STABILITY OF SELECTED BIOCHEMICAL ANALYTES IN STORED SERUM SPECIMEN AT KERICHO COUNTY REFERRAL HOSPITAL, KENYA

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A Thesis Submitted to School of Public Health Biomedical Sciences and Technology in Partial Fulfilment for the Requirement of the Award of Degree of Master of Medical Laboratory Sciences and Technology (Clinical Chemistry Option) of Masinde Muliro University of Science and Technology

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DEDICATION

I dedicate this work to my loving wife Rachel and Ethan for the support they gave me during the study period.

ACKNOWLEDGEMENTS

I enthusiastically appreciate the study participants who consented, hence enabling the study to be successful. I am also grateful to the management of Kericho County Referral hospital and their staff for giving me permission and participating fully during the study period.

To Dr. Sabella and Dr. Marera thank you so much for guiding me through in development of this thesis.

ABSTRACT

Accurate and reliable medical laboratory test results are essential for correct patient diagnosis and management. Pre-analytical variables, including samples storage, can adversely affect the reliability of medical laboratory results. Add-on tests, alongside unprecedented delays in testing, require that serum samples are stored in a manner that maintain their integrity and optimally preserve the various analytes. To ensure accurate results from stored samples, the determination of the stability of the analytes under various environmental conditions and storage periods is important in informing acceptable sample handling and storage. This study aimed at evaluating the stability of selected biochemical analytes in relation to storage time, exposure of light and temperatures to determine the maximum storage period at which accurate and reliable results can still be obtained at the Kericho County Hospital laboratory. Samples drawn from twenty healthy volunteering adult participants, randomly picked from the local population, were processed and analysed at baseline, then set of aliquots was made into 16 sterilized tube and stored at 2- 8°C, -20°C and other different environmental condition, expose light and other kept in the dark, in batches marked for analysis on the 7^{th}, 14^{th}, 21^{st} and 28^{th} days for aliquots refrigerated and frozen, for environmental condition was analysed at 2, 4, 8 and 24 hours. The data was coded and analysed on SPSS version 22. The statistical significance of the difference in the determined analyte concentrations in comparison to baseline levels was evaluated using student paired T-test and Wilcoxon rank test. From the findings, alkaline phosphate, creatinine, and aspartate aminotransferase were found to be stable up to 28 days in the refrigerated and frozen, with mean percentage differences less than calculated reference change value of 16.48%, 35.97% and 42.25% respectively. Sodium, total and direct bilirubin, were however unstable, as their mean percentage difference was more than the calculated reference change value of 2.16%, 0.657% and 1.14%. Urea and glucose showed stability at 2-8°C for up to the 14^{th} day, while potassium and chloride were only stable in refrigerated serum up to the 7^{th} day. Freezing improved the stability of all the analytes except bilirubin, alanine aminotransferese and sodium. Chloride, creatinine, glucose, potassium, sodium, urea, alkaline phosphates, alanine aminotransferese and aspartate aminotranferese were stable up to 24 hrs when serum sample left at room temperature as their calculated reference change value were 4.54%, 35.97%, 13.14%, 10.89%, 2.16%, 32.15%, 16.48%, 42.25% and 42.52% were less than mean percentage difference respectively only total and direct bilirubin were only stable for 4hrs and 2 hrs. respectively as their mean percentage difference of -0.48 and 0 were less than calculated RCV of 0.675 and 1.14. When serum samples were exposed to light and other aliquots kept in the dark, only total and direct bilirubin were unstable with reference change value of 1.14% and 0.675% respectively. Total bilirubin was only stable for 4 hours with calculated reference change value of -0.48% which is less than reference change value when serum samples were exposed to light but kept in the dark it was stable for 8hrs. This study recommends immediate analysis of serum for sodium, bilirubin, and alanine aminotransferese , which should never be part of add-on tests, and freezing of the samples when analysis is delayed for the other analytes. The findings of this study will help policy makers to make good decisions on health sectors on which laboratory equipment to procure in terms of refrigerators and freezers.

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LIST OF ABBREVIATIONS AND ACRONYMS

µmol	Micromole
AKI	Acute Kidney Injury
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
BIVAC	Biological Variation Data Critical Appraisal Checklist
BSL	Blood Sugar Level
BUN	Blood Urea Nitrogen
BV	Biological Variation
CBG	Capillary Blood Glucose
CKD	Chronic Kidney Disease
CLSI	Clinical and Laboratory Standards Institute
CO2	Bicarbonate
CV	Coefficient of Variation
CVD	Cardiovascular Disease
dl	Decilitre
EFLM	European Federation of Laboratory Medicine
eGFR	estimated Glomerular Filtration Rate
HDL	High Density Lipoprotein
HSP	Homeostatic Set point
ISE	Ion Selective Electrode
K	Potassium
K_3EDTA	Tripotassium Ethylenediaminetetraacetic Acid

KCR	Kericho County Referral Hospital
L	Litre
LDL	Low Density Lipoprotein
MDRD	Modification of Diet in Renal Disease
MPI	Maximum Permissible Instability
Na	Sodium
NACOSTI	National Commission for Science, Technology and Innovation
NADH	Nicotinamide Adenine Dinucleotide Hydrogen
PD%	Percentage Deviation
PT	Prothrombin Time
QC	Quality Control
RCV	Reference Change Value
SGOT	Serum Glutamic Oxaloacetic Transaminase
SS	Sample Size
TCA	Tricarboxylic Acid
TE	Total Allowable Error
UCB	Unconjugated Bilirubin

DEFINITION OF TERMS

Analytes- A substance whose chemical constituents are being identified and measured.

Colorimetry -A scientific technique that is used to determine the concentration of coloured compounds in solutions by the application of the Beer–Lambert law, which states that the concentration of a solute is proportional to the absorbance.

Serum-An amber-coloured, protein-rich liquid which separates out when blood coagulates.

Stability- Resistance to chemical change or to physical disintegration

Temperature- Is degree of hotness or coldness measured on a definite scale.

Turbidimetry - Is the measurement of light-scattering species in solution by means of a decrease in intensity of the incident beam after it has passed through the solution.

Venepuncture- The puncture of a vein with a needle to withdraw blood.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Laboratory medicine plays a crucial role in diagnosing, monitoring and managing human diseases by providing accurate and reliable test results. This discipline facilitates the identification of pathological conditions, the assessment of disease progression and the evaluation of treatment efficacy, ultimately guiding clinical decision-making and improving patient outcomes. A critical aspect of obtaining precise laboratory results is minimizing the time between sample collection and processing. In clinical chemistry, any delay in this process can lead to biochemical changes in the sample, affecting the concentration and activity of analytes. This, in turn, may result in inaccurate test results that can mislead clinicians in diagnosing and managing diseases. International guidelines such as those established by the Clinical and Laboratory Standards Institute (CLSI) and the Clinical and Laboratory Standards Institute (CLSI, 2010) provide standardized protocols for sample collection, handling, transportation and processing. However, implementing these guidelines in routine laboratory settings is often challenging due to logistical constraints, varying laboratory capacities and resource limitations. Among the primary factors necessitating the storage of clinical specimens are the lack of essential reagents, equipment malfunction and logistical constraints. In some cases, laboratory personnel may also need to store specimens for extended periods for future research purposes or additional confirmatory testing.

The stability limit of an analyte is typically defined as the point in time at which the deviation in its concentration or activity surpasses an acceptable threshold, leading to potential misinterpretation of clinical findings. If samples exceed their predefined stability period, the likelihood of degradation increases, affecting parameters such as

enzyme activity, electrolyte balance and metabolite concentrations. For instance, glucose levels may decrease due to ongoing glycolysis if blood samples are not processed in a timely manner. Similarly, haemolysis can lead to erroneous results by artificially increasing the concentration of certain analytes, such as potassium and lactate dehydrogenase (LDH), while decreasing others like bilirubin and glucose

One of the most common challenges in clinical laboratories is ensuring the stability of biological specimens, particularly serum and plasma samples. This challenge arises primarily during the pre-analytical phase, which includes the time from patient preparation to sample collection, processing and storage. Factors such as temperature fluctuations, delayed processing, hemolysis, contamination, incorrect sample handling and inappropriate use of anticoagulants can significantly impact the integrity of serum and plasma analytes, leading to unreliable test results (Gomez, 2019).

The pre-analytical phase encompasses all activities before actual laboratory analysis, including patient identification, proper specimen collection, handling, labeling, storage and transportation. This phase is particularly significant because any errors or inconsistencies occurring at this stage can severely impact the integrity of test results. While historically, most laboratory errors were thought to arise during the analytical phase where the sample undergoes chemical or biochemical testing using specialized instruments, recent studies have revealed that the majority of errors nearly 70%, actually occur in the pre-analytical phase (Emre *et al.,* 2021). These errors include inadequate patient preparation, improper collection techniques, incorrect sample handling, delays in transportation, contamination, hemolysis or exposure to unsuitable temperatures. Such pre-analytical errors can lead to the degradation or alteration of analytes, potentially producing unreliable test results that may cause misdiagnosis and inappropriate treatment.

The analytical phase is the stage where laboratory instruments and methodologies are employed to measure specific analytes in the sample. This phase involves the use of sophisticated technologies such as spectrophotometry, chromatography, immunoassays and molecular techniques. Though advancements in automation have significantly improved accuracy and precision in the analytical phase, errors may still arise due to instrument malfunctions, reagent instability, calibration errors or operator mistakes. However, recent research suggests that analytical errors contribute to a smaller proportion of overall laboratory errors accounting for less than 30% (Al Emre *et al.,* 2021). The post-analytical phase involves data interpretation, validation and communication of results to clinicians. This step is crucial in ensuring that results are accurate and reported correctly. Errors in this phase may include transcription mistakes, misinterpretation of data, or delays in delivering results to physicians, potentially leading to mismanagement of patients.

Given the significant impact of pre-analytical errors on laboratory test accuracy, it is imperative for clinical laboratories to implement strict quality control measures to standardize specimen collection, transportation, and handling processes (Alavi *et al.,* 2020). Several variables, including the time between blood collection and processing, temperature regulation and proper sample identification must be carefully monitored. For example, temperature fluctuations during transport can degrade labile analytes such as glucose, lactate and some enzymes, thereby compromising diagnostic accuracy. To mitigate such risks, laboratories must adhere to standardized operating procedures for blood collection and handling.

Maintaining the stability of collected specimens, particularly serum and plasma samples, is a critical concern in clinical chemistry laboratories. Once a sample is collected, immediate processing is ideal to preserve analyte integrity. However, delays

are sometimes unavoidable due to equipment failures, the backlog of samples, or the need for additional tests at a later stage. Improper storage conditions can lead to significant biochemical changes in analytes, which can affect test reliability and contribute to diagnostic errors. Therefore, an effective storage protocol is necessary to ensure that test results remain accurate and reflective of a patient's actual physiological condition. Typically, clinical laboratories store human specimens at refrigerated temperatures of 2–8°C for short-term storage, while longer-term storage requires freezing at -20°C or even -80°C, depending on the type of analyte being preserved (Gomez, 2019).

In conclusion, ensuring the reliability of laboratory test results is crucial for accurate disease diagnosis and effective patient management. Clinical laboratories must address pre-analytical, analytical and post-analytical challenges by implementing standardized protocols for sample collection, handling, transportation and storage. Given that a significant percentage of laboratory errors arise from the pre-analytical phase, laboratories must emphasize quality control measures to minimize these errors. Adhering to best practices and international guidelines will enhance the accuracy of laboratory test results, ultimately leading to improved patient care and clinical outcomes (Kristoffersen *et al.,* 2019).

In addition to the technical challenges associated with analyte stability, the consolidation of smaller laboratories into larger centralized facilities has introduced further complexities. This trend, observed globally and increasingly in Kenya, aims to optimize laboratory services, enhance cost-effectiveness, and improve operational efficiency. However, one of the unintended consequences of centralized laboratory systems is the increased transport time and handling steps required for sample processing. Patient samples must often travel longer distances before analysis, leading

to an elevated risk of analyte degradation due to exposure to fluctuating temperatures, prolonged storage and potential mishandling.

Kenya presents unique challenges for laboratory sample stability due to its diverse climatic conditions. Temperature variations between different regions in the country may significantly impact analyte stability, especially in areas with limited access to proper refrigeration and transportation infrastructure. Existing international guidelines on sample stability may not always be applicable to local conditions, making it difficult to ensure accurate and reproducible test results. Since laboratory test results form the basis of disease diagnosis, treatment decisions and patient monitoring, it is imperative to establish region-specific clinical guidelines for specimen handling.

Given these challenges, there is a strong need for further research and validation studies focused on pre-analytical and post-analytical sample handling under different environmental conditions specific to Kenya. This research would help define more applicable stability limits for key analytes under various storage and transportation conditions, ensuring that the diagnostic value of laboratory testing is not compromised. Standardized protocols tailored to local conditions would minimize pre-analytical errors, improve test result reliability and enhance the overall quality of patient care.

1.2 Problem Statement

Major issues in clinical chemistry laboratories, particularly in developing countries such as Kenya, Kericho County Referral Hospital laboratory included: Inadequate power supply for refrigeration, Equipment failure/malfunction and Delays in transportation due to logistical challenges. As a result, collected serum samples often need to be properly stored before analysis, to avoid analyte degradation and variability in test results.

As laboratory medicine continues to evolve, the importance of pre-analytical sample handling cannot be overstated. By addressing the challenges associated with specimen collection, transportation, and storage, clinical laboratories can enhance the precision of their analyses, reduce diagnostic errors, and ultimately improve patient care outcomes. The ongoing evaluation of storage conditions and analyte stability is essential in optimizing laboratory processes, especially in resource-limited settings such as Kenya, where maintaining ideal laboratory conditions remains a challenge. In referral hospitals like Kericho County Referral Hospital (KCRH), where a high volume of patient specimens is processed daily, ensuring consistent and standardized pre-analytical handling protocols is crucial. Efficient strategies to minimize pre-analytical variability will help enhance diagnostic accuracy and ensure the effectiveness of patient management strategies.

1.3 Study Objectives

1.3.1 General Objective

To determine the stability of selected biochemical analytes in stored serum at different conditions at Kericho County Referral Hospital laboratory.

1.3.2 Specific Objectives

The specific objectives for this study were:

i. To evaluate the influence of refrigeration, freezing, and room temperature on the stability of selected biochemical analytes on stored serum samples.
ii. To assess the effect of storage duration on the stability of selected biochemical analytes on stored serum samples.

iii. To evaluate the impact of light on the stability of selected biochemical analytes on stored serum samples.

1.4 Research Questions

i. What is the influence of refrigeration, freezing, and room temperature on the stability of selected biochemical analytes on stored serum samples?

ii. What is the effect of the storage time on the stability of selected biochemical analytes on stored serum samples?

iii. What is the impact of light on the stability of selected biochemical analytes on stored serum samples?

1.5 Significance of the Study

This study was done as part of creating knowledge for the future on how the environmental factors affect stability of stored serum specimen. From this information, it can be decided whether a delayed specimen stored appropriately can be used for future testing or research purposes. This study further gave insight into the specific clinical chemistry parameters that can be affected by serum storage.

1.6 Justification

In any clinical chemistry laboratory, it is vital to know, among others, what environmental factors affect the integrity of the specimen, quality assay results and this affects patient management. Environmental conditions are crucial for stability of analytes in biological material on storage and are bound to differ from those in Kenya, including Kericho County and neighboring counties who have similar climate. However, limited study has been done on the stability of biochemical analytes under different storage conditions at this health facility. Introduction of 'Add-on' tests are frequently demanding for specimen previously in the laboratory to be used for

additional test requested. This prevents additional venipuncture to the patients, as the initial specimen which was used during first analysis would have been stored in the right conditions. This study was carried out to assess the environmental conditions that can affect storage stability of biochemical analytes in specimens. However, a scanty literature is available about the longevity of commonly used biochemical analytes in human specimen (serum) including the impact of specimen kept as low as −20°C on serum hence prompting this study to be done.

1.7 Limitation of the study

The study could not determine all the clinical chemistry analytes due to financial challenge, thereby limiting the generalization ability of the findings to other serum biochemical analytes. Glucose analysis was done on serum rather than plasma hence causing glucose levels vary so much and are much lower when fluoride vacutainer is not used

1.8 Delimitation of the study

This study is limited to only three factors which can affect storage of serum (temperature, storage time and light). The study was conducted at Kericho County Referral hospital laboratory in Kenya, and the target population was healthy adult volunteer who are living within Kericho County. The Statistical Package for the Social Sciences (SPSS version. 22) were used to analyse the data. Summary statistics included mean, median level and standard deviation. To assess the statistical significance difference between serum aliquoted analysed at day 0 student pair t test and Wilcoxon ranked was used

1.9 Conceptual Framework

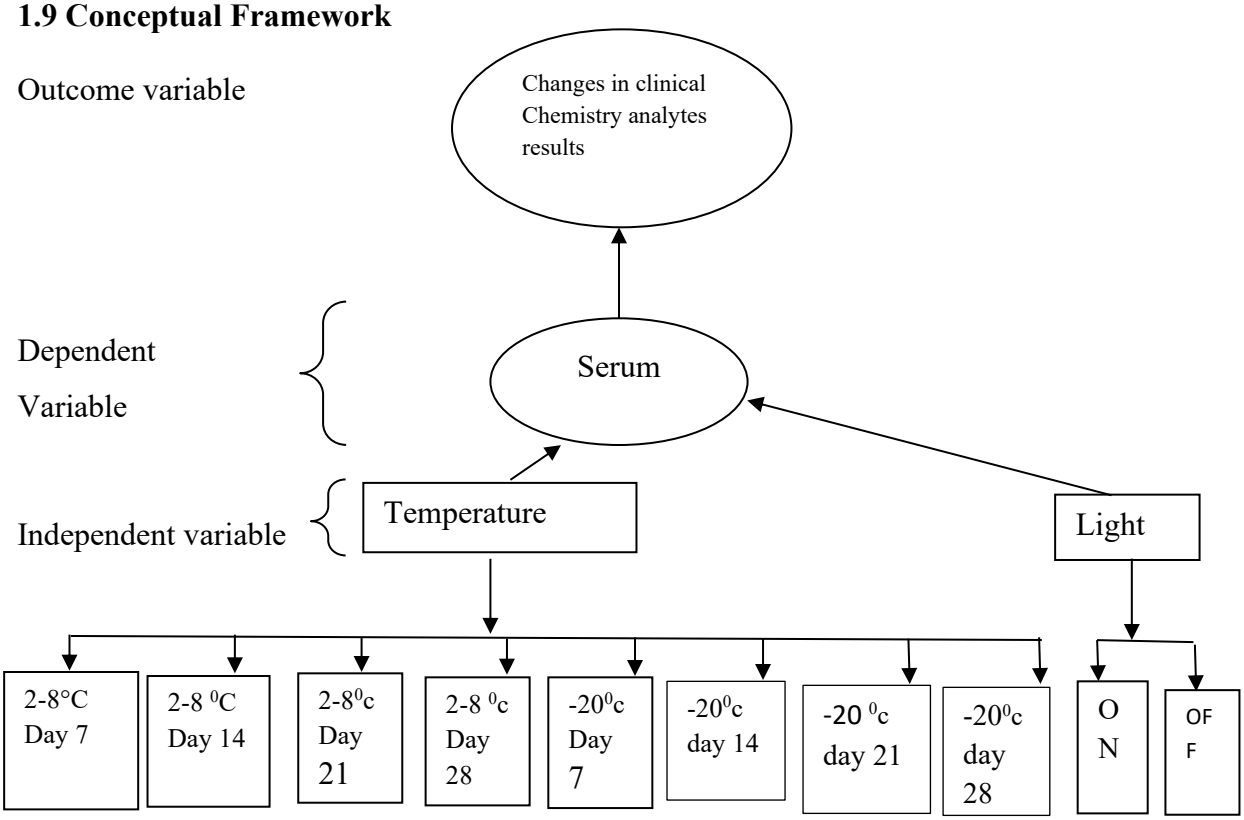


Figure 1.2 Conceptual Framework adapted from Analyte Stability during the total testing process: Studies of Vitamin A, D and E by LC-MS/MS (Albahrani *et al*., 2016)

1.9.1 Independent Variable

Temperature may affect serum specimen at various degrees. Stored specimen was measured at different temperature to determine if it has a significant change on the results compared to the baseline. The stored specimen was exposed to light and those kept at the dark to see the effect on the serum results before and after exposure for the study period occurrence.

