma is to be used as the specimen of choice,

then it has to be harvested immediately after collection of whole blood so as to avoid the reduction of glucose contents due to glycolysis that take place in blood cells with ultimate utilization of glucose. Serum has been found to have lower glucose concentration than plasma.

3. The two widely used analytical methods that are hexokinase and glucose oxidase either under electrochemical and spectrophotometric platforms can interchangeably be used in the analysis of glucose concentration. The two methods have been found to have the same diagnostic value in terms of result generation using one type of specimen (serum/plasma). Clinical laboratories can make use of reagent kits formulated with hexokinase and glucose oxidase analytical methods.

5.2 Recommendations

1. The study recommends use hexokinase based reagent kits or electrodes for blood sugar diagnosis since it has been found to be more superior than GOD based.
2. Similar studies to be carried out using whole blood and sodium fluoride anticoagulant plasma to determine whether there will be any similarities or differences with the current study findings.
3. The current study recommends that similar studies to be carried out to compare all three methods (glucose oxidase, glucose dehydrogenase and Hexokinase) under the two platforms at different time interval.

REFERENCES

Ajjan, R. A. (2017). *How Can We Realize the Clinical Benefits of Continuous Glucose Monitoring ? 19*, 27–36. https://doi.org/10.1089/dia.2017.0021

Ayyanar, K., Pichandi, S., & Janakiraman, P. (2018). Evaluation of Glucose Oxidase and Hexokinase Methods. *International Journal of Biotechnology and Biochemistry*, *14*(1), 51–58.

Coster, S., Gulliford, M. C., Seed, P. T., Powrie, J. K., & Swaminathan, R. (2000). Monitoring blood glucose control in diabetes mellitus: A systematic review. *Health Technology Assessment*.

Eldin Abdelsalam, K. A., Dirar, A. M., & Abdallah, D. A. (2010). Effect of Storage Time and Temperature on some Serum Analytes. In *International Journal of Pathology* (Vol. 8).

Frank, E. A., Shubha, M. C., & D'Souza, C. J. M. (2012). Blood Glucose Determination: Plasma or Serum? *Journal of Clinical Laboratory Analysis*, *26*(5), 317–320. https://doi.org/10.1002/jcla.21524

Freckmann, G., Schmid, C., Ph, D., Baumstark, A., Ph, D., Pleus, S., … Haug, C. (2012). *System Accuracy Evaluation of 43 Blood Glucose Monitoring Systems for Self-Monitoring of Blood Glucose according to DIN EN ISO 15197*. *6*(5), 1060–1075.

GLOBAL REPORT ON DIABETES WHO Library Cataloguing-in-Publication Data Global report on diabetes. (2016). Retrieved from http://www.who.int/about/licensing/copyright_form/index.html

Greenhaff, P. L., Hultman, E., & Harris, R. C. (2003). Carbohydrate Metabolism. *Principles of Exercise Biochemistry*, *46*, 108–151. https://doi.org/10.1159/000074367

Guariguata, L., Whiting, D. R., Hambleton, I., Beagley, J., Linnenkamp, U., & Shaw, J. E. (2014). Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Research and Clinical Practice*, *103*(2). https://doi.org/10.1016/j.diabres.2013.11.002

Hajian-Tilaki, K. (2014). Sample size estimation in diagnostic test studies of biomedical informatics. *Journal of Biomedical Informatics*, *48*, 193–204. https://doi.org/10.1016/j.jbi.2014.02.013

Jones, T. L. E. (2013). *Diabetes Mellitus : the increasing burden of disease in Kenya*. *6*(3).

Jorgetto, J. V., & Franco, L. J. (2018). *The impact of diabetes mellitus on quality of life – differences between genders*. 11–17.

Kori, A. A., Schmid-Priscoveanu, A., & Straumann, D. (2001). Vertical divergence and counterroll eye movements evoked by whole-body position steps about the roll axis of the head in humans. *Journal of Neurophysiology*.

Ministry of Health. (2015). *Kenya National Strategy for the Prevention and Control of Non-Communicable Diseases*. 80.

ministry of Public Health and Sanitation. (2010). *Kenya National Diabetes Strategy*.

Rising number of diabetes cases cause for concern. (2009). (August).