1.9.2 Dependent variable

Serum specimens were affected by different independent variables These included temperature and light as shown in the figure1.2 above. This was compared with the baseline results to record the changes during the study. Changes in Clinical Chemistry analytes results will be due to the changes on the

independent variables. This turn affected the outcome results thus bringing out the difference from the baseline results with either a significant increase or decrease.

CHAPTER TWO

LITERATURE REVIEW

2.1 General Background

In laboratory medicine, blood sample collection, handling and processing are crucial steps that directly impact the accuracy and reliability of test results. However, in rural and remote areas, blood samples often face unique challenges before reaching a laboratory for analysis. These challenges primarily stem from the environmental conditions they are exposed to the extended transportation times, and the lack of immediate access to fully equipped laboratories.

When blood samples are collected in remote locations, they may be subject to extreme temperatures, varying humidity levels, and potential delays in transportation. Such conditions can significantly affect the stability of various analytes, including enzymes, proteins, electrolytes and metabolites, which are highly sensitive to temperature fluctuations and prolonged exposure to external factors. If not properly stored and transported under optimal conditions, these biological samples may undergo hemolysis, coagulation, or biochemical degradation, leading to compromised test accuracy and unreliable diagnostic results. For instance, prolonged exposure to heat can lead to the breakdown of glucose, changes in enzyme activity and alterations in blood cell morphology, which can distort laboratory findings and potentially result in misdiagnosis or inappropriate medical interventions.

Once blood samples arrive at a clinical laboratory, they are typically received as whole blood and must undergo centrifugation to separate serum or plasma from cellular components. This separation is a vital step in clinical chemistry as many laboratory tests rely on the analysis of serum or plasma rather than whole blood. If the separation

process is delayed, intracellular enzymes, metabolites and proteins may leak into the plasma or serum, leading to falsely elevated or reduced analyte concentrations. To ensure accurate and clinically useful test results, it is imperative that the interval between blood collection and serum separation be strictly controlled. The Clinical and Laboratory Standards Institute (CLSI, 2010) provides recommendations for optimal pre-analytical procedures; however, adhering to these guidelines in resource-limited settings remains a significant challenge due to inadequate infrastructure, transportation delays and limited refrigeration facilities.

In some cases, laboratory analysis cannot be performed immediately due to logistical challenges, necessitating temporary storage of serum and other blood products in laboratories. This is common in healthcare facilities with limited testing capabilities or those that need to send samples to referral laboratories for advanced analysis. In such scenarios, it is crucial to store serum and plasma samples under appropriate temperature conditions to prevent the degradation of labile analytes such as enzymes, hormones and proteins, which may deteriorate if exposed to inappropriate storage conditions for extended periods (Cray *et al.,* 2002). Refrigeration at 2–8°C is commonly used for short-term storage, while freezing at -20°C or lower is required for long-term preservation. However, repeated freezing and thawing cycles should be minimized, as they can lead to the denaturation of proteins and other sensitive molecules, further compromising the integrity of test results.

Given the essential role of laboratory tests in disease diagnosis, monitoring and prognosis, it is critical for healthcare providers and laboratory personnel to adhere to standardized protocols to maintain sample integrity (Emre *et al.,* 2021). The implementation of strict quality control measures, investment in proper storage

facilities and optimization of transportation processes are necessary to minimize pre-analytical errors. By addressing these challenges, laboratory medicine can continue to provide accurate and reliable data that support clinical decision-making and improve patient outcomes.

2.1.1. Various types of biochemical analytes in serum

2.1.1.1 Alanine Aminotransferase (ALT)

Alanine aminotransferase (ALT), also known as serum glutamate-pyruvate transaminase (SGPT), is an essential enzyme predominantly found in the liver. It plays a crucial role in amino acid metabolism by catalyzing the reversible transfer of an amino group from L-alanine to α-ketoglutarate, a reaction that results in the formation of pyruvate and glutamate (Botros & Sikder, 2013). The produced pyruvate enters the tricarboxylic acid (TCA) cycle, also known as the Krebs cycle, where it contributes to cellular energy production through oxidative metabolism (Brosnan & Brosnan, 2006). Although ALT is primarily localized in hepatocytes, it is also present in lower concentrations in other tissues, including the kidneys, myocardium, skeletal muscles, brain, pancreas, spleen and lungs (Botros & Sikaris, 2013). However, the liver contains the highest concentration, specifically within hepatocyte cytoplasm, where ALT activity is reported to be up to 47 times higher than that found in cardiac muscle and 9 times higher than that in skeletal muscle (Dufour *et al.,* 2000).

The liver is a vital organ responsible for numerous metabolic, detoxification and biosynthetic processes crucial for homeostasis. It regulates blood composition, controls glucose metabolism, stores glycogen, synthesizes proteins such as albumin and clotting factors and is integral to the metabolism of lipids and amino acids (Trefts *et al.,* 2017). Furthermore, the liver serves as the body's primary detoxification center,

filtering toxins, metabolizing drugs and eliminating waste products. Any injury or dysfunction in the liver can have serious systemic consequences, making the evaluation of liver function a crucial component of disease diagnosis and patient management (Hall & Hall, 2021)

Because ALT is primarily localized in hepatocytes, its measurement serves as a reliable biomarker for liver health. Under normal physiological conditions, only small amounts of ALT are released into circulation. However, in the event of hepatocellular injury such as hepatitis, non-alcoholic fatty liver disease (NAFLD), drug-induced liver injury, alcohol-related liver disease or ischemic liver injury ALT is released in significantly increased concentrations, making it a sensitive marker of liver cell damage (Friedman *et al.,* 2003).

Given that ALT is a key marker of liver health, its accurate quantification in serum or plasma is critical for the proper diagnosis and monitoring of liver disease. However, ensuring precise ALT measurements is heavily dependent on appropriate pre-analytical sample handling, which encompasses all steps from patient preparation and blood collection to sample processing and storage. Several factors can affect ALT stability in biological specimens, including the time between blood collection and analysis, storage temperature, choice of anticoagulant and presence of hemolysis (Gomez, 2019).

Studies have shown that improper handling of blood samples can lead to enzyme degradation or alterations in analyte concentration, resulting in misleading laboratory values that can impact clinical decision-making (Emre *et al.,* 2021). For instance, prolonged storage of whole blood before serum separation can lead to leakage of intracellular enzymes such as ALT and aspartate aminotransferase (AST) from blood

cells into the plasma, falsely elevating their measured levels (Dufour *et al.,* 2000). Similarly, exposure of serum samples to high temperatures or prolonged storage at room temperature can lead to decreased enzymatic activity due to protein denaturation (Botros & Sikaris, 2013).

To ensure optimal stability of ALT in serum samples, laboratories must adhere to standardized procedures for blood collection, handling, and processing. Typically, venous blood is collected in a plain tube (without anticoagulants) allowed to clot at room temperature for approximately 30 minutes and then centrifuged to separate the serum from the clot. Once separated, the serum should be stored appropriately: at 2-8°C if analyzed within 48 hours or at -20°C to -80°C for extended storage (Carey *et al.,* 2020). Deviations from these conditions can lead to significant alterations in ALT levels, potentially affecting diagnostic accuracy.

Liver cell injury, regardless of its cause, is often accompanied by an elevation in serum alanine aminotransferase (ALT) levels, making it a crucial biomarker in the assessment of liver function. ALT is a hepatocellular enzyme predominantly found in the cytoplasm of hepatocytes and its release into the bloodstream occurs when there is damage to the hepatic cell membrane. While elevated ALT levels are commonly associated with specific hepatic diseases, it is important to recognize that there is no direct correlation between the absolute peak of ALT concentration and the severity of liver injury.

Marked elevations in ALT levels, particularly exceeding 1000 U/L, are typically observed in cases of acute liver injury. These extreme elevations are most frequently linked to acute ischemic liver injury (also known as shock liver), severe drug-induced liver injury (DILI) and acute viral hepatitis (McAllister & Good, 2015). Acute

ischemic liver injury occurs due to a sudden reduction in hepatic blood flow, leading to hepatocellular hypoxia and necrosis. Similarly, severe DILI can result from the toxic effects of medications such as acetaminophen overdose, certain antibiotics or nonsteroidal anti-inflammatory drugs (NSAIDs). Acute viral hepatitis, including hepatitis A, B and C infections, is another major cause of marked ALT elevation, particularly in cases of acute liver inflammation or hepatocellular damage. Additionally, other conditions such as hepatitis E virus infection and common bile duct obstruction due to gallstones can contribute to increased ALT levels, further complicating the differential diagnosis of liver dysfunction (McAllister & Good, 2015).

Therefore, while ALT is a valuable marker for liver injury, its elevation alone does not necessarily indicate the exact cause or severity of hepatic disease. A comprehensive clinical assessment, including patient history, imaging studies and additional liver function tests, is essential to accurately determine the underlying etiology and guide appropriate medical management.

2.1.1.2 Aspartate Aminotransferase (AST)

The Aspartate Aminotransferase (AST) test is a crucial laboratory examination that measures the concentration of the AST enzyme in the blood. This enzyme, also known as serum glutamic-oxaloacetic transaminase (SGOT), is predominantly found in various tissues and organs, including the liver, heart, skeletal muscles, kidneys, brain and red blood cells. AST plays a vital role in amino acid metabolism by facilitating the conversion of aspartate and α-ketoglutarate into oxaloacetate and glutamate, a process essential for cellular energy production and metabolism (Daniels *et al.,* 2022).

Under normal physiological conditions, only a small amount of AST is present in the bloodstream. This is because AST is typically contained within the cells of organs such as the liver and heart, and it is only released into the bloodstream when these tissues are damaged. Liver damage due to conditions such as hepatitis, cirrhosis, fatty liver disease, or excessive alcohol consumption can lead to an elevated release of AST into the blood. Similarly, myocardial infarction, muscle trauma, pancreatitis and kidney disease can also cause significant increases in AST levels. Studies indicate that AST levels begin to rise approximately 6 to 10 hours after tissue damage occurs, peak within 24 to 36 hours, and typically return to normal within 3 to 4 days (Daniels *et al.,* 2022).

To gain a comprehensive understanding of liver health, the AST test is often performed alongside the Alanine Aminotransferase (ALT) test. ALT is another enzyme found primarily in the liver, and its levels are also elevated in cases of liver injury. The ratio of AST to ALT can provide valuable diagnostic insights; for instance, a significantly elevated AST level relative to ALT may suggest conditions such as alcoholic liver disease or muscle damage, whereas a more pronounced elevation of ALT may indicate viral hepatitis or non-alcoholic fatty liver disease (NAFLD).The AST test is commonly ordered by healthcare providers to detect liver damage, diagnose and monitor liver diseases such as hepatitis and cirrhosis, evaluate the effectiveness of ongoing liver treatments, determine the cause of jaundice, and monitor the impact of certain medications that may affect liver function Certain drugs, including statins, nonsteroidal anti-inflammatory drugs (NSAIDs) and some antibiotics, can lead to hepatotoxicity, making regular AST monitoring crucial for preventing adverse effects. Additionally, the test is useful in assessing muscle-related disorders, such as muscular dystrophy or rhabdomyolysis, where muscle damage leads to the release of AST into the bloodstream (Ward *et al.,* 2021).

Given the sensitivity of AST levels to tissue damage, it is vital to minimize the interval between blood sample collection and processing. Delays in processing may result in cellular breakdown, leading to artificially elevated AST levels that do not accurately reflect a patient's true health status. To ensure sample stability and accuracy, clinical laboratories must adhere to rigorous pre-analytical protocols. Ideally, after blood collection, the sample should be left at room temperature for approximately 30 minutes to allow clot formation, followed by centrifugation to separate the serum from blood cells. Serum samples should be promptly analyzed or stored appropriately either refrigerated for short-term storage or frozen at -20°C or lower for long-term preservation (Carey *et al.,* 2016).

2.1.1.3 Alkaline Phosphatase (ALP)

Alkaline phosphatase (ALP) is an enzyme found in the blood that plays a crucial role in the breakdown of proteins. This enzyme is involved in various physiological processes within the human body, including the breakdown of proteins, bone mineralization and liver function. Abnormal levels of alkaline phosphatase, whether elevated or decreased may indicate the presence of several medical conditions, ranging from relatively benign issues such as gallstones and thyroid disease to more severe conditions such as hepatitis and cancer (Lowe *et al.,* 2022). The clinical measurement of serum alkaline phosphatase is particularly significant in the diagnosis of cholestatic liver disease, where elevated ALP levels are often observed. In patients suffering from cholestasis, ALP levels can rise substantially, with increases often reaching four times the upper limit of normal values. This elevation is seen in up to 75% of patients with either intrahepatic or extrahepatic cholestasis (Lowe *et al.,* 2022). Notably, the degree of elevation does not help differentiate between the two types of cholestasis, which can make diagnosis challenging without further investigation.

Elevations in alkaline phosphatase levels are also commonly associated with various conditions that cause biliary obstruction, such as cancers like cholangiocarcinoma, pancreatic head adenocarcinoma, or ampullary adenocarcinoma. Other conditions contributing to raised ALP include choledocholithiasis, biliary stricture and sclerosing cholangitis. Additionally, intrahepatic cholestasis can result from diseases such as primary biliary cholangitis, drug-induced liver injury, or chronic rejection of liver transplants (Brichacek & Brown, 2019). Furthermore, infiltrative liver diseases like sarcoidosis, amyloidosis, tuberculosis and liver metastasis may also lead to abnormal ALP levels. In some cases, severe alcoholic hepatitis, which leads to steatonecrosis, may result in elevated ALP concentrations. An interesting observation is that patients with acquired immunodeficiency syndrome (AIDS) often exhibit particularly high alkaline phosphatase levels, which can be attributed to cholangiopathy caused by opportunistic infections such as cytomegalovirus and cryptosporidiosis. Furthermore, granulomatous liver involvement, commonly due to tuberculosis, can also cause elevated ALP levels in these patients (Brichacek & Brown, 2019).

Alkaline phosphatase is primarily produced by the liver and bones, although it is also synthesized in smaller amounts by the intestines and kidneys. During pregnancy, the placenta also secretes alkaline phosphatase, contributing to increased ALP levels in pregnant individuals (Hirschmugl *et al.,* 2018). The distribution of alkaline phosphatase in various tissues varies, with the highest concentrations found in the placenta, ileal mucosa, kidney, bone, and liver. Of these, the liver and bones are the primary sources of ALP in the bloodstream, accounting for more than 80% of the total alkaline phosphatase in serum, with smaller contributions from the intestine (Sekaran *et al.,* 2021). Despite its widespread presence throughout the body, the precise

physiological function of alkaline phosphatase remains largely unknown, with ongoing research aiming to uncover its role in various cellular and metabolic processes.

In clinical practice, the measurement of alkaline phosphatase levels is an important tool in the diagnosis and monitoring of various diseases. Elevated levels of ALP can serve as an early indicator of liver dysfunction, bone disorders, and a range of other pathological conditions. However, due to the enzyme's presence in multiple tissues, interpreting ALP levels in isolation can be challenging, often necessitating further diagnostic testing to pinpoint the underlying cause of the abnormality.

2.1.1.4 Blood Glucose

A blood glucose test measures the amount of glucose present in the blood. Glucose is the primary source of energy for the body, essential for the proper functioning of cells, tissues and organs. The regulation of blood glucose levels is managed by a hormone called insulin, which facilitates the movement of glucose from the bloodstream into the cells, where it is used for energy. This delicate balance is critical because both excessive and insufficient levels of glucose in the blood can indicate serious health issues. When blood glucose levels are abnormally high (hyperglycemia), it may be a sign of diabetes mellitus, a chronic condition that can lead to severe complications, such as heart disease, kidney failure, blindness, nerve damage and other related health problems. Conversely, when blood glucose levels are too low (hypoglycemia), it can lead to dangerous consequences, including confusion, seizures or even brain damage if not treated promptly (Kesavadev *et al.,* 2021).

Blood glucose monitoring plays a pivotal role in observing the fluctuations in glucose levels over time, helping healthcare providers understand how a patient’s blood glucose responds to various factors such as diet, physical activity, medications and

underlying medical conditions like diabetes. These fluctuations can be an important indicator of how well the body is maintaining glucose homeostasis and whether any interventions are necessary. Persistently high or low blood glucose levels can result in both acute and chronic complications, potentially threatening the patient's life (Kesavadev *et al.,* 2021). Monitoring is particularly crucial in individuals with diabetes, as they are more likely to experience significant fluctuations in blood glucose levels and even small changes can have a substantial impact on their health and well-being.

Blood glucose levels can be measured in several ways, depending on the location and purpose of the test. Home-based monitoring typically involves capillary blood glucose (CBG) tests, which use a small blood sample obtained from a fingertip or another capillary site. Clinical facilities may use CBG tests as well, but they may also rely on venous blood samples to measure plasma glucose levels, which can provide a more accurate assessment of the body's glucose regulation. Regular monitoring is essential to managing diabetes effectively and preventing complications (Kesavadev *et al.,* 2021).