Roglic, G. (2015). World Diabetes Congress 2015: The Global Health Challenges Stream. *Diabetes Research and Clinical Practice*, *108*(2), 367–368. https://doi.org/10.1016/j.diabres.2015.03.010

Schmid, C., Baumstark, A., Pleus, S., Haug, C., Tesar, M., & Freckmann, G. (2014). *Impact of Partial Pressure of Oxygen in Blood Samples on the Performance of Systems for Self-Monitoring of Blood Glucose*. *16*(3). https://doi.org/10.1089/dia.2013.0184

Stanifer, J. W., Cleland, C. R., Makuka, G. J., Egger, J. R., Maro, V., Maro, H., … Philippin, H. (2016). Prevalence, risk factors, and complications of diabetes in the Kilimanjaro region: A population-based study from Tanzania. *PLoS ONE*, *11*(10). https://doi.org/10.1371/journal.pone.0164428

Steffes, M. (2005). *Fasting Glucose in Plasma NHANES Laboratory Procedure Manual Hexokinase-mediated reaction*. (January), 1–11. Retrieved from https://wwwn.cdc.gov/nchs/data/nhanes/2005-2006/labmethods/glu_d_met_fasting_glucose.pdf

Sundvall, J., Tuomilehto, J., Carstensen, B., & Lindstro, J. (n.d.). *Original Article Measurement of blood glucose : comparison between different types of specimens*.

Turchiano, M., Nguyen, C., Fierman, A., Lifshitz, M., & Convit, A. (2013). Impact of blood sample collection and processing methods on glucose levels in community outreach studies. *Journal of Environmental and Public Health*, *2013*, 1–4. https://doi.org/10.1155/2013/256151

Type, S., Guidelines, D., & Committee, E. (2017). *SEMDSA 2017 Guidelines for the*

Management of Type 2 diabetes mellitus. *22*(1).

Waithaka, S. K., Njagi, E. N. M., Ngeranwa, J. J. N., Mwangi, D. M., Chiuri, B. M., Njagi, L. J., & Gatua, W. K. (2010). Quantitative reference ranges for fasting profiles and oral glucose tolerance test for healthy adults in metropolitan region of Nairobi, Kenya. *International Journal of Health Research*, *3*(1), 13–19.

WHO. (2016). *Global Reports on Diabetes*. Retrieved from http://www.ijncd.org/article.asp?issn=2468,8827;year=2016;volume=1;issue=1;spage=3;epage=8;aulast=Roglic

APPENDICES

Appendix I: Ethical Approval letter

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School of Biomedical Science
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J.K.U.A.T

Dear Joyce,

REVISED RESEARCH PROPOSAL –COMPARATIVE ANALYSIS OF RANDOM BLOOD GLUCOSE LEVELS IN SERUM, PLASMA AND WHOLE BLOOD USING GLUCOSE OXIDASE, GLUCOSE DEHYDROGENASE AND HEXOKINASE METHODS (P360/07/2017)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above proposal. The approval period is from 23rd October 2017 –22nd October 2018.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
d) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

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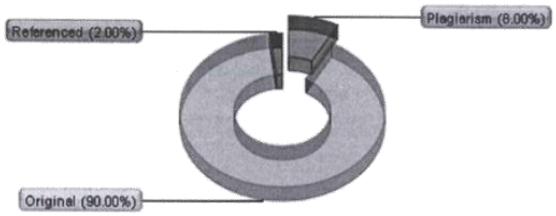

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Appendix III: Informed Consent Form

Introduction

Hello, My name is Joyce Gachoki from Jomo Kenyatta University of Agriculture and Technology. I am conducting a study on "comparative analysis of random blood glucose levels in serum, plasma and whole blood using glucose oxidase, glucose dehydrogenase and hexokinase methods". Diabetes, a condition associated with the impairment of the body's ability to produce or utilize the hormone insulin, leading to elevated levels of glucose in the blood and urine, is a serious non-communicable disease in Kenya and the whole world with many people not being aware of their condition because there are no serious symptoms associated with the disease. It is estimated that the prevalence in Kenya ranges from 2.7% (rural) and 10% (urban).In the USA, diabetes was the 7^{th} leading cause of death in 2010. Some people are not aware of the condition until it is too late because diabetes does not have major symptoms.

Purpose of the research

The study is aimed to establish the accuracy, sensitivity and specificity of glucose oxidase and glucose dehydrogenase used in the blood glucose level estimation using serum, plasma and whole blood as the specimens and comparing the results with hexokinase.

Type of Research Intervention

This research will involve your participation by signing a consent form and giving blood sample for estimation of glucose levels.

Participant Selection

You are being invited to take part in this study because your views will help me know what the gaps are and see what interventions can be put in place to address the problem.

Voluntary Participation

Your participation in this study is entirely voluntary. It is your choice whether to participate or not. If you choose not to participate all the services you receive at this clinic will continue and nothing will change.