Most of the foods we consume contain complex carbohydrates, which are broken down in the body to provide glucose as an energy source. Carbohydrates, once ingested, undergo digestion in the gastrointestinal system, where they are broken down into simpler sugars, primarily glucose. This glucose is then absorbed into the bloodstream through the walls of the small intestine. From there, it is transported via the bloodstream to various cells throughout the body, where it is either used immediately for energy or stored in the liver for later use. The body's ability to efficiently absorb and regulate glucose is crucial to maintaining healthy blood glucose levels. If this process is disrupted, whether by an underlying medical condition like diabetes or due

to other factors, it can result in abnormal blood glucose levels, which may have serious health consequences (Stanley *et al.,* 2019).

For individuals with diabetes, blood glucose monitoring is a fundamental aspect of disease management. Extreme fluctuations in blood glucose levels, whether too high or too low, can interfere with normal cellular functions, leading to significant health risks. Proper management through medication, diet, exercise and lifestyle changes is essential to prevent these fluctuations from reaching dangerous levels. Stress-related hyperglycemia, for example, is commonly observed in patients who have experienced acute medical or surgical events. This type of hyperglycemia can be temporary but requires careful monitoring and management to prevent further complications (Colberg *et al.,* 2016). Maintaining stable blood glucose levels helps optimize cellular functions, ensuring that the body's energy needs are met while avoiding the harmful effects of both hyperglycemia and hypoglycemia.

In conclusion, blood glucose monitoring is a vital tool in diagnosis, management, and prevention of complications in individuals with diabetes. By closely tracking blood glucose levels and understanding the factors that influence fluctuations, healthcare providers can help patients maintain more stable glucose levels, improve their quality of life, and reduce the risk of serious health issues associated with both high and low blood glucose.

2.1.1.5 Serum Urea Nitrogen (BUN)

Urea is a key nitrogenous end product of protein metabolism and is crucial for the body's nitrogen balance. It is primarily generated from the breakdown of dietary proteins and the turnover of tissue proteins. The urea molecule itself is relatively small, with a molecular weight of 60 Daltons, which allows it to distribute evenly throughout

the body's total water content. In clinical settings, urea is often measured in different ways depending on the region. In Europe, the entire urea molecule is assayed, while in the United States, the focus is typically on the nitrogen component of urea, commonly referred to as Blood Urea Nitrogen (BUN) or Serum Urea Nitrogen (SUN). The BUN value represents about half of the total urea level, with a rough ratio of 28/60, or approximately 0.446 of the blood urea concentration (Liu & Zeng, 2021).

The normal range of BUN in blood or serum is generally between 5 and 20 mg/dL, or 1.8 to 7.1 mmol/L of urea. This range can vary widely due to several physiological factors, including dietary protein intake, endogenous protein breakdown, hydration status, liver function (specifically urea synthesis), and renal function (specifically urea excretion). For example, a BUN value of 15 mg/dL may represent a normal finding in a typical adult but could indicate significantly impaired kidney function in specific populations, such as a pregnant woman in her thirtieth week of gestation. In this case, the woman's increased glomerular filtration rate (GFR), expanded extracellular fluid volume, and the anabolic processes associated with fetal development can result in a lower BUN level of around 5 to 7 mg/dL. In contrast, an individual with a high-protein diet, such as a rancher consuming over 125 grams of protein daily, may exhibit a normal BUN level of 20 mg/dL, despite possible kidney strain (Pagana, 2010).

Urea, or BUN, is produced in the liver through the urea cycle, a metabolic pathway that converts ammonia, a toxic byproduct of protein breakdown, into urea for safe excretion. Approximately 85% of urea is eliminated from the body via the kidneys, with the remainder excreted through the gastrointestinal (GI) tract (Shetty *et al.,* 2020). Elevated serum urea levels are often indicative of impaired renal function, particularly in cases of acute and chronic renal failure, where renal clearance is reduced. However, urea levels can also rise in non-renal conditions, such as upper GI bleeding,

dehydration, catabolic states, or the consumption of a high-protein diet. In contrast, serum urea levels may be decreased in conditions like starvation, a low-protein diet and severe liver disease, where the liver's ability to synthesize urea is compromised.

Although serum creatinine is generally considered a more accurate reflection of renal function, urea levels tend to rise earlier in the course of renal disease. In both acute and chronic renal conditions, elevated urea or BUN levels are commonly observed. To assess renal function more precisely, particularly in detecting and monitoring chronic kidney disease (CKD), estimated glomerular filtration rate (eGFR) equations are often used. These equations help determine the presence and stage of CKD, as well as track the effectiveness of treatments (Qian *et al.,* 2019).

2.1.1.6 Serum Creatinine

Creatinine is a by-product of the breakdown of creatine phosphate, primarily in muscle tissue. It is produced at a relatively constant rate by the body, with the rate of production being closely tied to muscle mass. Since it is produced by muscle metabolism, the amount of creatinine produced daily varies based on an individual’s muscle bulk. This means that there are differences in creatinine levels between individuals, with males generally having higher levels than females due to larger muscle mass. Children and individuals with reduced muscle mass, such as the elderly or those with malnutrition, tend to have lower serum creatinine values. Additionally, dietary factors, particularly the consumption of red meat, can influence serum creatinine levels. It has been reported that creatinine levels can change by as much as 30% following the ingestion of red meat, due to the creatine content in meat (Duong & Rhee, 2017).

Creatinine is primarily eliminated from the bloodstream by the kidneys and any decrease in the kidney's ability to filter blood leads to an increase in blood creatinine

levels. This is why serum creatinine is commonly used as a biomarker to assess kidney function. However, the relationship between serum creatinine and kidney function is not linear, and changes in serum creatinine may not immediately reflect changes in renal function. Notably, renal function must decrease by as much as 50% before a rise in serum creatinine is detectable. This makes serum creatinine a relatively late indicator of kidney dysfunction (Duong & Rhee, 2017).

During pregnancy, there is a physiological increase in the glomerular filtration rate (GFR), which results in lower serum creatinine values. This decrease is due to the increased renal filtration capacity in pregnancy, which helps in the clearance of waste products, including creatinine. As a result, the interpretation of creatinine levels in pregnant individuals must be carefully adjusted to account for these physiological changes (Daugirdas & Depner, 2017).

Serum creatinine (Scr) is the most widely used biomarker for evaluating kidney function, especially in assessing chronic kidney disease (CKD). In a steady-state, serum creatinine is used to estimate the glomerular filtration rate (GFR), a key indicator of kidney function. Two widely used formulas for estimating GFR from serum creatinine are the Modification of Diet in Renal Disease (MDRD) study equation and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. These formulas take into account factors such as age, sex, and race to provide an estimate of kidney function. While serum creatinine is commonly used for the diagnosis and monitoring of CKD, it is also a crucial criterion for defining acute kidney injury (AKI), an abrupt decline in kidney function that can occur due to a variety of causes, including dehydration, medication toxicity, or infections (Daugirdas & Depner, 2017).

In summary, while serum creatinine is an important tool in assessing kidney function, it has limitations. Its role as a biomarker for kidney impairment is most useful in chronic conditions but may not detect early kidney dysfunction, particularly in acute settings or in individuals with rapidly changing kidney function. Understanding the factors that influence serum creatinine levels, such as muscle mass, diet, and pregnancy, is essential for accurate interpretation in clinical practice.

2.1.1.7 Total bilirubin

Bilirubin is a yellow-orange pigment found in bile that results from the breakdown of heme-containing proteins, particularly during the catabolism of haemoglobin. The process begins with the degradation of heme, a component of haemoglobin, which is initially converted into biliverdin, a green pigment. Biliverdin is then further reduced to form unconjugated bilirubin (UCB), also known as indirect bilirubin. UCB is fat-soluble and, therefore, cannot be directly excreted in the urine. To facilitate its excretion, UCB binds to albumin in the bloodstream for transport to the liver. Once in the liver, glucuronic acid is added to UCB through a process called conjugation, converting it into direct bilirubin (also known as conjugated bilirubin), which is water-soluble. The conjugated bilirubin is either excreted into the bile and stored in the gallbladder or directly secreted into the intestines, where it aids in digestion. Some bilirubin may be reabsorbed back into the bloodstream and subsequently filtered by the kidneys, excreted in the urine (Creeden *et al.,* 2021).

Elevated levels of bilirubin in plasma, a condition known as hyperbilirubinemia, are commonly observed in both primary care and hospital settings. This condition is often associated with liver disorders that lead to a decrease in the number of functional hepatocytes, which can impair bilirubin metabolism and lead to an accumulation of

bilirubin in the bloodstream (Kunutsor *et al.,* 2017). Hyperbilirubinemia can occur at any stage of bilirubin metabolism, including excess production, impaired hepatic uptake, defects in bilirubin conjugation, or issues with biliary excretion. It is important to note that the elevation of bilirubin levels is not specific to liver dysfunction and may also arise from disorders involving the red blood cells, such as haemolysis, or obstructions in the bile ducts (Kamisako *et al.,* 2000).

Bilirubin is a well-established and routinely measured biomarker in patients suspected of having liver dysfunction. It is included in routine biochemical tests, such as liver function tests, as part of the diagnostic process. However, while bilirubin levels provide valuable information, it is not a highly sensitive or specific marker of liver function. Consequently, careful interpretation of bilirubin test results is essential for accurate diagnosis. Factors such as the patient's medical history, the degree of bilirubin elevation, and the presence of other biochemical markers should be taken into account. The diagnostic value of bilirubin is often more informative when combined with other clinical indicators, such as liver enzyme levels, albumin, and clotting factors (Vítek, 2019).

The clinical significance of altered bilirubin concentrations is linked to the underlying condition causing the disturbance in bilirubin metabolism. Disorders or diseases that interfere with bilirubin metabolism can lead to an increase in serum bilirubin levels. When bilirubin levels in the blood exceed 1 mg/dL, it can cause bilirubin to accumulate in tissues, especially those rich in elastic fibers, such as the palate and conjunctiva (Ichihara *et al.,* 2017). Jaundice, a clinical manifestation of hyperbilirubinemia, occurs when serum bilirubin concentrations exceed 2.5 mg/dL. This condition is characterized by the yellowing of the skin and mucous membranes, including the sclera of the eyes. Jaundice itself does not necessarily indicate poor prognosis, as the body has highly

effective detoxification mechanisms to process bilirubin, except in neonates, where the immature liver may not be able to handle bilirubin efficiently (Vítek, 2019). However, persistent hyperbilirubinemia is indicative of underlying liver dysfunction, impaired biliary flow, or hemolytic disease, requiring further investigation to identify the cause of the elevated bilirubin levels.

In conclusion, bilirubin testing is a critical tool in assessing liver function, diagnosing liver diseases, and evaluating the impact of various conditions on bilirubin metabolism. Understanding the causes of bilirubin elevation and interpreting its levels in conjunction with other diagnostic markers are essential steps in providing accurate diagnoses and developing effective treatment plans for patients with liver-related or other systemic disorders. The careful management of bilirubin concentration is paramount in identifying conditions such as hepatitis, cirrhosis and gallstone disease, among others (Creeden *et al.,* 2021; Kamisako *et al.,* 2000).

2.1.1.8 Sodium, Potassium and Chloride.

Sodium, potassium, and chloride are key electrolytes in the body, and their measurements play a vital role in the diagnosis, treatment and monitoring of various diseases. Each of these electrolytes serves as an essential indicator in clinical settings, providing critical information about a patient's health status. In particular, sodium measurements are crucial in diagnosing and managing a range of disorders, including aldosteronism, diabetes insipidus, adrenal hypertension, Addison's disease, dehydration and syndromes of inappropriate antidiuretic hormone secretion (Berend *et al.,* 2012). Aldosteronism, characterized by excessive secretion of aldosterone, leads to sodium retention and potassium excretion, which can result in hypertension and electrolyte imbalances. Similarly, in diabetes insipidus, the kidneys' inability to

concentrate urine results in the excretion of large volumes of diluted urine, leading to significant sodium and fluid imbalances. For these reasons, the measurement of sodium levels is essential for both diagnosis and therapeutic monitoring.

In the case of potassium, accurate measurements are essential for managing conditions that involve abnormal blood potassium levels. Potassium is vital for maintaining normal cellular function, and its concentration is tightly regulated in the body. Both hypokalemia (low potassium levels) and hyperkalemia (high potassium levels) can be life-threatening, affecting the function of muscles, nerves and the heart. Therefore, potassium measurements are commonly used in the diagnosis and management of diseases that lead to potassium imbalances, such as renal disease, diabetic ketoacidosis and the use of medications that affect potassium levels, such as diuretics (Shrimanker *et al.,* 2021). Monitoring potassium levels is critical to prevent complications such as arrhythmia, muscle weakness and metabolic disturbances.

Chloride, another key electrolyte, is often measured alongside sodium and potassium as part of a comprehensive electrolyte panel. Chloride levels are particularly useful in the diagnosis and management of electrolyte and metabolic disorders, such as cystic fibrosis and diabetic acidosis. Cystic fibrosis, a genetic disorder that affects the lungs and digestive system, results in elevated chloride levels in sweat. Therefore, measuring chloride levels in sweat can be a diagnostic tool for cystic fibrosis. In diabetic acidosis, an excess of acidic metabolites in the blood leads to metabolic acidosis, which can cause disturbances in chloride balance. Therefore, chloride measurements assist in understanding the body’s acid-base status and can provide insights into underlying metabolic conditions.

In clinical practice, urine electrolytes, including sodium, potassium, and chloride, are primarily used as nutritional indicators in healthy individuals. They are typically measured in critical care settings, such as intensive care units (ICUs), where precise monitoring of a patient's electrolyte balance is essential. While these measurements are not diagnostic of any particular disease by themselves, they can be valuable in certain clinical scenarios. For example, in the case of acute renal failure, monitoring urinary electrolytes helps clinicians assess kidney function and fluid balance. In some rare cases, abnormal urinary electrolyte levels can provide additional evidence to support the diagnosis of specific clinical conditions (Berend *et al.,* 2012). However, it is important to note that urinary electrolyte measurements are not typically used as standalone diagnostic tools in routine clinical practice.

Beyond individual patient care, urinary electrolyte data plays an important role in public health studies. Specifically, urine sodium measurements serve as a significant biomarker of dietary sodium intake, which has been linked to cardiovascular health. High sodium intake is a major risk factor for hypertension and cardiovascular diseases, making urine sodium levels a valuable tool in population-based studies that aim to assess the relationship between dietary habits and health outcomes. These data contribute to the development of public health guidelines on sodium consumption and its impact on health (Shrimanker *et al.,* 2021). Furthermore, urinary potassium and chloride data also provide insights into broader nutritional and health trends, especially in populations with known dietary or metabolic conditions.

In summary, the measurement of electrolytes such as sodium, potassium and chloride is essential for diagnosing, treating, and monitoring various medical conditions. While their use in clinical settings can provide critical information for individual patient care, their role extends to broader public health initiatives, offering valuable insights into

nutrition, disease prevention, and health promotion. Thus, accurate and timely measurements of these electrolytes are indispensable in both clinical and public health contexts.

2.1.2. Electrolytes and Ions

Electrolytes and ions are essential for maintaining various physiological functions in the human body, contributing significantly to metabolic pathways, acid-base balance, enzyme reactions, muscular function, and the contraction of nervous tissue. The proper regulation of electrolyte levels is critical for ensuring optimal bodily functions. This regulation is closely tied to pH balance and water homeostasis, which are primarily controlled by the kidneys through complex processes, including active transport, osmosis, and passive diffusion in the renal system. Specifically, active transport in the proximal convoluted tubules allows for the movement of electrolytes like sodium and potassium, while osmosis and passive diffusion further assist in maintaining the body's fluid balance and ion concentrations (Arneson, 2014).

In the context of human cells, the sodium-potassium pump, which operates using adenosine triphosphate (ATP), plays a critical role in maintaining intracellular and extracellular sodium and potassium levels. This pump helps to move sodium ions out of the cell and potassium ions into the cell, thus ensuring the proper ionic gradient that is essential for many cellular processes, including nerve transmission and muscle contractions (Ackerman, 1990). The function of the ATPase pump is pivotal in ensuring that cells can maintain a stable environment despite fluctuations in external conditions, which highlights the importance of electrolyte regulation in overall cellular function.

Electrolytes themselves are small, charged particles, either positively charged (cations) or negatively charged (anions), that contribute to the body's ionic balance. These charged particles, which include elements such as sodium (Na^+), potassium (K^+), and chloride (Cl^-), are integral components of an elaborate physiological mechanism responsible for maintaining water balance and ionic equilibrium within the human system (Burtis *et al.,* 2015). The kidneys, as the primary organs involved in maintaining this balance, regulate the concentrations of these electrolytes, ensuring that the body's internal environment remains stable a process known as homeostasis (Arneson, 2014).

Beyond maintaining osmotic pressure and fluid balance, electrolytes are involved in various metabolic processes that support normal body functions. They are essential for generating electrical impulses required for nerve function, ensuring proper muscle contraction, and contributing to enzymatic reactions that facilitate biochemical processes within cells. Calcium (Ca^{2+}), magnesium (Mg^{2+}), phosphorus (PO_4^{3-}), and iron (Fe^{2+}) are examples of important ions that, while also crucial for bodily functions, differ slightly in their roles compared to the classic electrolytes like sodium and potassium (Burtis *et al.,* 2015). These ions are involved in processes such as bone formation (calcium and phosphorus), oxygen transport (iron) and enzymatic regulation (magnesium), each contributing to the overall metabolic network that sustains life.

The kidneys play a central role in regulating electrolyte homeostasis through filtration, reabsorption, and secretion. As blood passes through the kidneys, various electrolytes are filtered by the glomerulus, after which they are reabsorbed back into the bloodstream in the renal tubules. This selective reabsorption is vital to prevent the loss of essential electrolytes and ions while maintaining an appropriate balance for optimal

body function. Furthermore, excess electrolytes or ions are secreted into the urine, thus preventing toxic accumulation and maintaining the overall ionic balance of the body (Guyton & Hall 2021).

In summary, electrolytes and ions are indispensable for maintaining the integrity of human physiology. Through a complex interplay between the kidneys, cellular processes, and metabolic pathways, the body is able to regulate these substances with remarkable precision. Their roles extend beyond merely maintaining fluid balance to participating in a variety of processes that are fundamental to life, including muscle function, enzyme activation and the transmission of electrical signals in the nervous system. The homeostatic regulation of electrolytes is, therefore, essential for ensuring that the human body functions in a stable and efficient manner.

2.1.3. Small molecules and metabolites

Living organisms rely on molecules to perform essential biological functions, including providing energy, serving as structural components of cells and tissues, and acting as metabolic sensors to regulate overall metabolic processes. Within these dynamic systems, millions of seemingly insignificant molecules are constantly being formed and broken down as part of ongoing metabolic activities. These molecules, particularly those circulating in the blood or excreted in the urine, serve as vital indicators of an individual's health and metabolic status (Gounden & Jialal, 2019). Through the measurement of specific small molecules, healthcare providers can gain insight into an individual's nutritional status, the efficiency of waste elimination, and the overall control of metabolic processes, all of which are integral to maintaining homeostasis.