Risks/Discomforts

The procedure will involve drawing of blood from your arm and it will inflict some pain but it should take less than 20 minutes.

Benefits

Your participation is likely to help us formulate targeted strategies aimed at addressing diabetes diagnosis and management.

Confidentiality

The information that we collect from this study will be kept private. Any information about you will be reported anonymously and no names will be used. You can ask me any questions about any part of the study, if you wish to.

Storage of samples

The samples will be stored for 5 years and after that they will be destroyed and disposed of. If you don't want your sample to be stored, you are given a period a period of 3 months to contact us. If you will not come, it will be assumed that you have agreed that your samples be stored for further analysis.

Whom Do I Call if I Have Questions or Problems?

For questions about the study, call or contact Joyce M. Gachoki, Jomo Kenyatta University of Agriculture and Technology, Mobile: 0721-246967 or Dr. Amos Mbugua of JKUAT, Mobile: 0702-961963, or Dr. S.K. Waithaka of MKU, Mobile 0722362719. For questions about your rights as a volunteer, contact the Secretary of the Ethics Committee at, Kenyatta National Hospital/ University of Nairobi Ethical committee. Tel 254-020-2726300-9

INFORMED CONSENT FORM

I,(name of the volunteer)_____

Of (address)_____

I have read the foregoing information, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Participant: Print Name: _____

Signature/mark or Thumb print _____

Date _____

Person Obtaining Consent:

I have explained the nature and demands of the above study to the volunteer and answered her questions:

Print Name: _____

Signature: _____

Date: _____

Impartial Witness: (only necessary if the volunteer was not able to read and understand the Consent Information sheet and informed Consent Document):

I affirm that the Informed Consent Document has been read to the volunteer and she understands the study, had her questions answered, and I have witnessed the volunteer's consent to study participation.

Print Name: _____

Signature/mark or Thumb print _____

Date _____

Appendix IV: Publication

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Research Article

Comparative Analysis of Random Blood Glucose Levels in Serum, Plasma and Whole Blood Using Glucose Oxidase and Hexokinase Methods under Spectrophotometric and Electrochemical Platforms

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Abstract
Diabetes is, a condition associated with the impairment of the body's ability to produce or utilise the hormone insulin. This leads to abnormal metabolism of carbohydrates and elevated levels of glucose in the blood. This serious non-communicable disease has been on the rise in Kenya partly because many people are not aware that they are diabetic since there are no serious early symptoms associated with the disease. It is estimated that the prevalence of diabetes in Kenya ranges from 2.7% (rural settings) and 10% (urban areas) affecting both the affluent and non-affluent population. It is expected that the actual numbers could be higher since many cases go unreported for lack of regular screening in the general population. In Kenya there is no study that has been conducted to compare the performance of current blood glucose testing methods being used. The suitability of serum or plasma as alternatives to whole blood has also not been well researched. The sample of choice for glucose testing has always been whole blood, either in fluoride or from a finger prick. The aim of the study was to compare the results obtained from these three sample types (whole blood, serum and plasma) using two methods: glucose oxidase and hexokinase methods. The investigation was carried out using 300 study subjects that included 150 diabetic patients attending diabetic clinic either for management, diagnosis or monitoring of blood sugar and 150 healthy individuals in the blood donation centre at Kenyatta National Hospital Nairobi County. The analytical instruments used were glucometers and a spectrophotometer to compare blood glucose levels in serum, plasma and whole blood using electrochemical and spectrophotometric platforms. While comparing the serum and plasma glucose concentration using hexokinase method under electrochemical and spectrophometric platforms, the mean difference for the two protocols was 0.145 which was found to be statistically insignificant (p=0.342) using paired T-test. Similarly while comparing the same using glucose oxidase method under the electrochemical and spectrophotometric platform, the mean difference for the two protocols was 0.012 which was found to be statistically insignificant (p=0.135) using paired T-test. The results revealed that the two methods used in glucose concentration analysis were similar irrespective of the method or sample used. There was no significant difference in their means. Either of the two methods can be used interchangeably in the analysis of glucose and either serum or plasma in fluoride can be used as a specimen of choice.
Keywords: *Glucose oxidase, Hexokinase, electrochemical , spectrophotometric.*

Introduction

Diabetes (commonly called diabetes mellitus) is a metabolic condition (non-communicable disease/ lifestyle disease) in which the body's ability to produce or respond to the hormone insulin is impaired. This results in abnormal metabolism of carbohydrates leading to elevated glucose levels in blood (hyperglycaemia) which marks the diagnosis of the disease. Diabetes affects a large population in the world many of whom are not aware of their condition with diagnosis being done either too late, done through medical outreach camps or on arrival to the hospital with related complications with hyperglycaemia being the hallmark of the disease [1].

It was estimated that 415 million people lived with diabetes worldwide as of 2015 with diabetes Type 2 estimated to have 90% of the cases. This represented 8.3% of the total adult population for both male and female [2]. The number has quadrupled since 1980 thus calling for health systems to be able to diagnose, treat and care for diabetic patients. There is an estimated 40 million living with Type 1 diabetes[3]. The disease doubles ones risk of early death with an approximate 1.5 to 5.0 million deaths in every year from 2012 to 2015 resulting from the disease. Globally in 2014 it was estimated that the economic cost for diabetes was US$612 billion, out of this 80% was found in the developing world [2]. This figure was expected to rise to around 18.6 million by 2030 if no interventions are done to try and curb the disease. The economic burden results from medical costs and this can impose burden not only to the people living with the disease and their families but also on the health-care system and the national economy[4]. In Kenya about 1% of the deaths in 2012 were directly attributed to diabetes (WHO, 2012) though this could be an underestimate since patients with diabetes do not die directly with the disease but from complications [2].

There are various methods used in the laboratory for analysing glucose levels in blood these include glucose oxidase, glucose dehydrogenase and hexokinase methods. In the hexokinase method, nicotinamide adenine dinucleotide (β-NADH) is measured and it is directly proportional to the glucose concentration in the sample. When using glucose oxidase the glucose concentration is measured indirectly by detecting the levels of quinoneimine. According to Freckmann blood glucose levels vary among the GOD oxygen-sensitive system. For proper diagnosis and management of diabetes, there is need to compare screening methods performance and also selection of sample. Different researchers have conducted studies on effect of oxygen on glucose using different methods[5] but none has compared the performance of the methods which is the purpose of our study.

Materials and Methods

This cross-sectional comparative study was carried out at the medical outpatient clinic and the blood donation centre after obtaining an informed consent form duly signed by the subject. All samples collected were divided in to three, prepared and analysed for glucose levels using glucose oxidase and hexokinase methods under electrochemical and spectrophotochemical platforms. In any case analysed total confidentiality was observed and no significant harm was reported during the whole study time.

For relative suitability of serum, plasma and whole blood for blood glucose determination, fluoride plasma, whole blood and serum samples were compared for the same patient. The analysis of these samples was carried out within the first 30 minutes of drawing the blood.8ml of whole blood was drawn from each of the participants through venipunture. This blood was then separated into three different tubes which were labelled with letters and serial numbers specific to each participant as follows: contained 2mls, B,3mls and C, 3mls of the aliquoted blood sample Tubes A and B contained fluoride as an anticoagulant and were for the unprocessed whole blood and serum preparation, respectively. The aliquots in tube C which was a plain tube (no anticoagulant) were for plasma preparation.The tube containing 3ml of sample (un-anticoagulated) was let to stand for 10mins and spun at 2500RPM for 5

minutes; serum separated and put in a sample vial. The tube containing 3ml of sample (anticoagulated) was let to stand for 10mins and spun at 2500RPM for 5 minutes; plasma separated and put in a sample vial. The samples were then analysed by glucose oxidase and hexokinase methods under electrochemical and spectophotochemial platforms for glucose levels.

Results

A total of 300 adult participants both male and female aged 18 years and above were recruited for the study. Out of the 300 recruited one declined to allow their blood sample to be drawn. Therefore blood samples were obtained from 299 participants. From these 299, 149 (49.8%) were diabetic whereas 150 (50.2%) were non-diabetic randomly selected from diabetic clinic and blood donation centre respectively. The total number of participant's distribution according to gender was 49.7% for males and 50.3% for females, with a percentage of 40.7% males and 59.3% females for the diabetic and 59.3% male and 40.7% female for the non-diabetics. The mean serum glucose concentrations for electrochemical technique using glucose oxidase and hexokinase analytical methods were 7.337 and 7.452 respectively. The means difference for the two protocols was 0.115 which was found to be statistically insignificant (p=0.431) using paired T-test. The mean serum glucose concentration for spectrophotometric technique using glucose oxidase and hexokinase analytical methods were 7.325and 7.445 respectively. The means difference for the two protocols was 0.12 which was found to be statistically insignificant (p=0. 0.532) using paired T-test. The mean plasma and serum glucose concentration using glucose oxidase method under spectrophotometric technique were 7.325 and 7.415 with mean difference of 0.09 and statistical t value of 1.097 which was statistically significance at p=0.018.On the other hand, the mean plasma and serum glucose concentration using hexokinase method under spectrophotometric technique were 7.469 and 7.445 with mean difference of 0.024 and statistical t value of 1.041 which was statistically significance at p=0.039. Serum and plasma specimens from two hundred and ninety-nine study subjects were also used to determine whether the two types of specimens have any difference in sensitivity and specificity when run using the two methods under different platforms. The sensitivity and specificity for the GOD method were 100% and 70% respectively with a PPV and NPV was 51% and 100% respectively.