The body’s metabolic processes are highly intricate, involving the continuous synthesis and breakdown of substances like proteins, lipids, carbohydrates, and nucleic acids,

which contribute to energy production and the regulation of bodily functions. During these metabolic processes, end products are generated, which are then either used for other biochemical pathways or eliminated from the body. These metabolic by-products, when measured, can provide crucial information about organ function and the efficiency of various physiological processes.

For instance, the measurement of small molecules such as urea, nitrogen, and creatinine in the blood is commonly used to assess kidney function. The kidneys play a central role in filtering and excreting waste products, including nitrogenous waste like urea and creatinine, as well as regulating fluid and electrolyte balance. Elevated levels of these substances in the blood may indicate kidney dysfunction, as the kidneys are no longer able to efficiently filter and remove these waste products (Ejaz *et al.,* 2019). Creatinine levels, in particular, are widely used as a marker for renal impairment, as it is a product of muscle metabolism that is normally cleared by the kidneys. When kidney function declines, creatinine accumulates in the blood, serving as a reliable indicator of glomerular filtration rate (GFR), a key measure of kidney function.

Similarly, the measurement of uric acid levels in the blood is an important diagnostic tool, as elevated uric acid concentrations can reflect a variety of health issues, including kidney disease, gout and tissue damage. Uric acid is a by-product of purine metabolism, and its excess accumulation in the body can lead to crystal formation in joints, causing painful inflammation characteristic of gout. Furthermore, persistently high levels of uric acid may indicate impaired kidney function, as the kidneys are responsible for excreting most of the body's uric acid. If the kidneys are not functioning properly, uric acid can accumulate in the bloodstream, increasing the risk of gout and other complications (Ejaz *et al.,* 2019).

Beyond kidney function, other metabolic end products can provide insights into the function of various organs. For example, the levels of glucose and lipids in the blood can reflect metabolic control and indicate conditions such as diabetes or dyslipidemia. Regular monitoring of these small molecules helps healthcare providers assess how effectively the body is utilizing and storing energy, as well as its ability to eliminate waste products. These measurements can also reflect the underlying health of other organs, such as the liver and pancreas, which are central to maintaining energy balance and metabolic regulation.

In conclusion, the study of small molecules in the bloodstream and urine is an invaluable tool for assessing the functionality of various metabolic systems in the body. These molecules not only reflect an individual's nutritional status and waste elimination efficiency but also serve as critical biomarkers for detecting metabolic disorders, organ dysfunction, and the overall health status of an individual. Accurate measurements of metabolic end products, such as urea, creatinine, uric acid, glucose, and lipids, provide essential information that guides clinical decision-making and helps to monitor the progression of diseases (Gounden & Jialal, 2019; Ejaz *et al.,* 2019).

2.1.4. Proteins

Proteins are large, complex molecules that play a crucial role in the proper functioning of cells and are essential for maintaining overall health. The basic building blocks of proteins are amino acids, and the sequence and structure of these amino acids determine the protein's function. Proteins are integral to the structure and function of body cells, tissues and organs, regulating vital processes such as cell growth, repair and the functioning of enzymes and hormones (Nordqvist, 2018). The body's cells rely heavily

on proteins to maintain their integrity, perform biochemical reactions and communicate with other cells, ensuring the body's homeostasis.

Plasma proteins, which are found in the blood, are particularly vital in regulating several physiological functions. They play an essential role in maintaining osmotic pressure, which helps to regulate the balance of fluids within and outside cells. Plasma proteins also carry lipids, metabolic byproducts, trace elements and chemical messengers like hormones, ensuring that essential substances are transported throughout the body efficiently. Furthermore, these proteins serve as carriers for drugs and nutrients and are critical for detoxification and waste elimination processes in the body (Ling & Murali, 2019).

Antibodies, which are a type of plasma protein, are integral to the immune system's defense mechanism. Their primary function is to recognize and neutralize foreign invaders such as bacteria, viruses, and toxins. In addition to their direct role in immunity, antibodies support other immune proteins like complement proteins and C-reactive protein (CRP), which are among the earliest proteins to have evolved within the immune system, playing critical roles in immune responses (Ling & Murali, 2019).

The levels of proteins in the body are not only vital for maintaining normal bodily functions but also serve as important biomarkers in diagnosing various health conditions. Deviations in protein levels can indicate underlying issues such as malnutrition, metabolic disorders, and even certain oncological (cancerous) conditions. For example, serum total protein and albumin levels are often measured to assess the health of the liver and kidneys, as well as to diagnose malnutrition. Low levels of albumin may suggest liver disease, kidney disease, or nutritional deficiencies. Similarly, globulin analysis, along with the albumin-to-globulin ratio, can help

diagnose a variety of conditions. An imbalance in this ratio is frequently observed in cases of infection, inflammation, autoimmune diseases, and certain blood-related cancers (Lv *et al.,* 2018).

The albumin-to-globulin ratio (A/G ratio) is a useful diagnostic tool, with low A/G ratios often associated with chronic infections, inflammatory conditions and specific cancers such as multiple myeloma. Elevated globulin levels may point to immune system disorders or blood cancers, while abnormalities in serum protein levels can reflect metabolic imbalances or organ dysfunction. Therefore, careful monitoring and analysis of plasma protein levels are essential for clinicians in detecting, diagnosing and managing a wide range of health conditions.

In conclusion, proteins are indispensable to life, performing essential roles in maintaining cell structure, metabolic processes, immune defense, and overall physiological balance. Monitoring the levels of serum proteins such as albumin and globulin is crucial in diagnosing diseases related to liver, kidney, immune function, and nutritional status, among others. Ensuring proper protein function and balance is essential for maintaining health and detecting early signs of disease.

2.1.5. Enzymes

When analyzing enzymes, several critical factors must be carefully controlled to ensure accurate and reliable results. These factors include temperature, pH, ionic strength, and the appropriate levels of key components, such as substrates and enzymes themselves. Maintaining the consistency of these parameters is essential for obtaining precise measurements, as slight variations in any of them can lead to significant changes in enzyme activity. However, due to the vast diversity of enzyme types, particularly with

respect to their structural and functional characteristics, it is challenging to standardize assay conditions across all enzymes.

For many enzymes, particularly those derived from mammalian sources, there is a general pH optimum close to the physiological pH of approximately 7.5, which reflects the pH found in the human body (Bisswanger, 2011). Similarly, the body temperature, typically around 37°C, is often used as the standard assay temperature. However, in experimental settings, a temperature of 25°C is more commonly preferred for practical reasons, as it can be easier to maintain consistently throughout the experiment and is less likely to induce thermal instability in some enzymes.

Despite these general guidelines, the unique characteristics of individual enzymes often necessitate specific assay conditions that can deviate significantly from these default parameters. This is particularly true for enzymes that are highly sensitive to changes in temperature, pH, or ionic strength, which can cause their activity to either diminish or become erratic. In such cases, customized assay conditions are required to optimize enzyme performance and ensure the accuracy of the results.

Another challenge in enzyme analysis is determining the precise concentrations of assay components, such as substrates and enzymes. While it is difficult to provide exact concentrations applicable to all enzyme assays, general rules can be derived based on the degree of saturation of the enzyme's active site. This saturation level influences the rate of the reaction, which is critical for accurate measurement. Typically, enzyme assays aim to operate under conditions where the enzyme is neither oversaturated nor undersaturated with its substrate, ensuring that the enzyme's activity is measured accurately (Bisswanger, 2011).

In addition to establishing the right assay conditions, there are several methodological considerations that need to be addressed during the enzyme assay. Proper handling of samples, preparation of assay mixtures and blanks, and the choice of assay duration all play a role in minimizing errors. Careful attention must also be paid to avoid both systematic and trivial errors that could skew the results. Systematic errors may arise from consistent issues in the experimental setup, such as inaccurate temperature control or improper substrate concentrations, while trivial errors might involve issues like inconsistent sample handling or contamination. Researchers must be vigilant about these factors to ensure that the results are truly reflective of the enzyme's activity and not influenced by extraneous variables.

Some enzyme assays are particularly complex and involve additional challenges. These include assays for enzymes that catalyze reactions in dynamic environments, such as those requiring coupled reactions, where the activity of one enzyme is linked to the activity of another enzyme. These types of assays require careful consideration of the interactions between the enzymes involved and the conditions under which these reactions occur. For instance, the presence of cofactors, inhibitors, or activators can alter the activity of one enzyme and thereby affect the overall reaction rate, making it essential to optimize conditions for all enzymes involved (Bisswanger, 2011).

2.2. Methods of analysing biochemical analytes in serum

Photometry is widely regarded as one of the most commonly used analytical techniques in clinical chemistry due to its effectiveness in measuring the concentration of analytes in biological samples. It operates based on the interaction of radiant energy (light) with substances in a sample, primarily through the absorption or emission of specific wavelengths. This interaction, governed by Beer-Lambert's law, enables precise quantification of clinically relevant compounds such as glucose, cholesterol, and

creatinine (Tietz, 2021; Linné & Ringsrud, 2010). Its simplicity, cost-effectiveness, and compatibility with automation make it essential in modern clinical laboratories (Comprehensive Analytical Chemistry, 2008).

The technique of photometry encompasses several subtypes, each tailored to measure different properties of light and its interaction with substances. One of the most fundamental subtypes is colorimetry, or absorption photometry, which is based on the absorption of light by a sample at specific wavelengths. When light passes through or is reflected by a sample, certain wavelengths are absorbed depending on the concentration of the analyte present, which is then measured and used to calculate concentration levels. Another widely used subtype is spectrophotometry, which extends colorimetry by employing a range of wavelengths, allowing for more precise measurements of analytes in complex biological matrices (Gómez-Rioja *et al.,* 2019).

In addition to this, potentiometry is another related technique used in clinical chemistry, which measures the potential (voltage) between two electrodes in a solution. This is particularly useful for determining the concentration of ions in a sample. Fluorometry, another subtype of photometry, measures the fluorescence emitted by a sample after it absorbs light, making it especially useful in detecting specific compounds or molecules that naturally fluoresce or have been tagged with fluorescent markers (Gómez-Rioja *et al.,* 2019).

These techniques, through their ability to measure specific interactions between light and analytes, form the backbone of many clinical laboratory tests. They provide accurate, quantitative data that clinicians rely on for diagnosing, monitoring, and treating various health conditions. Each technique has its own set of applications

depending on the type of sample and analyte being studied, making photometry a versatile and indispensable tool in the field of clinical chemistry.

2.2.1 Colorimetry

Colorimetry is a widely used analytical technique for determining the concentration of colored compounds in solutions. The fundamental principle behind colorimetry is the Beer–Lambert law, which establishes that the absorbance of light by a solution is directly proportional to the concentration of the solute, provided the light path length and the wavelength of light used remain constant. In simple terms, the Beer–Lambert law allows for the quantitative measurement of a solute's concentration by analyzing how much light is absorbed by the solution. This makes colorimetry a valuable method in various fields, including chemistry, biology, and environmental science, where the concentration of specific substances needs to be accurately determined based on their color (Präbst *et al.,* 2017).

Colorimetry as a scientific discipline has evolved through several key stages, which have contributed to its widespread application in both qualitative and quantitative analysis. The development of colorimetry can be divided into three main phases: color matching, color difference evaluation, and prediction of color appearance. The first phase, color matching, involves comparing the color of a sample to standard color references, often utilizing standardized color charts or systems like the CIE (Commission Internationale de l'Eclairage) system. This phase is crucial for ensuring that color measurements are consistent and accurate when comparing samples (Präbst *et al.,* 2017).

The second phase, color difference evaluation, focuses on determining the variation in color between samples, which is essential in many industrial applications such as paint

production, food processing, and textile manufacturing. This phase often involves the use of advanced instruments such as colorimeters and spectrophotometers, which quantify color differences using mathematical models, such as the CIEDE2000 formula, to express how the human eye perceives these differences. Accurate color difference evaluation is particularly important in ensuring product quality and consistency, as even slight deviations in color can be perceptible and affect consumer satisfaction (Präbst *et al.,* 2017).

The final phase, prediction of color appearance, seeks to predict how a sample will appear under different lighting conditions or when subjected to various environmental factors. This is especially important in areas like design, art, and manufacturing, where the final color of a product may be influenced by factors such as lighting, surface texture, and material properties. The ability to predict color appearance accurately requires an understanding of both the physical properties of light and the complex ways in which humans perceive color (Präbst *et al.,* 2017).

Colorimetry has evolved into a sophisticated field of study that combines elements of chemistry, physics, and human visual perception. The advances in color matching, color difference evaluation, and color prediction have made colorimetry an indispensable tool in many industries and scientific research areas, providing valuable insights into both the composition and the aesthetic qualities of materials.

2.2.2 Turbidimetry

Turbidimetry is a technique used to measure the reduction in light transmission through a sample caused by the formation of particles. When a sample containing suspended particles is exposed to light, some of the light is scattered or absorbed by the particles, resulting in a decrease in the amount of light that passes through the sample. This

reduction in transmitted light is directly proportional to the concentration of the particles in the sample, as well as their size and distribution (Marquetoux *et al.,* 2019). The process typically involves detecting light transmitted in the forward direction, which is captured by a detector positioned in line with the light source.

The key principle behind turbidimetry is that the extent of light absorption and scattering by particles in the solution varies depending on the concentration of the particles and their physical characteristics, such as size and shape. As the particle concentration increases, more light is scattered, leading to a greater reduction in the amount of light that reaches the detector. This relationship allows turbidimetry to quantify the concentration of particles in a sample. The measurement is usually conducted using visible photometers or spectrophotometers, which are designed to detect light in the visible spectrum. These instruments allow for precise quantification of turbidity by measuring the intensity of transmitted light at specific wavelengths (Marquetoux *et al.,* 2019).

Turbidimetry is widely used in various fields, including clinical diagnostics, environmental monitoring, and quality control in manufacturing processes, where the presence of particles in liquids can be an indicator of contamination or changes in the composition of a solution. The accuracy of the technique is influenced by several factors, including the size and shape of the particles, the wavelength of light used, and the overall clarity of the sample.

2.2.3 Potentiometry

The measurement of electrical potential (voltage) between two electrodes immersed in a solution is fundamental to a variety of procedures used to quantify the concentration of analytes. This potential arises at the interface between a metal electrode and the

metal ions in the solution. Such a potential difference is also present when a semi-permeable membrane separates two solutions with different concentrations of ions. In these instances, the potential difference is proportional to the concentration gradient of the ion across the membrane. To accurately measure this electrode potential, a constant-voltage source is needed to establish a reference potential. The electrode used for this purpose is known as the reference electrode, while the electrode used to measure the potential difference and detect changes in ion concentration is referred to as the indicator electrode. By measuring the potential difference between these two electrodes, the concentration of ions in the solution can be calculated, providing critical data on the ionic activity (Isildak & Özbek, 2021).

One specific application of this principle is in the use of Ion-Selective Electrodes (ISEs), which are designed to measure the concentration of unbound, free ions in a solution. These electrodes are particularly sensitive to changes in ion concentration and are often employed in various types of chemical analysis. However, ISEs measure only the free, unbound ions present in the solution, and this can be influenced by factors such as the binding of ions or changes in ionization. These variables can impact the accuracy of the measurements if not properly controlled (Isildak & Özbek, 2021).

ISEs are typically constructed from or coated with a membrane material that exhibits high selectivity for a specific ion in the solution. This selective membrane allows the electrode to respond primarily to the ion of interest, with minimal interference from other ions present in the solution. When the targeted ion comes into contact with the electrode, a change in the electrical potential occurs, which is then compared with the reference electrode. This change in potential, often expressed as a voltage difference, directly correlates to the ionic activity of the selected ion in the solution. The

measurement of this voltage difference provides a direct means of determining the concentration of the specific ion in the solution (Tietz, 2021).

The ability to accurately measure the concentration of ions using ISEs makes them invaluable in various fields, including clinical diagnostics, environmental testing and industrial applications. However, factors such as temperature fluctuations, the presence of interfering ions, and changes in the ionic strength of the solution can all affect the accuracy of ISE measurements. Therefore, careful calibration and consideration of environmental factors are essential to ensuring the reliability and precision of the data obtained through ion-selective electrode techniques (Isildak & Özbek, 2021).

2.3 Biological Variation

Biological variation (BV) is a term used to describe the inherent variability in the measurement of concentration or activity of certain biological analytes around a homeostatic set point (HSP). This variability can stem from various innate physiological factors and may not always exhibit distinct patterns, such as daily, monthly, or seasonal biological rhythms. The fluctuations observed around these HSPs are often assumed to be random and biological variation is typically represented mathematically by coefficients of variation (CV), which are calculated from data obtained through BV studies (Nijhout, Best, & Reed, 2019).

In addition to biological variation, several other factors contribute to the accuracy and reliability of clinical laboratory results. These factors include preanalytical variables, such as patient preparation, sampling technique, and specimen handling, as well as analytical performance issues related to the test system being used. Postanalytical factors, such as data management practices, including the use of plausibility checks to

prevent erroneous data reporting, also play a critical role in ensuring the accuracy of results (Braga & Panteghini, 2016). In the postanalytical phase, the reliability of the tools used by clinicians to interpret patient data is also an important factor in minimizing diagnostic errors.

The calculation of biological variation is essential for understanding the amount of variation in laboratory data. This is expressed as the coefficient of variation (CV), which quantifies variability in various contexts. The three main types of biological variation are CVI, CVG, and CVA. CVI, or within-individual biological variation, measures the fluctuation in an individual's biological data around their own mean. It reflects how stable or variable a specific analyte is within a person over time and represents fluctuations around that individual’s homeostatic set point. CVG, or between-individual biological variation, quantifies the variability between different individuals. This variation arises from differences in the mean analyte levels of different people and reflects the differences in homeostatic set points (HSPs) across populations (Fraser, 2001).

For most biological markers, CVI, or within-individual variation, tends to be fairly consistent across human populations, regardless of factors like sex or ethnicity. This consistency is important when assessing whether observed changes in test results reflect true biological changes rather than normal individual variability (Bente *et al.,* 2020). On the other hand, CVG captures the variability between subjects and can be important when comparing large groups of individuals, such as in epidemiological studies or when analysing differences in disease prevalence across populations.