Table 1 Comparison of serum and plasma glucose concentration using hexokinase analytical method under electrochemical and spectrophotometric platforms.

Sample type	platform	N	Mean	SD	SEM	95% CI		MD	t-value	Sig. (2tailed)
						Lower	Upper			
Serum	electrochemical	299	7.452	4.311	0.249	0.085	0.281	0.017	1.980	0.193
	spectrophotometric	299	7.469	4.194	0.243					
Plasma	electrochemical	299	7.590	4.303	0.249	0.024	0.344	0.145	1.685	0.342
	spectrophotometric	299	7.445	4.216	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference

Table 2 Comparison of serum and plasma glucose concentration using glucose oxidase analytical method under electrochemical and spectrophotometric platform

Sample type	technique	N	Mean	SD	SEM	95% CI of the difference		MD	t-value	Sig. (2tailed)
						Lower	Upper			
Serum	electrochemical	299	7.561	4.244	0.361	3.6889	4.3442	0.084	1.126	0.185
	spectrophotometric	299	7.445	4.216	0.244					
Plasma	electrochemical	299	7.337	4.057	0.350	3.5724	4.2098	0.012	1.028	0.135
	spectrophotometric	299	7.325	4.116	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference

Table 3 Comparison of Hexokinase and Glucose Oxidase analytical methods

Sample type	platform	Method	N	Mean	SD	SEM	95% CI of the difference		MD	t-value	Sig. (2tailed)
							Lower	Upper			
serum	electrochemical	GOD	299	7.337	4.244	0.361	3.256	4.111	0.115	1.282	0.431
		HK	299	7.452	4.311	0.249					
	spectrophotometric	GOD	299	7.325	4.216	0.244	3.421	4.251	0.12	1.244	0.532
		HK	299	7.445	4.194	0.243					
plasma	electrochemical	GOD	299	7.561	4.057	0.350	3.419	4.418	0.029	1.372	0.751
		HK	299	7.590	4.303	0.249					
	spectrophotometric	GOD	299	7.415	4.116	0.244	3.221	4.331	0.054	1.333	0.673
		HK	299	7.469	4.216	0.244					

Key: N=number, SD=standard deviation, SEM =standard mean error, CI=confidence interval, MD=means difference, HK= hexokinase, GOD= glucose oxidase

Table 4 Difference between glucose concentration in plasma and serum specimens used for glucose analysis

Specimen	Method	platform	N	mean	SD	SEM	95% CI of the difference		MD	t	sig
							L	U			
P	GOD	electrochemical	299	7.561	4.244	0.361	3.111	4.212	0.224	1.324	0.013
S			299	7.337	4.057	0.350					
P	HK	electrochemical	299	7.59	4.311	0.249	3.108	4.123	0.138	1.124	0.044
S			299	7.452	4.303	0.249					
P	GOD	spectrophotometric	299	7.415	4.216	0.244	3.042	4.202	0.09	1.097	0.018
S			299	7.325	4.116	0.149					
P	HK	spectrophotometric	299	7.469	4.194	0.243	3.117	4.531	0.024	1.041	0.039
S			299	7.445	4.358	0.341					

KEY:S=serum, P=plasma, GOD=glucose oxidase, HK=hexokinase, N=number,SD=standard deviation, SEM=standard mean error, CI=confidence interval, L=lower limit, U= upper limit, MD= mean difference, t=t value, sig= significance difference.

Table 5 Evaluating the performance characteristics of the glucose oxidase method Vs hexokinase method under electrochemical and spectrophotometric platforms.

Glucose analysis Method	Performance characteristics			
	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Hexokinase	100	100	100	100
Glucose oxidase	100	70	51	100

Discussion

Results generated from the clinical laboratory are mostly utilized either to make a diagnosis or determine the prognosis of any pathological disorder. It is of paramount important therefore that these results are reliable and goes a long way in ensuring that the clients get the benefit of the services offered in the laboratory. The current study incorporated internal quality control procedure in every step of the undertaking in the generation of study results. The internal quality control results produced in this study were within the expected range according to the assigned glucose reagent kits manufacturer values. The internal quality control was found to be accurate and precise for both dry and wet chemistry glucose analytical procedures undertaken in the current study. Other qualitative and quantitative studies undertaken here in Kenya are in agreement with this good laboratory practice.[6] in a study on quantitative reference ranges for fasting profiles and oral glucose tolerance test for healthy adults from metropolitan region of Kenya emphasized the need to carry out internal quality control procedure prior to analysing specimens from clients as a measure of ensuring that the results generated are accurate and precise.

The current study was able to make use of whole blood, plasma and serum as the specimens of choice since these are the type of specimens used for analysis of glucose concentration in a routine clinical laboratory. Other studies carried out elsewhere have widely used the three specimens in

undertaking similar studies on glucose concentration[7]. The choice of the two categories of study population that is the diabetic and non-diabetic study subjects was made to ensure that the analysis is not baised. Normal study subjects enabled the study to use glucose concentration that is within the normal reference ranges using the studied techniques that is wet and dry chemistry. The anticipation of the current study was to reveal whether the glucose concentration in the three types of specimen is affected by the type of technique applied in a clinical laboratory. Studies carried out in other parts of the world are in agreement with the current study as far as the use of normal subjects referred in this study as non-diabetic study subjects. On the other hand, the current study had to use diabetic patients as study subjects, so as to determine whether the application of dry or wet chemistry analytical techniques in the analysis of high concentration of glucose is affected by the type of specimen used from a single subject. The utilization of diabetic patients as study subjects in an effort to establish the effect on the techniques applied on the three specimens under study is in agreement with a study carried out on a similar subject by[8].

It is very clear from the current study findings that the two methods applied in glucose analysis, are able to pick high glucose concentration in diabetic study subjects as well as picking normal glucose concentration in non- diabetic study subjects. This picture of glucose concentration is irrespective of the type of glucose analytical method applied. The current study has established that the glucose concentration in whole blood is lower than the glucose concentration in both serum and plasma of the study subjects. This trend is expressed in the two methods used that is glucose oxidase and hexokinase methods. The reason attributed to this low glucose concentration in whole blood is that glycolysis taking place in the blood cells utilizes glucose therefore lower the glucose concentration in the whole blood specimen. The current study findings are similar to other studies carried out elsewhere in the world.[9] researching on the impact of blood sample collection and processing methods on glucose levels in community outreach studies established similar findings of low glucose levels due to glycolysis taking place in whole blood specimen. Serum and plasma specimens when analysed for glucose concentration produce similar results irrespective of the type of method used for analysis. This similarity in glucose concentration in both serum and plasma is expressed in glucose concentration for both diabetics and non-diabetics study subjects. The current study findings on the similarities of glucose concentration in serum and plasma concur with a similar study by Hye Soon Kim2016 which aimed at establishing whether serum and plasma from the same individual using same or different glucose conventional methods generates similar or different results.

The study compared the means of the two methods and we found that there was no significant difference between the means in GOD in relation to HK method. This finding was similar to other studies conducted by Ayyaanar et al, 2018 in their study that showed that there was good correlation between GOD and HK methods.

Due to liberation of reagent market in our country today, clinical laboratories are at liberty to choice which reagent kit to use for analysis of glucose. The current study considered that these reagents kits are representation of the conventional methods widely used in our clinical chemistry laboratories today. The current study also considered the possibilities of health workers making request for glucose analysis using either plasma or serum as the specimen of choice. It is evident from the current study that when plasma specimen is used for the analysis of glucose concentration using either the hexokinase or glucose oxidase analytical methods, the results generated are similar. Likewise, serum specimen generates similar results when the two analytical methods are used for analysis of glucose concentration. The current study finding is in agreement with a study carried out by Jia and Zhang, 2010, which evaluated serum and plasma glucose concentration results generated by routine glucose analytical methods.

For any particular test reliability and validity must be tested. Validly is testing how accurate the results from a particular test are compared to another method. The current study tested the validity of GOD comparison to HK method in the determination of glucose levels. Measure of validity was done by testing the tests sensitivity, specificity, positive predictive value and negative predictive values in relation to the gold standard when using serum and plasma samples. The sensitivity and specificity of the GOD method was 100% and 70% respectively while for hexokinase the sensitivity and specificity was 100% and 100% respectively.

These were found to be in linewith other studies conducted by[10], in their study that showed that there was good correlation of both GOD and HK methods. This also correlates with the WHO 2003 guidelines on diabetes type 2 screening methods for venous blood sugar.