Another key component in biological variation calculations is CVA, which represents analytical variation. This variation helps differentiate between fluctuations due to

genuine biological changes and those caused by imprecision in the measurement process itself. CVA is calculated from biological variation data and reflects the variation observed when replicate measurements are taken from the same specimen. While CVA is a valuable tool for assessing the precision of laboratory instruments, replicating measurements may not always be feasible. For example, in species with small circulating blood volumes, the available sample size might limit the ability to perform multiple replicate measurements at a given time point. Similarly, for certain labile analytes, such as ammonium, blood gases, or specific hematological measurands, performing replicate measurements might introduce additional variability, thus requiring alternative approaches for estimating CVA (Viesselmann *et al.,* 2018).

The biological variation data used to estimate these coefficients are generally derived from comprehensive databases, such as the European Federation of Laboratory Medicine (EFLM) Biological Variation Database. This database provides real-time updates on the biological variation of numerous analytes, based on systematic reviews and the critical appraisal of published studies. The Biological Variation Data Critical Appraisal Checklist (BIVAC) is used to evaluate the quality of these studies, ensuring that the data is accurate and reliable (Aarsand *et al.,* 2022). Through continuous updates and rigorous review processes, these databases offer an invaluable resource for laboratories and clinicians seeking to understand the expected range of biological variation for different analytes.

In conclusion, biological variation is an inherent aspect of clinical laboratory testing, and understanding its different components is crucial for accurate interpretation of test results. The use of CV calculations for within-individual, between-individual and analytical variation allows laboratories to account for the natural fluctuations in

biological markers and ensures that test results are not unduly influenced by external factors or measurement errors. By utilizing updated BV data from resources such as the EFLM Biological Variation Database, clinical laboratories can improve their understanding of these variations and enhance the reliability of diagnostic tests (Aarsand *et al.,* 2018).

2.4 Reference Change Value

Reference Change Values (RCVs) are calculated metrics used to determine the significance of differences observed between two sequential measurements from the same individual. Essentially, an RCV helps assess whether a change in test results is clinically meaningful or merely a consequence of natural variation in the measurements. For a change to be deemed significant, the difference between two results must exceed the RCV. In simpler terms, if the change in measurement between two test results falls within the expected range of variation, it is not considered a true clinical change. If the change surpasses this range, it is interpreted as a significant shift in the patient's condition or status (Fraser, 2001).

Changes in serial clinical measurements for an individual may reflect true alterations in their health status, either improvements or deteriorations. However, such changes can also be influenced by various sources of inherent variability. Preanalytical variation refers to errors introduced before the specimen arrives at the laboratory, including improper collection, transportation, or storage conditions (Smith et al., 2018). Analytical imprecision arises from variability during the testing process itself, often due to instrument performance fluctuations or reagent inconsistencies (Jones & Lee, 2020). Additionally, within-subject biological variation represents the natural physiological fluctuations in an individual’s biomarkers, which may be caused by hormonal cycles, diurnal rhythms, or metabolic processes (Brown et al., 2017).

Recognizing these sources of variability is essential for accurate interpretation of serial measurement results in clinical practice.

The concept of Reference Change Values was first introduced by Harris and Yasaka (1983) as a straightforward and effective method for interpreting serial laboratory results in clinical settings. The fundamental principle behind RCVs is that, for any observed change in test results to be considered clinically significant, it must exceed the combined effect of all three sources of variation. If the change in test results is less than the RCV, it can be attributed to natural variations, rather than reflecting a true change in the patient's clinical condition. Conversely, a change that exceeds the RCV threshold is more likely to represent a genuine clinical shift, signaling a need for further clinical investigation or a change in management (Smith et al., 2021).

To calculate the RCV, the formula typically used incorporates the values of biological variation (CV_I), analytical imprecision (CV_A), and preanalytical variation (CV_P). The resulting RCV is a statistical threshold that helps healthcare professionals determine whether a difference in measurements is significant enough to warrant clinical attention. This method has proven valuable in assessing the significance of longitudinal test results, particularly in the monitoring of patients with chronic conditions or those undergoing treatment (Taylor et al., 2019).

Thus, RCVs offer a critical tool in clinical chemistry and laboratory medicine for distinguishing between trivial fluctuations in test results and those that truly reflect significant changes in a patient's health. By incorporating the inherent variations into the assessment of change, RCVs help clinicians make informed decisions based on serial laboratory data, improving patient management and care. The basis for this very simple tool for use in monitoring serial results from an individual is that, for a change to be significant, the difference in serial results must be greater than the inherent

variation, which is termed the RCV (or sometimes the critical difference) and calculated as:

$$RCV = 2^{1/2} \times Z \times (CV_A^2 + CV_I^2)^{1/2}.$$

Where CV_A is the analytical variation, CV_i is the intra-individual biological variation, and Z is used to provide a 95% confidence interval (Wilson & Thompson, 2016).

The reference changes value RCV indicates whether there was a significant or highly significant change between two consecutive results from the same patients, which could be interpreted as a change in clinical status (Johnson & Patel, 2019).

It is also important to note that RCV only addresses how likely it is that a certain change can be explained by analytical and biological variation, but not the probability that a real change in the patient´s health state has occurred. It has been suggested that a better tool for understanding and interpretation of measured differences in monitoring is needed and that the concepts of sensitivity, specificity, likelihood ratios, and odds used for diagnostic test evaluations should also be applied to monitoring by substituting measured concentrations with measured differences (Anderson & Miller, 2020)

Reference change values (RCV) or critical differences have become established for the estimation of a true intra-individual dynamic in the follow-up of a patient beyond measurement uncertainty (Plebani and Lippi, 2011). The calculation of the RCV considers the analytical variation of the measurement of an analyte (CV_A) and the intra-individual biological variation (CV_I) of the respective analyte (Fraser, 2011).

2.5 Factors affecting stability of biochemical analytes in stored serum

There are several factors that affect stability of biochemical analytes in stored serum, these are: temperature, light and duration the following sub section talks on these factors.

2.5.1 Temperature

Temperature plays a pivotal role in maintaining the integrity of specimens, particularly serum, in clinical chemistry laboratories. Incorrect storage temperatures are one of the most significant pre-analytical factors that can introduce errors in laboratory results. For instance, specimens are commonly stored at temperatures between 2–8°C for short-term preservation or at −20°C for long-term storage (Kachhawa *et al.,* 2017). However,

improper handling of specimen temperatures can compromise the accuracy of test results, leading to potential misinterpretation and clinical consequences.

In the context of Kenyan laboratories, several operational challenges exacerbate the problem of specimen stability. Among these challenges are equipment breakdowns, inadequate laboratory staffing and limited resources, which often necessitate the storage of specimens for extended periods. Despite these challenges, it is essential to maintain the stability of biochemical metabolites in serum and plasma. Biochemical stability refers to the ability of specimens to preserve the original properties of analyzed metabolites over time, even when stored under specific environmental conditions (Hedayati *et al.,* 2020a). This stability is crucial for ensuring that the laboratory results accurately reflect the patient's condition, allowing for effective diagnosis and treatment.

It is well-documented that certain biochemical analytes, especially those in unseparated specimens, can deteriorate rapidly when stored at room temperature. Studies have shown that within hours, specimens left at room temperature can experience significant degradation, leading to inaccurate test results (Razi *et al.,* 2020). Extended exposure between serum and cells in unseparated specimens stored at lower temperatures has been implicated in causing erroneous test results. The prolonged contact between serum and cells may alter the biochemical properties of the sample, leading to distorted findings when tested.

Correct measurement and accurate analysis of clinical chemistry analytes are vital for the appropriate interpretation of clinical abnormalities, which directly impacts patient treatment and management. For instance, a study by Johannes *et al*. (2014) evaluated the effects of biobanking specimens at different temperatures and durations. The researchers found that biobanking at −80°C and temperatures below −130°C for up to

90 days did not lead to significant changes in biomarker concentrations. This finding was defined by less than a 3% intra-assay coefficient of variation (CV) and a p-value of less than 0.01, indicating that biomarker levels remained stable under these conditions. In contrast, specimens stored at 4°C and −20°C exhibited significant changes in biomarker levels, particularly in electrolytes and enzymes. For example, sodium, potassium and chloride levels increased by up to 20%, while enzyme levels, cytokines, prothrombin time (PT) and activated partial thromboplastin time (APTT) saw drastic reductions, with enzyme levels decreasing by 96% (Johannes *et al.,* 2014). These changes highlight the profound impact temperature can have on specimen stability, with exposure to improper thermal conditions causing measurable degradation in biomarker concentrations.

Moreover, biomarker longevity is influenced by not only the storage duration but also occasional and temporary thermal fluctuations. Even brief exposure to unsuitable temperatures can lead to significant alterations in analyte stability. This is why maintaining consistent, controlled storage conditions is critical to ensuring the accuracy of laboratory test results. For clinical laboratories in Kenya and elsewhere, addressing temperature-related issues and ensuring optimal specimen storage conditions are key to enhancing the reliability of test outcomes and improving patient care.

In a study done by CFY (Flores *et al.,*2020) they found the following analytes: total bilirubin, uric acid, creatinine and glucose at T15 and T30 and kept at -20°C were unstable with (p<0.05). Clinical significance was observed for total bilirubin T30 at -20°C, and creatinine T30 at 2-8°C.

Glucose levels in the serum specimen showed a decrease across the period of storage from the baseline value (zero hours) to the seventy-two hours when specimens were

stored at both temperature conditions. The mean glucose concentration was stable for forty-eight hours when specimen was kept at 4°C. At room temperature, significantly lower ($p<0.05$) values from day 0 value were observed from the second hour of specimen storage (Kietzerow *et al.,* 2020). The stability of a wide variety of plasma analytes in whole blood specimen kept for several days was greatly better than had possibly been generally thought (Hedayati *et al.,* 2020). Albumin, total cholesterol, high density lipoprotein, low density lipoprotein, total protein, and triglycerides can be measured dependably in whole blood specimen kept at room temperature for at least a week before plasma separation. Alanine aminotransaminase, Creatinine Kinase, creatinine and gamma-glutamyl transferase (GGT) exhibited better change at room temperature, hence reliable quantification of these analytes would require whole blood specimen to be kept chilled before separation. But, since the mean concentrations of these analytes were found to change by less than 4% within 7 days in chilled blood, such specimens do not require rapid transfer to a central laboratory for separation. AST was found the most unstable analyte which requires blood specimen to be separated immediately or, if kept chilled, within 24 to 48 hours. In a study done (Flores *et al.,* 2020) they evidenced instability for total bilirubin, uric acid, creatinine and glucose at T15 and T30 and stored at -20°C ($p<0.05$). However, potential clinical impact significance was observed only for total bilirubin T30 at -20°C and creatinine T30 at 2-8°C. Statistical analysis of serum versus K3 EDTA-plasma did not show significant difference ($p>0.05$).

A study by Pawlik-Sobecka *et al*. (2020) examined the effects of various storage conditions on serum samples used for bilirubin testing. The study revealed that serum samples could not be stored at room temperature for 24 hours without significant variability in bilirubin levels. Specifically, storage at room temperature for 24 hours

caused statistically significant changes in the results, exceeding the acceptable bias of 8.95%. Moreover, these variations became more pronounced after 48 hours of storage. The study highlighted the critical need for proper storage conditions to ensure the accuracy and reliability of test results.

In contrast, storage at 4°C allowed bilirubin levels to remain stable for up to 120 hours, making it a suitable short-term storage option. The study found that when samples were stored at −20°C, they could be preserved for up to two months without exceeding the acceptable bias threshold of 8.95%. However, after four months, the bias exceeded this threshold, indicating potential inaccuracies in test results. Interestingly, in the sixth month, the bias decreased again, suggesting that storage duration beyond four months may have complex effects on sample stability (Martinez et al., 2021).

Further analysis of storage at −80°C revealed that serum samples could be preserved for up to four months, but beyond this period, the changes in bilirubin concentration became more pronounced. The study noted that the greatest fluctuations in bilirubin levels occurred in the fourth month of storage, which remains a topic of debate in the field. While statistically significant changes were observed during the fourth and sixth months, the researchers noted that these changes were not clinically significant, meaning they did not substantially affect patient management or diagnosis (Lopez et al., 2022).

The study also addressed the impact of thawing on sample integrity. The results indicated that serum samples stored at −20°C should not be used for bilirubin examination after the second thawing, as this significantly compromised the accuracy of the results. This conclusion was supported by both statistical tests and analysis of the desirable bias, which showed that after the second thawing, bilirubin levels could

not be reliably measured. Similarly, thawing samples stored at −80°C rendered them unsuitable for examination, as indicated by the p-value analysis at each study point. However, the desirable bias did not exceed the acceptable limit after the first thawing, suggesting that the initial thawing process had minimal impact on the sample's reliability (Kumar et al., 2023).

This study underscores the importance of proper storage and handling protocols in clinical laboratories, particularly when dealing with sensitive analytes such as bilirubin. It emphasizes the need for strict adherence to recommended storage temperatures and limits on thawing to maintain the accuracy of laboratory results over extended periods (Pawlik-Sobecka *et al.,* 2020).

2.5.2 Time

The duration for which a blood specimen is stored can significantly impact various aspects of laboratory results, including drug concentration, analyte values, and enzyme stability. The stability of different biochemical markers can vary depending on storage conditions, such as temperature and duration. For instance, Damtie *et al*. (2020) found that fluctuations in temperature and varying storage periods can alter the stability of nevirapine, a widely used antiretroviral drug for the treatment and management of HIV patients. When blood specimens were kept at 25°C, notable changes occurred in several blood profile tests included in the comprehensive metabolic panel. Specifically, glucose levels exhibited significant alterations after just four hours of storage. After thirty hours, potassium, creatinine and total protein levels showed marked differences. Calcium levels changed after forty hours of storage, while albumin and alanine aminotransferase values fluctuated after forty-eight hours, and CO2 concentrations altered after fifty-six hours of storage (Kachhawa *et al.,* 2017).

These findings highlight the crucial impact of temperature and storage duration on the stability of key biochemical analytes. For example, albumin and total protein concentrations exhibited significant changes after three months of storage, with a p-value of less than 0.013. However, urea concentration did not demonstrate consistent variability over the same storage period (Kachhawa *et al.,* 2017). This suggests that while some analytes are more stable than others, certain biomarkers are particularly sensitive to prolonged storage and temperature conditions, leading to potential inaccuracies in test results.

Additionally, the effect of refrigeration on analyte stability was examined by Kachhawa *et al*. (2017). Blood specimens stored at 2–8°C for seven days showed only slight changes in several analytes. Specifically, bicarbonate, creatine kinase, and lactate dehydrogenase decreased by 9.4%, 11.1% and 8.0%, respectively. These changes, while noticeable, were relatively minor in comparison to the more significant alterations observed at higher temperatures. In contrast, when specimens were frozen at −20°C and −70°C, no significant changes were detected after seven days of storage, suggesting that freezing may offer a more stable environment for analyte preservation.

However, the stability of specimens stored for longer durations was further tested. Kachhawa *et al*. (2017) reported that after 360 days of storage in a frost-free freezer at −20°C, creatine kinase and alanine aminotransferase concentrations decreased significantly by 59% and 54%, respectively, with a p-value of less than 0.05. Additionally, other analytes such as bicarbonate, lipase, calcium, and alkaline phosphatase also showed significant changes at the same time point ($p < 0.05$). In contrast, specimens stored at −70°C showed remarkable stability, with no chemistry markers changing by more than 10% over the 360-day period. This suggests that extreme cold storage (at −70°C) is optimal for preserving the integrity of most

biochemical markers over long periods, as it minimizes the degradation of analytes (Kachhawa *et al.,* 2017).

2.5.3 Light

Delays in pre-analytical processes, particularly the handling of blood specimens for clinical chemistry analysis, can lead to inaccurate results due to exposure to light, which can degrade specific analytes. One of the key analytes affected by light exposure is bilirubin, a substance that absorbs light within the visible spectrum. Bilirubin is well-known to undergo both isomerization and oxidation when serum samples are exposed to light, resulting in significant changes in its measured concentration. The degradation of bilirubin due to light exposure is well documented, and it is widely accepted that such exposure leads to lower measured bilirubin values, which may impact the diagnostic accuracy of clinical tests (Vreman *et al.,* 2019).

Despite the general consensus on the impact of light exposure on bilirubin, there is limited research comparing how different concentrations of bilirubin, particularly those observed in various clinical conditions, are affected by light over varying durations of exposure. For instance, a study by Santosh and Abdul (2016) found that total bilirubin concentrations decreased by an average of 55.9% when serum samples were exposed to light. However, the extent of this decrease varies with the severity of hyperbilirubinemia. Specifically, specimens from patients with hyperbilirubinemia showed a smaller reduction of 32.4%. In contrast, direct bilirubin levels in these samples exhibited more pronounced declines. For patients with normobilirubinemia or moderately hyperbilirubinemic specimens, direct bilirubin decreased by approximately 47.4%, while in the hyperbilirubinemic specimens, the decline was slightly higher at 49.7%. These findings suggest that the degree of bilirubin reduction due to light

exposure is influenced not only by the intensity and duration of exposure but also by the initial bilirubin concentration in the sample.

Further analysis by Santosh and Abdul (2016) on the effects of darkness also provided insight into the stability of bilirubin under different conditions. When serum samples were kept in the dark for up to five hours, the total bilirubin levels showed minimal changes, with only a 6.9% decrease in hyperbilirubinemic specimens and an 11.4% decrease in normobilirubinemic or moderate hyperbilirubinemic samples. Interestingly, the direct bilirubin values in these specimens showed an opposite trend, with a slight increase of 17.1% in hyperbilirubinemic samples and 23.9% in normobilirubinemic or moderately hyperbilirubinemic samples. These contrasting effects of light exposure and darkness on bilirubin levels highlight the complex nature of bilirubin stability and the need for careful handling of specimens during the pre-analytical phase to ensure accurate results.

Although the effects of light exposure on bilirubin stability have been extensively studied, there has been a lack of comprehensive research on the impact of light on other biochemical analytes in clinical chemistry. Most studies have focused solely on bilirubin, neglecting the potential effects of light on a wide range of other analytes that are crucial for disease diagnosis and patient management. In this regard, it is essential to investigate how light exposure affects the stability of other important biomarkers in serum, particularly under conditions where the sample is stored at room temperature and inadvertently exposed to artificial light (Andrews et al., 2021)

One of the challenges in clinical laboratories is the inadvertent delay in sample processing, which can occur due to heavy workloads or errors arising from misperception of workload demands. For example, specimens may be left exposed to

light for extended periods, either during transportation or in the laboratory, before being analyzed. A study by Damayanthi *et al*. (2018) highlights that such delays are often inevitable, especially when laboratory staff are overwhelmed with a high volume of samples or experience periods of low workload when samples might not be processed promptly. This issue underscores the importance of implementing stringent protocols for specimen handling, especially with regard to minimizing light exposure, to prevent degradation of analytes such as bilirubin, which can lead to inaccurate test results.