In this study no significant difference was found between GOD method vis a vie Hexokinase while using plasma sample. But when using serum sample there was a significant difference (p=0.093) when using serum sample and this could be attributed to the fact that glycolysis could have taken place prior to analysis. This is in agreement with other studies carried out carried out by Eldin Abdelsalam, Dirar, & Abdallah, 2010)

It is desirable to have tests that are both highly sensitive and highly specific to the analyte being analysed, though at times this is not usually possible. When choosing the cut-off point there is need to trade-off between sensitivity and specificity since when one is increased the other reduces. When sensitivity increases there is little decrease in specificity up until when high levels of sensitivity is reached. Screening methods should be valid, reliable and reproducible in the population where screening is taking place (WHO, 2003). To ensure this, uniform procedures and methods, standardized techniques, proper functioning equipment and quality assurance were used in this study.

Predictive values of a test are determined by sensitivity, specificity of the test and the prevalence of the disorder in the population being screened. In Kenya the prevalence of diabetes was estimated to be 3.3% (*WHO 2016)* and was projected to increase to 4.5% by 2025 though most of the cases undiagnosed. It is evident from our current study that the two methods are highly sensitive and specific on glucose. This evident on the fact that they were able to pick both high and low glucose levels on the study subjects used during the study. This was in comparison with hexokinase which is considered as the gold standard

The level of agreement between GOD and hexokinase was determined using kappa statistics. A 2X2 contingency table was used to tabulate the results. The Kappa value was 0.52 and this therefore depicts that the level of agreement between the two techniques is substantial. The two methods are able to pick hyper and hypoglycemic status of individuals since they have shown to be having high specificity and sensitivity. Therefore either of the two methods can be used interchangeably for diagnosis, screening and management of diabetes Hexokinase which was used as the gold standard in glucose screening diagnosis and management reported 68 true positive and 231 true negatives 0 false negatives and 1 false positive.

Conclusion

Glucose analysis in our clinical chemistry laboratories can be achieved by the use electrochemical and spectrophotometric analytical techniques with equal chance of generating similar results. This is an assurance that there is no bias in the results generated. Neither of the two techniques has an advantage over the other in relation to the techniques application. Clinical chemistry laboratories can interchangeably use the two techniques in glucose analysis.

Sodium fluoride plasma and serum can be used as specimens of choice for the analysis of glucose concentration for the purpose of diagnosing and monitoring glucose concentration of an individual. The current study has established that these two specimens generates different results. If plasma is to be used as the specimen of choice, then it has to be harvested immediately after collection of whole

blood so as to avoid the reduction of glucose contents due to glycolysis that take place in blood cells with ultimate utilization of glucose. Serum has been found to have lower glucose concentration than plasma.

The two widely used analytical methods that are hexokinase and glucose oxidase can interchangeably be used in the analysis of glucose concentration. The two methods have been found to have the same diagnostic value in terms of result generation using one type of specimen (serum/ plasma). Clinical laboratories can make use of reagent kits formulated with hexokinase and glucose oxidase analytical methods.

Recommendations

1) Similar study to be carried out using the same analytical techniques and methods on other specimens such as cerebral spinal fluids.
2) Similar studies to be carried out using whole blood and heparinized plasma to determine whether there will be any similarities or differences with the current study findings
3) The current study recommends that results generated using plasma as specimen of choice should be interpreted using glucose reference ranges established using plasma of healthy individuals. On the other hand, results generated using serum as specimen of choice should be interpreted using glucose reference ranges established using serum of healthy individuals

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Conflict of interest: None declared