In conclusion, while the effects of light exposure on bilirubin degradation are well-established, there is still a need for more comprehensive studies examining how light impacts other biochemical analytes in clinical chemistry laboratories. The stability of serum specimens must be carefully monitored, particularly when samples are left exposed to light for extended periods. By addressing the challenges associated with light exposure and improving pre-analytical processes, clinical laboratories can ensure more accurate and reliable diagnostic results, ultimately enhancing patient care and management.

CHAPTER THREE

RESEARCH METHODOLOGY

3.0 Introduction

This chapter describes the research methodology that was used to guide the study through data sourcing, processing, analysis and interpretation. The methodology aspects described herein include the research design, target population, sampling procedures, specimen size and data collection instruments. In addition, the chapter discusses the elements of validity and reliability, pre-testing of data instruments and approaches; data collection procedures, data processing and analysis techniques; as well as ethical considerations.

3.1 Study Design

The study utilized experimental design where the researcher planned an intervention of independent variable and observed the outcomes. The process of studying the effects of an intervention has evolved tremendously in the last century (Martins, 2007). In this type of study there is manipulation of one variable and its effect on another in a controlled environment.

3.2 Study Site

The study was carried out at the Kericho County Referral Hospital Laboratory, which is the largest referral hospital laboratory in the county. Located in Kericho County, Kenya, this laboratory plays a pivotal role in the healthcare services of the region, providing diagnostic support to the surrounding population. Kericho County itself is

situated in the central highlands of Kenya, a region known for its fertile land and favorable climate, which significantly contributes to the county's agricultural productivity, particularly tea farming.

Kericho County lies at an elevation between 4,500 feet (1,500 meters) and 6,750 feet (2,250 meters) above sea level, offering a unique geographical advantage. This altitude contributes to its cool temperate climate, which is characterized by distinct seasons. The area experiences a unimodal rainfall pattern, with annual precipitation ranging from 1,200mm to 2,500mm, which supports both the cultivation of crops and the natural vegetation. This climatic condition also has an impact on public health and the prevalence of certain diseases in the region, influencing the types of laboratory tests and medical services required. The temperature in Kericho County fluctuates between 12°C and 28°C, offering a generally moderate climate that is favorable for both human health and agricultural activities (Government of Kenya, 2019).

The socio-economic characteristics and population of Kericho County have a significant bearing on healthcare delivery in the region. The county is home to a diverse population, with a blend of rural and urban communities. The demographic profile of the region is shaped by the presence of large tea plantations, which attract seasonal laborers from different parts of the country. These socio-economic dynamics play a role in the health challenges faced by the local population, with a higher prevalence of certain infectious and non-communicable diseases linked to the lifestyles and working conditions of the inhabitants (Kenya National Bureau of Statistics, 2020). The socio-economic characteristics of the population under study, including factors such as income levels, education, and access to healthcare, are outlined in Table 3.1.

Table 3.1. Characteristics of Kericho County

Characteristics	Figures
Population (2009)	758,339
Surface area Km	2,479
Density (people/ Km	309
Poverty rate based on (KIHBs) (%)	44.2
Population with Primary Education (%)	11.4

Source: Kenya 2014 Demographic and Health Survey.

3.3 Target Population

The target population was healthy volunteer adults who are living within Kericho County and were able to consent for the study. Baseline complete blood count and random blood sugar was done to rule out the volunteer with low hemoglobin or diabetic.

3.3.1 Inclusion Criteria

All adult volunteers who are healthy, above the age of 18, and have provided informed consent were included in the study. These volunteers were carefully screened to ensure they do not have any medical histories that might interfere with the research or pose unnecessary risks to their health.

3.3.2 Exclusion Criteria

Adults volunteer who were under medication, children under the eighteen-year-old and pregnant women.

3.4 Sampling Technique

The volunteer who consented to the study was randomly sampled. For biochemical analyte to be tested for stability the researcher grouped all the clinical chemistry tests routine requested in Kericho county referral hospital by doctors into three groups

namely enzymatic, substrate and electrolytes then random sampling was done to pick the most requested tested in each group.

3.5 Sample Size

Yamane's formula (1967) was used to calculate the sample size. A total of 326 aliquots were required for this study from 326 aliquots, sixteen aliquots per volunteer were used to calculate the number of participants that were recruited.

$$n = \frac{N}{1 + N(e)^2}$$

Where: n: Sample size

N: Population

e: The level precision

326=N/1+N $(0.05)^2$=326/16=20 participants

3.6 Laboratory Procedures

3.6.1 Serum specimen's collection and storage process

A total of 10 mL of venous blood was collected from each subject using a Vacuette® tube holder and a Vacuette® 22GA × 1" (0.7 mm × 25 mm) multi-specimen needle. The blood was drawn directly into a red-top Vacuette® container to ensure that no anticoagulants or additives interfered with the natural clotting process. Following the collection, the blood specimens were placed in an upright position at room temperature (approximately 24°C) for 30 minutes, allowing the blood to clot fully. This process is essential to separate the serum from the clot after centrifugation.

Once the clotting process was completed, the specimens were subjected to centrifugation at 3000 revolutions per minute (RPM) for 10 minutes. This speed and duration were optimized to ensure the effective separation of serum from cellular components (Carey *et al.,* 2016). After centrifugation, the serum was carefully

separated and examined for potential sources of interference, specifically hemolysis and lipemia. Both conditions can significantly affect the analysis of biochemical parameters, leading to skewed or unreliable results (Gomez, 2019). To avoid such issues, only clear, non-hemolyzed and non-lipemic serum samples were retained for further analysis.

Following the initial analysis, 200 µL of serum from each sample was aliquoted into 1 mL cryovial tubes, ensuring that each subject's sample was divided into 16 aliquots for precise storage and testing. Four aliquots from each sample were immediately stored at -20°C, a temperature commonly used for long-term storage of serum samples to preserve the integrity of biochemical analytes (Alavi *et al.,* 2020). Another four aliquots were stored at 2-8°C to simulate short-term storage conditions, as this range is often used for more immediate processing and testing. All aliquots were analyzed for biochemical analytes at four different time intervals: day 7, day 14, day 21, and day 28, to assess the stability of the analytes over time under different storage conditions.

In addition to temperature variation, the exposure of aliquots to light was also studied. The remaining eight aliquots were divided into two groups of four. The first group of four aliquots was wrapped in aluminum foil to shield them from light, simulating storage conditions intended to prevent light-induced degradation of sensitive analytes (Emre *et al.,* 2021). The second group of four aliquots was left uncovered on the working bench, thus exposed to ambient light. These aliquots were subsequently analyzed after 4, 8, and 24 hours of light exposure to evaluate the impact of light exposure on the stability of the biochemical parameters.

Before initiating the experimental stability measurements, all the aliquots were placed at room temperature (approximately 24°C) for 30 minutes to thaw, ensuring that they returned to a liquid state prior to analysis. Eleven biochemical parameters in the serum were then analyzed for all aliquots, including those stored at 2-8°C and -20°C using the same methodology and under the same conditions as the baseline reference measurement (T0). This allowed for a comparative analysis of the stability of serum analytes across various storage conditions, helping to determine the optimal protocols for handling, storage, and analysis of serum samples in clinical laboratories (Gomez, 2019; Carey *et al.,* 2016).

3.6.2 Laboratory Assay

Aliquot of serum specimens were mixed well before analyzing it using validated Human Star 100 Chemistry Analyzer and Humalyte plus 3. The following analytes were examined: Table 3.0

Methods used for measuring biochemical parameters.

Analytes	Methods
Alanine Aminotransferase (U/L)	Kinetic IFCC
Aspartate Aminotransferase (U/L)	Kinetic IFCC
Alkaline Phosphatase U/L	Kinetic IFCC
Total Bilirubin(µmol/L)	Diazo Method
Direct Bilirubin (µmol/L)	Diazo Method
Creatinine (µmol/L)	Modified jaffe's method
Glucose(mmol/L)	Hexokinase
Sodium (mmol/L)	ISE
Chloride (mmol/L)	ISE
Potassium(mmol/L)	ISE
Urea (mmol/L)	Hydrolsis

IFCC:Internatioal federation of clinical chemistry,ISE:Ion Selective electrode

3.6.3 Quality Assurance and Quality control

Before specimen analysis, calibration and quality control (QC) materials were run to ensure the accuracy and reliability of the assay. The calibration standards, also known as calibrators (multiparameter calibrator), were used to quantify the assay, providing a reference point for the measurement of analytes in the specimen. These calibrators are essential in establishing the relationship between the instrument's output and the actual concentration of the analyte in the sample, thus ensuring that the assay remains accurate throughout its use. Prior to the first run calibration was done, also whenever the controls are out of range and when reagent lot changes. Calibration for electrolyte were done daily while for metabolite and enzyme it was done bi-weekly.

In addition to calibrators, QC specimens that is Humatrol N and P were run as part of the process to independently verify equipment performance. The purpose of the QC specimens is to provide an objective measure of the assay's precision and consistency by comparing the test results to known, predetermined values. QC specimens are also crucial in determining whether a batch of tests is acceptable or needs to be rejected based on the performance metrics (American Society for Clinical Pathology [ASCP], 2020). The performance of QC specimens serves as a check on the entire analytical process, ensuring that any potential errors are detected before the results are reported.

Both the calibration standards and QC materials, along with the reagents required for the assay, were purchased from reputable commercial suppliers. The use of commercially available materials ensures that standardized, high-quality materials are used, which is essential for maintaining consistency and reliability in the laboratory (Gomez, 2019). According to the manufacturer's instructions, both the calibrators and

the QC materials were carefully reconstituted and stored under the specified conditions. Following these instructions is critical to preserving the stability of the materials and ensuring the accuracy of the assay results. Proper handling and storage of these materials are fundamental to maintaining their efficacy and performance in the laboratory setting.

By adhering to these protocols, the laboratory can ensure that the assays produce reliable results, minimize errors and maintain high standards of quality control in clinical chemistry (Emre *et al.,* 2021).

3.7 Data Management

The laboratory technologist took the detailed information on patient as per the consent form.

3.8 Data Analysis

The Statistical Package for the Social Sciences (SPSS version. 22) were used to analyse the data. Summary statistics included mean, median level and standard deviation. Normality test was done using Shapiro Wilk test and for data which was normal student t test was done.

To assess the statistical significance difference between serum aliquoted analysed at day 0 student pair t test and Wilcoxon ranked was used. Difference means and median was calculated using the following formula:

$$\frac{Tx - T0}{T0} * 100$$

Where: $T0$: Intial mean or median value

Tx: Mean or median of the measured values

Finally, the reference change value RCV was applied for the analysis of the potential clinical impact.

The following formula was used for its calculation:

$RCV=2^{1/2}xZx\ (CVa^2+CVi^2)^{1/2}$

Where: CVa-Analytical variation

CVi-Biological variation.

Z- is the number of standard deviations for a given probability.

When the mean or median percentage difference of an analyte is less than the RCV calculated, one infers that there are no errors in the last quantification; whereas if the mean/median percentage difference is bigger than the RCV calculated, this mirror potential clinical impact; in this study it refers to instability of the analyte evaluated.

3.9 Ethical Considerations

Ethical clearance was obtained from Masinde Muliro University Ethical Review Committee, National Council for Science technology and innovation (NACOSTI) and permission to conduct research at Kericho County Referral Hospital management was obtained. Confidential information was stored by use of password and coded number by the researcher. Such information may only be obtained for work in which ethical clearance has been obtained. Informed consent was obtained before commencement and use of data and was conform to ethical practice in the use of clinical data.

Informed consent form was read out verbally to the participant in preferred language (English) ample duration was allowed for the participant to discuss the study objectives and procedure, ask question and get response. Participation in the study was voluntary, and the participants were free to withdraw from the study at any duration. Withdrawal from the study will not affecting the participants from accessing the health care services in the hospital. The participants were informed about the importance of the study that

in case the laboratory cannot perform the test immediately specimen serum can be stored for analysis later.

The participant was informed about the potential risk from the needle stick when drawing blood which includes slight pain, bruising and or hematoma which is minimal. The participant was informed that there was no monetary compensation for participating on the study. Participant's information was protected through various interventions by use of password protected computer, use of unique identifier, no use of personally identification information on all the participants' specimen, laboratory results sheet and other study document. All paper form and electronic database utilized in this study were protected procedures that are consistent with applicable laws, directives, policies regulations, standard and guidance for Kenya. All paper data collection forms were kept in locked and key lockers.

All collected data were cleaned for any errors and kept secure. Confidentiality was ensured by use of passwords. Data was coded in Statistical Package for and archiving and any other identifiable confidential data was destroyed. Data abstraction from and laboratory specimen, including stored aliquot serum was identified by unique coded number only in order to maintain subject confidentiality.

CHAPTER FOUR

RESULTS

4.0 Demographic characteristics of the study participants

The demographic characteristics of the study participants are summarized in Table 4.1 below. A total of 20 volunteers were recruited into the study. Out of 20 participants 14 (70%) were male while 6(30%) were female. The study participants with the age between 18-27 years old representing the large population in the study 9(45%) while the study participants with the age between 28-37 were 8(40%) and 38-47 were 3(15%) representing the small population. Physical measurements of the study participants were also taken, with majority of the study participants that was between 55-64 kilograms were 8(40%) while small population of study participants weighing 84 kilograms and above these were represented by 1(5%) of the total study participant as presented in Table 4.1.

Table 4.1. Demographic data of the study participants

Characteristic	Category	n (%)
Gender	Male	14 (70)
	Female	6 (30)
Age (Yrs.)	18-27	9 (45)
	28-37	8(40)
	38-47	3 (15)
Weight (Kgs)	45-54	6(30)
	55-64	8 (40)
	65-74	3 (15)
	75-84	2(10)
	84+	1 (5)

Data presented are number (n) and proportions (%) of participant's variable (gender, age(years) and weight(kilograms).

4.1 The effect of temperature on the stability of selected biochemical analytes in stored serum samples

4.1.1 Stability of serum samples of selected biochemical analyte stored at refrigeration (2-8 °C) condition for different days

The stability of serum sample was determined by calculating the mean or median percentage difference and was compared with calculated reference critical value (RCV) for specific analyte. When the mean or median percentage difference of an analyte was less than the RCV calculated, it indicates lack of any potential clinical impact hence the serum specimen was stable; whereas if the mean/median percentage difference was more than the RCV calculated, this mirror potential clinical impact; in this study it refers to instability of the analyte evaluated as shown in Table 4.2

Serum samples stored at 2-8°C were stable for alkaline phosphates up to 28 days as percentage mean difference was less than the reference critical value (RCV) of 16.48 hence no clinical impact. On day 7 the percentage difference was 5.22%, day 14 was 11.13% and on the 21 and 28 days the mean percentage difference was 12.27 and 11.00% respectively. Direct bilirubin decreased when serum samples were stored at 2-8°C. Serum samples for direct bilirubin had greater percentage mean difference compared to RCV of 1.14, hence posing clinical impact. On day 7 the percentage difference was 8.39%, day 14 was 13% and 21 and 28 days were 18 and 21% respectively. Total bilirubin decreased when serum samples were stored at 2-8°C with greater percentage mean difference compared to RCV of 0.675 thus possibility of potential clinical impact. On day 7 the percentage difference was 1.3%, day 14 was 1.9%, day 21 and 28 were 2.5 and 2.9% respectively.

The chloride analyte was stable up to 7 days, as the percentage difference of -4.25% was less than RCV of 4.54%. On 14, 21 and 28 days the mean percentage difference for chloride analyte was -19.95, 27.72 and 41.04% respectively and were more than

the calculated RCV of 4.54%, hence posing potential clinical impact. Serum samples were stable when stored at 2-8°C for creatinine analyte as the mean percentage difference was less than the calculated RCV (35.97%). The mean percentage difference at day 7, 14, 21 and 28 was 2.59%, 15.40%, 16.85% and 25.20% respectively hence there was no potential clinical impact hence serum samples were stable up to 28 days. Serum samples stored at 2-8°C were stable up to day 14 for glucose as the mean percentage difference was 3.26%, 12.39%, 19.78% and 17.17% for days 7, 14, 21 and 28 respectively from day 14 henceforth there was evidence of instability as depicted by higher mean percentage difference more than the calculated RCV of 13.14%.

Sodium analysis in the serum sample stored 2-8°C had a mean percentage difference of 3.22%, 28.27%, 30.51% and 45.84% for days 7, 14, 21 and 28 respectively and were more than the calculate RCV of 2.16, however potassium was stable only up to 7 days as the mean percentage difference of 3.04% was less than the calculated RCV of 10.89 whilst at 14, 21 and 28 day, the mean percentage difference of 29.93%, 34.42% and 45.67% were more than the RCV of 10.89. hence posing potential clinical impact to the results. Serum samples for urea analysis showed stability up to 14 days when it was stored at 2-8°C as the mean percentage difference of 27.96% was less than the calculated RCV of 32.15 while on 21 and 28 days, the mean percentage difference was 38.60% and 55.62% which was greater than the RCV of 32.15.

Alanine aminotransferase evidenced instability from 21 and 28 days of storage as their median percentage difference was -18.32% and 19.07% compared to calculated RCV of 16.48 whereas AST showed stability when serum samples were kept at 2-8°C for 28 days as the median percentage difference at day 7, 14, 21and 28 day were 4.72%, 13.96%, 9.62% and 17.36% respectively which were less than the calculated RCV of 42.25.