References

1. Ajjan, R. A. (2017). How Can We Realize the Clinical Benefits of Continuous Glucose Monitoring ?, *19*, 27–36. http://doi.org/10.1089/dia.2017.0021
2. Ackermann, R. T., Liss, D. T., Finch, E. A., Schmidt, K. K., Hays, L. M., Marrero, D. G., &Saha, C. (2015). A randomized comparative effectiveness trial for preventing type 2 diabetes. American Journal of Public Health, 105(11), 2328–2334. http://dx.doi.org/10.2105/AJPH.2015.302641
3. Ahmad OB, Boschi-Pinto C, Lopez AD, Murray CJL, Lozano R, Inoue M. Age Standardisation of rates: a New WHO standard. Global program on evidence for health policy discussion paper series: No 31. Geneva: World Health Organisation; 2001.
4. Ali, M. K., Echouffo-Tcheugui, J., & Williamson, D. F. (2012). How effective were lifestyle interventions in real-world settings that were modeled on the Diabetes Prevention Program? Health Affairs, 31(1), 67–75. http://dx.doi.org/10.1377/hlthaff.2011.1009
5. American Diabetes Association. (2016). Standards of medical care in diabetes—2016. Diabetes Care, 39(Suppl 1), S4–S5. http://dx.doi.org/10.2337/dc16-S003
6. Arias, E. (2014). United States life tables, 2010. National Vital Statistics Reports, 63(7), 1– 62. Retrieved from http://www.cdc.gov/nchs/data/nvsr/nvsr63/nvsr63_07.pdf
7. Ayyanar, K., Pichandi, S., & Janakiraman, P. (2018). Evaluation of Glucose Oxidase and Hexokinase Methods. *International Journal of Biotechnology and Biochemistry*, *14*(1), 51–58.
8. Ayah, R., Joshi, M. D., Wanjiru, R., Njau, E. K., Otieno, C. F., Njeru, E. K., &Mutai, K. K. (2013). A population-based survey of prevalence of diabetes and correlates in an urban slum community in Nairobi, Kenya. *BMC Public Health*, *13*, 371. http://doi.org/10.1186/1471-2458-13-371
9. Barte, J. C. M., terBogt, N. C. W., Bogers, R. P., Teixeira, P. J., Blissmer, B., Mori, T. A., & Bemelmans, W. J. E. (2010). Maintenance of weight loss after lifestyle interventions for overweight and obesity, a

systematic review. Obesity Reviews, 11(12), 899–906. http://dx.doi.org/10.1111/j.1467-789X.2010.0074

10. Berg JM, Tymoczko JL, Stryer L. Biochemistry. 5th edition. New York: W H Freeman; 2002. Section 30.2, Each Organ Has a Unique Metabolic Profile. Available from: https://www.ncbi.nlm.nih.gov/books/NBK22436/
11. Burrin, J. M., Price, C. P. (1985) Measurement of blood glucose. Ann. Clin. Biochem. 22, 327–342. Chromý, V., Fischer, J., Havel, J., Votava, M. (2002) Bioanalytics – Analytical Chemistry in Laboratory Medicine. Masaryk University, Brno. (in Czech)
12. Burtis, Carl A et al. Tietz Fundamentals of Clinical Chemistry, 6th ed. Saunders: St Louis, Missouri, 2008.
13. Carstensen B, Lindström J, Sundvall J, Borch-Johnsen K, Tuomilehto J, (2008). Measurement of blood glucose: comparison between different types of specimens. Annals of Clinical Biochemistry.45(2):140-8.
14. Comparison of Three Methods for Determination of Glucose Dohnal L.1 , Kalousová M.2 , Zima T.2
15. Dempír, J., Dohnal, L. (2005) Some robust procedures for determination of mean values and deviation of data and their usage. Klin. Biochem. Metab. 13(34), 139–144. (in Czech) *Diabetes Care. 2004 May; 27(5):1047-53.*
16. Evaluation and comparison of 10 glucose methods and the reference method recommended in the proposed product class standard (1974). Clin. Chem. 23, 131–139. Sacks, D. B. (2006) Carbohydrates.
17. Evaluation of Glucose Oxidase and Hexokinase Methods Mrs. Kavitha Ayyanar1, Dr. Suresh Pichandi2*, Janakiraman P3. International Journal of Biotechnology and Biochemistry ISSN 0973-2691 Volume 14, Number 1 (2018) pp. 51-58 © Research India Publications http://www.ripublication.com
18. Giampletro, O., Pilo, A., Buzzigoli, G., Boni, C., Navalesi, R. (1982) Four methods for glucose assay for various glucose concentrations and under different clinical conditions. Clin. Chem. 28(12), 2405–2407.
19. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030.
20. healthy adults from metropolitan region (Nairobi) of Kenya .International Journal of Health
21. Hye Soon Kim(2016). Blood Glucose Measurement: Is Serum Equal to Plasma? journal of diabetes metabolism. 40(5)
22. In: Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition, eds. Burtis, C. A., Ashwood, E. R., Bruns, D. E., pp. 37–901, Elsevier Saunders, St. Louis.
23. Jia KK and Zhang J.(2010). Evaluation of five routine glucose methods on an Olympus AU5400 analyzer using the CDC hexokinase reference method. Clinical Chemistry and Laboratory Medicine.48(3):361-4
24. Waithaka, S.K, Njagi, E.N, Ngeranwa, J.N, Muturi, D.M,, Chiuri B.M, Njagi .LJ, Gatua, K.G *Wild S, Roglic G, Green A, Sicree R, King H.*