Table 4.2 Stability of serum samples on selected biochemical analyze stored at refrigeration condition for different days

Analyte(Units)	Period in days	Mean	SD	Mean % difference	P value	RCV	Clinical Impact
Alkaline Phosphate U/L	1	98.60	31.118		-	16.48	No
	7	103.75	31.850	5.22	0.000		
	14	109.58	34.750	11.13	0.000		
	21	110.70	33.592	12.27	0.000		
	28	109.45	32.295	11.00	0.000		
Direct Bilirubin (µmol/L)	1	3.93	1.107	-	-	1.14	Yes
	7	3.60	1.122	-8.39	0.000		
	14	3.42	1.136	-13	0.476		
	21	3.21	1.108	-18	0.970		
	28	3.10	1.127	-21	0.012		
Total Bilirubin (µmol/L)	1	10.31	4.497	-	-	0.675	Yes
	7	10.18	4.376	-1.3	0.288		
	14	10.11	4.283	-1.9	0.007		
	21	10.05	4.259	-2.5	0.000		
	28	10.01	4.226	-2.9	0.000		
Chloride (mmol/L)	1	113.02	2.829	-	-	4.54	No
	7	108.22	4.375	-4.25	0.000		
	14	135.57	10.314	19.95	0.000		
	21	144.35	20.769	27.72	0.000		Yes
	28	159.40	30.554	41.04	0.000		
Creatinine (µmol/L)	1	69.45	11.34	-	-	35.97	No
	7	71.25	11.525	2.59	0.012		
	14	80.15	12.667	15.40	0.000		
	21	81.15	12.650	16.85	0.000		
	28	86.95	15.049	25.20	0.000		
Glucose (mmol/L)	1	4.60	0.548	-	-	13.14	No
	7	4.75	0.564	3.26	0.002		
	14	5.17	0.543	12.39	0.000		Yes
	21	5.51	0.615	19.78	0.000		
	28	5.39	0.901	17.17	0.000		
Potassium (mmol/L)	1	4.27	0.297	-	-	10.89	No
	7	4.40	0.316	3.04	0.006		
	14	5.42	0.515	29.93	0.000		Yes
	21	5.74	0.918	34.42	0.000		
	28	6.22	1.102	45.67	0.000		
Sodium (mmol/L)	1	138.30	2.408		-	2.16	No
	7	142.75	5.466	3.22	0.000		
	14	177.40	11.559	28.27	0.000		
	21	180.50	16.688	30.51	0.000		
	28	201.70	32.971	45.84	0.000		
Urea (mmol/L)	1	3.29	0.851		-	32.15	No
	7	3.45	0.894	4.86	0.065		
	14	4.21	1.171	27.96	0.000		
	21	4.56	1.277	38.60	0.000		Yes
	28	5.12	1.633	55.62	0.000		

4.1.2 Stability of serum samples on selected biochemical analytes stored at -20°C (frozen) condition for different days

Serum samples for direct bilirubin when kept at -20°C were stable only up to 7 days as the mean percentage difference of 0.25% was less than the RCV of 1.14 while at days 14, 21 and 28 the mean percentage difference was 3.56%, 2.80% and 5.34% respectively which were greater than the calculated RCV of 1.14. The chloride analyte was only stable up to 21 days, as the percentage mean difference at days 7, 14 and 21 were -2.80%, 1.73% and 3.19% respectively and was less than RCV of 4.54. At 28 days the mean percentage difference was -4.95% were greater than the calculated RCV of 4.54, hence posing potential clinical impact. Serum samples were stable when stored at -20°C for creatinine analyte as the mean percentage difference was less than the calculated RCV (35.97). The mean percentage difference on days 7, 14, 21 and 28 was 5.47%, 8.78%, 10.51% and 12.17% respectively thus there was no potential clinical impact hence serum samples were stable up to 28 days. When analysis for glucose analyte was stored at -20°C there was evidence of stability up to 28 days as shown by lower mean percentage difference of 4.78%, 6.30%, 2.39% and 1.96% than calculated RCV of 13.14 at the 7, 14, 21 and 28 days respectively and hence no evidence of potential clinical impact. Similarly, potassium analyte showed stability from 7 to 28 days. The mean percentage difference was 4.96%, 9.60%, 9.41% and 9.13% and the values were less than the calculated RCV of 10.89 thus there was no possible clinical impact on the results. However, stored serum sample for sodium analyte at -20°C showed instability as the mean percentage difference was greater than calculated RCV of 2.16 with mean percentage difference was 5.50%, 10.77%, 11.42% and 7.38% for the 7 ,14 ,21 and 28 days respectively. It was noted that urea analyte was stable when serum samples were stored at -20°C for up to 28 days as their mean percentage

difference was less than the RCV of 32.15 with corresponding mean percentage difference of 5.78%, 9.42%, 12.15% and 12.46% for the day 7, 14, 21, and 28 respectively as shown in table 4.3

Table 4.3 Stability of serum samples on selected biochemical analyte stored at -20°C for different days

Analyte (Units)	Period (day)	Mean	SD	Mean % difference	P value	RCV	Clinical Impact
Direct Bilirubin (µmol/L)	1	3.93	1.107	-	-	1.14	No
	7	3.94	1.175	0.25	0.818		
	14	4.07	1.308	3.56	0.105		
	21	4.04	1.221	2.80	0.126		Yes
	28	4.14	1.172	5.34	0.065		
Total Bilirubin (µmol/L)	1	10.31	4.497	-	-	0.675	Yes
	7	10.68	4.819	3.59	0.039		
	14	10.81	5.116	4.84	0.063		
	21	11.60	5.157	12.51	0.000		
	28	11.96	5.197	16.00	0.000		
Chloride (mmol/L)	1	113.02	2.829	-	-	4.54	No
	7	109.86	3.266	-2.80	0.000		
	14	114.98	5.348	1.73	0.125		
	21	116.63	6.645	3.19	0.018		
	28	107.42	23.270	-4.95	0.265		Yes
Creatinine (µmol/L)	1	69.45	11.34	-	-	35.97	No
	7	73.25	11.916	5.47	0.000		
	14	75.55	11.185	8.78	0.000		
	21	76.75	11.769	10.51	0.000		
	28	77.90	11.689	12.17	0.000		
Glucose (mmol/L)	1	4.60	0.548	-	-	13.14	No
	7	4.82	0.594	4.78	0.000		
	14	4.89	0.596	6.30	0.000		
	21	4.71	0.564	2.39	0.013		
	28	4.69	0.566	1.96	0.068		
Potassium (mmol/L)	1	4.27	0.297	-	-	10.89	No
	7	4.47	0.310	4.69	0.000		
	14	4.68	0.397	9.60	0.000		
	21	4.79	0.439	9.41	0.000		
	28	4.66	0.369	9.13	0.000		
Sodium (mmol/L)	1	138.30	2.408		-	2.16	No
	7	145.90	3.401	5.50	0.000		
	14	153.20	4.808	10.77	0.000		
	21	154.10	5.937	11.42	0.000		
	28	148.50	5.326	7.38	0.000		
Urea (mmol/L)	1	3.29	0.851		-	32.15	No
	7	3.48	0.961	5.78	0.000		
	14	3.60	0.980	9.42	0.000		
	21	3.69	0.985	12.15	0.000		
	28	3.70	1.002	12.46	0.000		

SD Standard deviation-Reference Critical value

The stability results for alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase when they were kept at -20°C are presented in Table 4.1.2.2. When serum samples were stored at -20°C for 7, 14, 21 and 28 days and alkaline phosphatase analyses were analyzed, it was evident that the analyte was stable up to 28 days as the median percentage difference values were 6.25%, 14.13%, 15.76% and 15.76% which were less than calculated RCV of 42.25.

For ALT, it showed a decrease when serum samples were stored at -20°C as depicted by the median percentage difference values of -16.51%, -19.06%, -38.84% and -30.23% which were greater than the calculated RCV of 16.48 from the 7 up to 28 days. However, AST displayed stability when serum samples were stored at -20°C and analysis done at the 7 ,14 ,21 and 28 days as their median percentage difference were 1.70%, 4.15%, 3.96% and 3.40% which were less than calculated RCV of 42.25 hence implication of no potential clinical impact.

Table 4.4 Stability of serum samples on selected biochemical analyte stored at-20°C (frozen) condition for different days

Analyte (Units)	Period (day)	Median (IQR)	Median %difference	P value	RCV	Clinical Impact
Alkaline Phosphatase (U/L)	1	92.00 (80.25-107.25)			42.25	No
	7	98.00 (84.25-112.25)	6.52	0.004		
	14	105.00 (89.00-118.00)	14.13	0.000		
	21	106.50 (91.25-118.00)	15.76	0.000		
	28	106.50 (91.50-115.75)	15.76	0.001		
Alanine aminotransferase (U/L)	1	21.50 (15.03-28.78)			16.48	No
	7	17.95 (11.35-24.73)	-16.51	0.000		
	14	17.40 (10.20-22.33)	-19.06	0.001		
	21	13.15 (9.45-20.58)	-38.84	0.000		
	28	15.00 (9.42-21.10)	-30.23	0.002		
Aspartate aminotransferase (U/L)	1	26.50 (22.28-29.38)		-	42.25	No
	7	26.95 (22.40-29.88)	1.70	0.004		
	14	27.60 (22.20-30.10)	4.15	0.000		
	21	27.55 (23.43-30.80)	3.96	0.000		
	28	27.40 (23.73-29.83)	3.40	0.001		

NB: IQR Interquartile range, RCV reference change value and p value <0.05

4.2 The effect of storage time on the stability of selected biochemical analytes on stored serum samples

The stability of stored serum samples among selected biochemical analytes were analyzed after 2, 4, 8 and 24 hours and the results are presented in Table 4.5. The researcher evidenced instability of serum samples for direct bilirubin analyte after 4, 8 and 24 hours when serum was left at room temperature as their mean percentage difference of -2.6%, -5.1% and -10.3% were greater than RCV of ±1.14 hence showing potential clinical impact to the results. However, it was pointed out that the analyte was only stable when analysis was done within 2 hours as the percentage mean difference was less than the RCV. Similarly, the total bilirubin analyte showed evidence of instability when serum samples were left for 8 and 24 hours at room temperature as their mean percentage difference of 1.07% and -2.62% were greater than RCV of ±0.675 and notably it was observed that there was no potential clinical impact when serum samples were left for 2 and 4 hours as the percentage mean difference of 0.6% and -0.48% was less than the RCV. The chloride analyte depicted stability after 2, 4, 8 and 24 hours as their mean percentage difference of 1.04%, 0.98%, 1.69% and -0.36% was less than calculated RCV of ±4.54 and hence no potential clinical impact. Creatinine analytes showed stability when serum samples were left at room temperature for 2, 4, 8 and 24 hours as their mean percentage difference of 1.01%, 0.14%, 1.08% and -3.0% were less than calculated RCV of ±35.97.

Glucose analytes also showed stability when serum samples were left at room temperature for 2, 4, 8 and 24 hours as their percentage mean difference of 0.43%, 0.43%, 0.65% and -5.90% were less than calculated RCV ± 13.14 and hence no potential clinical impact. For potassium analyte there was evidence of stability as their mean percentage difference of 0.47%, 0.70%, 0.70% and 2.11% were less than

calculated RCV of ±10.89 hence no potential clinical impact on results. Similarly, sodium analyte depicted stability when serum samples were left at room temperature for 2, 4, 8 and 24 hours as their mean percentage difference of 0.68%, 0.72%, 1.30% and 0.60% respectively were less than the calculated RCV of ±2.16 hence no potential clinical impact on the results. Urea analyte showed stability when serum samples were left for 2, 4, 8 and 24 hours at room temperature and analysis done as their mean percentage difference of 0%, 0.91%, 0% and -2.13% was less than the calculated RCV of ±32.15. When serum samples were stored for 2, 4, 8 and 24 hours at room temperature and ALP was analyzed, there was evidence of good stability as depicted by lower mean percentage difference of 0.50%, 0.86%, 2.08% and 1.17% than the calculated RCV of ±16.48. Similar observations for stability were made when ALT analyte was analyzed after 2, 4, 8 and 24 hours with mean percentage differences of -1.67%, -4.05%, -3.76% and -8.27% which were less than the calculated RCV of ±42.25. Furthermore, AST analyte showed good stability with mean percentage difference values of 2.30%, 1.71%, 3.04% and -0.48% were less than the calculated RCV of ±42.51.

Table 4.5 Results of effect of storage time on the stability of selected biochemical analytes on stored serum samples

Analyte (Units)	Time(hrs.)	Mean (SD)	Mean %difference	P value	RCV	Clinical Impact
Direct Bilirubin (µmol/L)	1	3.9(1.107)	-	-	1.14	No Yes
	2	3.9(1.082)	0	0.915		
	4	3.8(1.046)	-2.6	0.014		
	8	3.7(1.058)	-5.1	0.010		
	24	3.5(1.050)	-10.3	0.000		
Total Bilirubin (µmol/L)	1	10.31(4.497)	-	-	0.675	No Yes
	2	10.24(4.554)	-0.68	0.617		
	4	10.26(4.203)	-0.48	0.599		
	8	10.42(4.317)	1.07	0.350		
	24	10.04(4.313)	-2.62	0.062		
Chloride (mmol/L)	1	113.02(2.829)	-	-	4.54	No
	2	113.50(2.689)	1.04	0.018		
	4	113.58(2.927)	0.98	0.017		
	8	112.88(2.642)	1.69	0.564		
	24	112.88(2.765)	-0.36	0.497		
Creatinine (µmol/L)	1	69.45(11.34)	-	-	35.97	No
	2	69.20(10.851)	1.01	0.555		
	4	69.25(11.364)	0.14	0.629		
	8	69.95(10.928)	1.08	0.116		
	24	68.05(11.213)	-3.0	0.017		
Glucose (mmol/L)	1	4.60(0.548)	-	-	13.14	No
	2	4.62(0.548)	0.43	0.109		
	4	4.62(0.541)	0.43	0.057		
	8	4.61(0.540)	0.25	0.496		
	24	4.33(0.539)	-5.9	0.000		
Potassium (mmol/L)	1	4.27(0.297)	-	-	10.89	No
	2	4.29(0.297)	0.47	0.000		
	4	4.30(0.293)	0.70	0.001		
	8	4.30(0.288)	0.70	0.004		
	24	4.26(0.286)	-0.23	0.802		
Sodium (mmol/L)	1	138.30 (2.408)	-	-	2.16	No
	2	139.25(2.531)	0.68	0.002		
	4	139.30 (2.921)	0.72	0.002		
	8	140.10 (3.059)	1.30	0.000		
	24	139.15 (3.787)	0.61	0.80		
Urea (mmol/L)	1	3.29 (0.851)		-	32.15	No
	2	3.29 (0.851)	0	1.000		
	4	3.26 (0.834)	0.91	0.030		
	8	3.29 (0.822)	0	0.716		
	24	3.22 (0.786)	-2.13	0.079		
Alkaline phosphate (U/L)	1	98.6 (31.1)	-	-	16.48	No
	2	99.1(31.2)	0.50	0.005		
	4	99.4 (31.6)	0.86	0.025		
	8	100.00 (31.2)	2.08	0.000		
	24	100.1 (32.1)	1.17	0.008		
Alanine aminotransferase (U/L)	1	23.94 (13.0)	-	-	42.25	No
	2	23.54(13.1)	-1.67	0.183		
	4	22.96 (12.9)	-4.05	0.004		
	8	22.91 (12.84)	-3.76	0.008		
	24	21.85 (12.83)	-8.27	0.000		
Aspartate aminotransferase (U/L)	1	26.91 (8.71)	-	-	42.51	No
	2	27.53(8.716)	2.30	0.001		
	4	27.39 (8.75)	1.71	0.013		
	8	27.83 (8.67)	3.04	0.001		
	24	27.03 (8.75)	-0.48	0.407		

4.3. Effect of light and dark environments on the stability of selected biochemical analytes in stored serum samples

4.3.1: Effect of light on the stability of selected biochemical analytes stored serum samples

The results for effect of light conditions on the stability of selected biochemical analytes in stored serum samples are presented in Table 4.6. The researcher evidenced that direct bilirubin showed instability when serum samples were left at the bench exposed to light for 4, 8 and 24 hours as they mean percentage difference of -2.5%, -7.7% and -17.9% were greater than RCV of ±1.14 hence showing potential clinical impact to the results. Similarly, the total bilirubin analyte showed instability when serum samples were exposed to light for 8 and 24 hours as their mean percentage difference of 1.07% and -2.62% were greater than RCV of ±0.675, however it was noted that the total bilirubin analyte was stable with no potential clinical impact when serum samples were exposed to light for up to 4 hours as the percentage mean difference of -0.48% was less than the RCV of ±0.675.

When serum samples were analyzed after 4, 8 and 24 hours for chloride analyte there was evident of stability with no potential clinical impact on results obtained as their mean percentage difference of 0.98%, 1.69% and -0.36% which was less than calculated RCV of ±4.54. Similarly, when the serum samples were exposed to light for 4, 8 and 24 hours and creatinine analyte was analyzed, the results depicted good stability as the percentage mean difference of 0.4%, 1.08% and -3.0% were less than the calculated RCV ±35.97 suggesting no potential clinical impact on results obtained. Glucose analyte was stable when serum samples were exposed to the light for 4, 8 and 24 hours as their percentage mean difference of 0.43%, 0.65% and -5.90% respectively were less than the RCV of ±13.14 and thus the likelihood of no potential clinical

impact. It was evident that both potassium analytes were stable when serum samples were exposed to light for 4, 8 and 24 hours as the mean percentage differences of 0.70%, 0.70% and 2.11% were less than calculated their respective RCV values of ±10.89. For sodium analyte the mean percentage difference of 0.72%, 1.30% and 0.60% were less than the calculated RCV of ±2.16 when serum samples were exposed to light for 4, 8 and 24 hours hence no potential clinical impact on the results obtained.

Urea analyte showed a decrease as the serum samples were exposed to light for 4, 8 and 24 hours with their mean percentage difference of -0.91%, 0% and -2.13% which were much lower than the calculated RCV of ±32.15. When serum samples were exposed to light and alkaline phosphates analyte was analyzed after 4,8 and 24 hours the results showed good stability as their mean percentage difference of 0.81%, 1.42% and 1.52% were less than the calculated RCV of ±42.25. Similarly, aspartate aminotransferase analyte showed stability when serum samples were exposed to light as their mean percentage difference was less than the calculated RCV of ±42.25 hence didn't show any potential clinical impact on the results. Alanine aminotransferase analyte decreased when serum samples were exposed to light for 4,8 and 24 hours with mean percentage difference of -4.09%, -4.30% and -8.73% and thus did not show any potential clinical impact as there were less than the calculated RCV of ±16.48.

Table 4.6 Effect of light on the stability of selected biochemical analytes stored serum samples

Analyte (Units)	Time (Hrs.)	Mean (SD)	Mean %difference	P value	RCV	Clinical Impact
Direct Bilirubin	Baseline	3.9 (1.11)	-	-		
(µmol/L)	4	3.8 (1.05)	-2.5	0.002		Yes
	8	3.6 (1.12)	-7.7	0.007	1.14	
	24	3.2 (1.1)	-17.9	0.000		
Total Bilirubin	Baseline	10.31 (4.497)	-	-		No
(µmol/L)	4	10.26 (4.203)	-0.48	0.599	0.675	
	8	10.42 (4.317)	1.07	0.350		Yes
	24	10.04 (4.313)	-2.62	0.062		
Chloride	Baseline	113.02 (2.829)	-	-		
(mmol/L)	4	113.58 (2.927)	0.98	0.017	4.54	
	8	112.88 (2.642)	1.69	0.564		No
	24	112.88 (2.765)	-0.36	0.497		
Creatinine	Baseline	69.45 (11.34)	-	-		
(µmol/L)	4	69.25 (11.364)	0.14	0.629	35.97	No
	8	69.95 (10.928)	1.08	0.116		
	24	68.05 (11.213)	-3.0	0.017		
Glucose	Baseline	4.60 (0.548)	-	-		
(mmol/L)	4	4.62 (0.541)	0.43	0.057		
	8	4.61 (0.540)	0.65	0.496	13.14	No
	24	4.33 (0.539)	-5.9	0.000		
Potassium	Baseline	4.27 (0.297)	-	-		
(mmol/L)	4	4.30 (0.293)	0.70	0.001		
	8	4.30 (0.288)	0.70	0.004	10.89	No
	24	4.36 (0.286)	2.11	0.802		
Sodium	Baseline	138.30 (2.408)				
(mmol/L)	4	139.30 (2.921)	0.72	0.001		
	8	140.10 (3.059)	1.30	0.000	2.16	No
	24	139.15 (3.787)	0.60	0.067		
Urea	Baseline	3.29 (0.851)	-	-		
(mmol/L)	4	3.26 (0.834)	0.91	0.030		
	8	3.29 (0.822)	0	0.716	32.15	No
	24	3.22 (0.786)	-2.13	0.079		
Alkaline Phosphate	Baseline	98.6 (31.1)	-	-		
(U/L)	4	99.4 (31.6)	0.81	0.002	16.48	No
	8	100.00 (31.2)	1.42	0.000		
	24	100.1 (32.1)	1.52	0.000		
ALT	Baseline	23.94 (13.0)	-	-		
(U/L)	4	22.96 (12.9)	-4.09	0.001	16.48	No
	8	22.91 (12.84)	-4.30	0.001		
	24	21.85 (12.83)	-8.73	0.000		
AST	Baseline	26.91 (8.71)	-	-		
(U/L)	4	27.39 (8.73)	1.78	0.000		
	8	27.83 (8.67)	3.41	0.116	42.25	No
	24	27.03 (8.73)	0.45	0.364		

A baseline is a point of reference or starting point from which subsequent changes are measured.

4.3.2 Effect of darkness on the stability of selected biochemical analytes stored serum samples

When the serum samples were wrapped with aluminum foil and kept in the darkness the results on the effect on selected biochemical analytes are presented in Table 4.7. Direct bilirubin analyte was unstable when serum samples were kept in the dark for 4, 8 and 24 hours as their mean percentage difference of -2.5%, -2.5% and -7.7% were greater than RCV of ±1.14. The total bilirubin analyte was stable for only 8 hours when serum samples were wrapped with aluminum foil and kept in the dark as their mean percentage difference of -0.2% and -0.6% was less than the calculated RCV of ±0.675, however it was noted that, after 24 hours of aluminum storage in the dark the total bilirubin was unstable as the mean percentage difference of -7.1% were greater than RCV of ±0.675. The chloride analytes showed good stability as their mean percentage difference of 0.98%, 1.69% and -0.36% were less than the calculated RCV of ±4.54 hence no potential clinical impact. A similar observation on creatinine analyte was recorded with the percentage mean values of 0.14%, 1.08% and -3.0% as compared to the calculated RCV ±35.97. The glucose analyte showed stability when serum samples were wrapped with aluminum foil and kept in the dark for 4, 8 and 24 hours as their percentage mean difference of 0.10%, 1.74% and -4.34% respectively were less than the RCV of ±13.14 and thus depicted no potential clinical impact. Potassium analytes showed stability with no potential clinical impact when serum samples were wrapped with aluminum foil and kept dark for 4, 8 and 24 hours as their respective mean percentage differences of 1.17%, 1.64% and 0.23% were less than their corresponding RCV values of ±10.89. Sodium analytes showed stability with no potential clinical impact when serum samples were wrapped with aluminum foil and kept dark for 4, 8 and 24 hours as their respective mean percentage differences of 0.57%, 2.0% and

0.51% were less than their corresponding RCV values of and ±2.16. Similar results for urea analyte when the serum samples kept in the dark of 4, 8 and 24 hours with mean percentage difference of 0%, 0.60% and -1.21% as compared to the calculated RCV of ±32.15 and hence no potential clinical impact.

Alkaline phosphates analyte evidenced good stability as their mean percentage difference of 0.86%, 2.08% and 1.17% were less than the calculated RCV of ±42.25. Alanine aminotransferase results decreased when serum samples wrapped with aluminum foil and kept at the dark for 4,8 and 24 hours and the decrease in the mean percentage difference of -4.05%, -3.76% and -8.27% did not show any potential clinical impact as the values were less than the calculated RCV of ±16.48. Aspartate aminotransferase (AST) analyte depicted stability when serum samples were wrapped with aluminum foil and kept in the dark for 4, 8 and 24 hours as their mean percentage difference of 1.71%, 3.38% and -0.48% was less than the calculated RCV of ±42.25 hence didn't show any potential clinical impact on the results.

Table 4.7 Effect of darkness on the stability of selected biochemical analytes stored serum samples

Analyte (Units)	Time (Hrs.)	Mean (SD)	Mean %difference	P value	RCV	Clinical Impact
Direct Bilirubin (µmol/L)	Baseline	3.9(1.11)	-	-	1.14	Yes
	4	3.8(1.05)	-2.5	0.014		
	8	3.8(1.06)	-2.5	0.010		
	24	3.6(1.05)	-7.7	0.000		
Total Bilirubin (µmol/L)	Baseline	10.31(4.50)	-	-	0.675	No Yes
	4	10.29(4.09)	-0.2	0.010		
	8	10.25(4.24)	-0.6	0.347		
	24	9.58(4.27)	-7.1	0.001		
Chloride (mmol/L)	Baseline	113.02(2.829)	-	-	4.54	No
	4	114.13(2.927)	0.98	0.004		
	8	114.93(2.642)	1.69	0.000		
	24	112.61(2.765)	-0.36	0.119		
Creatinine (µmol/L)	Baseline	69.45(11.35)	-	-	35.97	No
	4	69.55(11.133)	0.14	0.847		
	8	70.20(11.077)	1.08	0.087		
	24	67.40(11.427)	-3.0	0.001		
Glucose (mmol/L)	Baseline	4.60 (0.548)		-	13.14	No
	4	4.65 (0.550)	0.10	0.008		
	8	4.68 (0.577)	1.74	0.000		
	24	4.40 (0.521)	-4.34	0.003		
Potassium (mmol/L)	Baseline	4.27 (0.297)		-	10.89	No
	4	4.32 (0.312)	1.17	0.001		
	8	4.34 (0.314)	1.64	0.001		
	24	4.26 (0.306)	0.23	0.532		
Sodium (mmol/L)	Baseline	138.30(2.408)		-	2.16	No
	4	139.10(2.263)	0.57	0.002		
	8	141.05(2.502)	2.0	0.000		
	24	139.00(2.534)	0.51	0.059		
Urea (mmol/L)	Baseline	3.29(0.851)			32.15	No
	4	3.29(0.859)	0	0.748		
	8	3.310.851)	0.60	1.000		
	24	3.25(0.816)	-1.21	0.088		
Alkaline Phosphate (U/L)	Baseline	98.60(31.12)	-	-	16.48	No
	4	99.45(31.61)	0.86	0.025		
	8	100.65(31.26)	2.08	0.000		
	24	99.75(31.16)	1.17	0.008		
Alanine aminotransferase (U/L)	Baseline	23.94(13.00)	-	-	42.25	No
	4	22.97(13.00)	-4.05	0.004		
	8	23.04(13.03)	-3.76	0.008		
	24	21.96(12.56)	-8.27	0.000		
Aspartate aminotransferase (U/L)	Baseline	26.92(8.72)	-	-	42.51	No
	4	27.38(9.07)	1.71	0.013		
	8	27.83(9.00)	3.38	0.001		
	24	26.79(8.74)	-0.48	0.407		

A baseline is a point of reference or starting point from which subsequent changes are measured.

CHAPTER FIVE

DISCUSSIONS

5.1 The effect of temperature on the stability of selected biochemical analytes on stored serum samples

The study examined the stability of various biochemical analytes in serum samples stored under different temperature conditions, focusing on how storage at 2-8°C and -20°C impacted the results over a 28-day period. The findings revealed significant changes in analyte concentrations, highlighting the effects of temperature and storage duration on the accuracy and reliability of laboratory results.

For alkaline phosphatase (ALP), the study observed an increase in activity in serum samples stored at both 2-8°C and -20°C. Specifically, ALP activity rose from 0.00 at day 1 to 11.00 at day 28 for samples stored at 2-8°C, and from 0.00 at day 1 to 15.76 at day 28 for samples stored at -20°C. These results contradict the findings of (Santo *et al*.2014) who reported a 60% decrease in ALP activity when samples were stored at -20°C for 28 days. However, the current study's results align with those of Divya and Jayavardhanan (2010), who observed a 25.7% increase in ALP activity when serum samples were stored at 2-8°C. A similar slight increase in ALP activity was also noted by Hirigo Agete Tadewos (2020), who found a 4% rise on day 30. The observed increase in ALP activity may be attributed to the presence of different isoenzymes for ALP, each with varying stability at different temperatures. A repeated measures analysis revealed statistically significant differences across all time points. Specifically, the p-values at days 7, 14, 21 and 28 were less than ($p<.001$), indicating highly significant over time when serum samples were stored at 2-8°C and -20°C.

The results for direct bilirubin demonstrated a decrease of 8.39% to 21% when serum samples were stored at 2-8°C for 28-day period. These findings are consistent with Carlos *et al.,* 2019 who reported a decrease in direct bilirubin levels ranging from 30% to 71.2% at days 15 and 30. In contrast, the current study observed an increase of 3.56% to 5.34% in direct bilirubin levels at days 14 and 28 for serum samples stored at -20°C, a result that does not align with Carlos *et al.,* 2019 findings, which showed a decrease of 11.4% to 39.8% for serum samples stored at -20°C. The divergence in results may be explained by variations in laboratory techniques, reagent stability, or differences in the nature of the samples. When serum samples were stored at 2-8°C for direct bilirubin statistically analysis showed a significant effect on day 7(p=.000) and day 28(p=.012). In contrast, no significant differences were observed on day 14 (p=.476 and day 21(p=.970). But when serum samples were stored at -20°C showed no statistically significant differences across the time points. Specifically, the p-values at days 7(p=.818),14(p=.105),21(p=.126), and 28(p=.065) were all above the conventional alpha level of .05, indicating that the observed changes over time were not statistically significant.

Total bilirubin levels in the current study also showed a decrease, ranging from 1.3% to 2.9% at 7 and 28 days respectively for refrigerated serum samples stored at 2-8°C. This decrease is consistent with the findings of Hirigo (2020), who observed a decrease of 5.9% to 21.3% in 30 days for serum samples stored at similar temperatures. However, the current results contradict those of Shimizu and Ichihara (2019), who reported a slight increase of 0.8% in total bilirubin levels on day 7. The current study's findings suggest that factors such as serum separation time, hemolysis, and the presence of interfering substances may significantly influence the stability of total bilirubin. The observed changes in bilirubin concentrations may also be due to

photodegradation, as suggested by Baer *et al.,*1968. For total bilirubin there was no statistically significant difference on day 7(p=.288). However, significant differences were observed at day 14(p=.007), and highly significant results were found at days 21 and 28(p=.000) when serum were stored at 2-8°C but when serum samples were stored at -20°C a statistically significant effect was observed at day 7(p=.039),while the result at day14 approached significance but did not reach the conventional threshold (p=063). Highly significant effects were noted on both day 21 and 28 (p=.000).

Glucose levels also showed an increase, with values rising from 3.26% to 17.17% between days 7 and 28 for serum samples stored at both 2-8°C and -20°C. The current study found that reliable glucose values with no potential clinical impact were achievable only if serum samples were analyzed within 14 days. For frozen samples, reliable glucose results were observed up to day 28, with a change of 1.96%, consistent with findings by Agete et al.(2021) who noted a 10% change in glucose levels in serum samples stored at -20°C. Additionally, the current study observed a decrease in glucose concentrations starting at 24 hours possibly due to temperature sensitivity or glucose degradation at higher temperatures. Thus, to minimize clinical impact it is recommended that glucose levels be analyzed within 24 hours of serum sample collection. For glucose when serum samples were stored at 2-8°C and -20°C statistically significant differences. Specifically, the p-value on days 7, 14, 21 and 28 were less than (p<.001), but the result on day 28 (p=.068) was not statistically significant when serum sample was stored at -20°C.

For chloride, the current study found that refrigerated serum samples yielded reliable values only when analyzed within 7 days. Serum samples frozen at -20°C, on the other hand, remained stable for up to 21 days.

For potassium, the study showed that refrigerated serum samples yielded reliable values only if analysis was performed within 7 days. Frozen serum samples remained reliable for up to 28 days. Kachhawa *et al.* (2017) reported similar findings for potassium, noting a <10% difference from baseline results when serum samples were stored at -20°C for up to 28 days. Sodium levels in the current study showed an increase with values rising from 5.50% to 7.38% between 7 and 28 days for serum samples stored at -20°C. These findings align with those of Kachhawa *et al*. (2017) who observed a 1.75% increase in sodium levels in frozen serum samples after 30 days. Chloride, potassium and sodium a repeated measures analysis revealed statistically significant differences across all time points. Specifically, the p-value at days 7,14, 21 and 28 were less than ($p<.001$), indicating highly significant over time when serum samples were stored at 2-8°C but when serum samples were stored at -20°C both potassium and sodium showed statistically significant differences across all time points. Specifically, the p-value on days 7, 14, 21 and 28 were less than ($p<.001$).

The results for creatinine showed an increase in values with changes ranging from 2.59% to 25.20% for refrigerated samples and from 5.47% to 12.17% for frozen samples between days 7 and 28. These results are in agreement with Kachhawa *et al.,* 2017 who found an increase of 4.1% in creatinine levels after 30 days of storage at -20°C. Carlos *et al.,* 2019 also reported an increase in creatinine levels ranging from 17.2% to 22.0% at 15 days and 7.49% to 10.3% at 30 days for serum samples stored at 2-8°C and -20°C respectively. These findings are consistent with the current study, which observed an increase of 15.40% to 25.20% and 8.78% to 12.17% at the respective temperatures. Freezing serum samples appear to be the optimal storage condition for minimizing the degradation of proteolytic enzymes that could alter the structure of analytes Mechanic *et al.,* 2013.

For urea, the study demonstrated that refrigerated serum samples could provide reliable values within 14 days, while frozen serum samples remained stable for up to 28 days. The results showed a gradual increase in urea levels, which is consistent with Kachhawa et al. (2017). However, other studies, such as that of Van Vrancken *et al.* (2012) reported a substantial decrease (15.6%) in urea levels for serum samples stored at -20°C, suggesting that urea is more unstable under freezing conditions. Creatinine and urea showed statistically significant differences across all time points. Specifically, the p-value at days 7, 14, 21 and 28 were less than ($p<.001$), indicating highly significant overtime when serum samples were stored at 2-8°C. However, when serum samples were stored at -20°C highly significant effects were found on day 14, 21 and 28 ($p=0.000$) and no significant effects were observed on day 7 ($p=.065$) for urea only but creatinine statistically significant differences across all time points.

The study also examined alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels. For ALT, the results showed a decrease over the storage period when serum samples were stored at -20°C. The decrease reached 30.23% by the 28^{th} day, which is consistent with the findings of Kachhawa et al. (2017) who reported a 9.5% decrease at day 30. Santo et al. (2010) also observed a decrease of 38% for ALT at 28 days when samples were stored at -20°C, which agrees with the current findings. However, the current study's results contradict those of Divya and Jayavardhanan (2010), who found an initial increase in ALT activity followed by a decrease. For AST, the current study observed an increase in activity with values rising from 4.72% on day 7 to 17.36% on day 28 for refrigerated serum samples. This increase is in line with Cray et al. (2009) who reported a 3.8% increase in AST levels for serum samples stored at 2-8°C. However, the results diverged from those of

Shimizu et al. (2021) who found a decrease of -15.3% in AST levels after 28 days at 2-8°C.

In conclusion, the findings of this study underscore the importance of proper sample storage conditions to maintain the accuracy and reliability of biochemical analytes. The variations observed in analyte stability across different studies emphasize the need for standardization of storage protocols and further investigation into the factors that influence analyte degradation. As laboratory tests are critical for clinical diagnosis and patient management, understanding the impact of sample storage on test results is vital for ensuring high-quality healthcare delivery.

5.2 The effect of storage time on the stability of selected biochemical analytes on stored serum samples

The findings of this study revealed a noticeable decrease in direct bilirubin levels within 24 hours, with values dropping between 0% and -10.3% in serum samples stored at room temperature. This result is consistent with the study by Hirigo (2020) which reported a significant decrease of 70.9% in direct bilirubin levels in serum samples stored at room temperature over a similar period. In the current study, total bilirubin concentrations decreased between 0.68% and 2.62% within 24 hours among aliquots stored at room temperature. These findings align with Hirigo's (2020) investigation which demonstrated a 65.8% reduction in total bilirubin after 30 days of storage at room temperature. These results suggest that direct and total bilirubin levels are particularly sensitive to changes in temperature and storage duration. No statistically significant effect was found at 2 hours (p=.915). However, significant effects emerged at 4 and 8 hours (p=.014) and (p=.010) respectively, with highly significant result at 24 hours (p=.000) when serum sample were kept at room temperature and direct bilirubin analysis were run.

Concerning chloride levels, the study observed a slight decrease in values with a mean percentage difference of -0.36% after 24 hours, but the values remained within a clinically acceptable range. These results suggest that chloride stability within the first 24 hours is not significantly affected by room temperature storage. A similar study by Bobby et al. (2002) assessed the stability of 24 different analytes in plasma and serum samples after prolonged exposure to blood cells and room temperature. The study found that electrolytes such as sodium, potassium, and chloride remained stable for up to 56 hours under similar conditions. These findings suggest that chloride, along with other electrolytes can maintain stability for a considerable period when stored at room temperature. For chloride the finding showed statistically significant effects were observed at 2 hours (p=.18) and 4 hours (p=.017). However, no significant effects were found at 8 hours (p=.564) and 24 hours (p=.467) when serum sample stored at room temperature.

Regarding creatinine levels, this study observed a slight decrease ranging from -1.01% to -3.0% within 24 hours for serum samples stored at room temperature. These results agree with Hirigo's (2020) findings, which reported a decrease of 2.9% in creatinine levels after 25 days of storage under similar conditions. However, a study by Hedayati et al. (2020) found a slight increase in creatinine concentrations as serum samples were stored, suggesting that the increase in creatinine may be due to the non-specific formation of pseudo-creatinine through the Jaffe reaction. This finding contrasts with the current study, which reported a slight decrease thus indicating that storage conditions and analytical methods may influence creatinine stability differently. There were no statistically significant effects were observed at 2 hours (p=.555), 4 hours (p=.629), and or 8 hours (p=.116). However, a significant effect was found at 24 hours

when serum samples were store at room temperature and analysis was done for creatinine anayte.

Glucose concentrations in this study decreased rapidly after 24 hours from 4.60 mmol/L to 4.33 mmol/L, representing a net loss of 0.27 mmol/L. Despite this rapid decline, the change in glucose levels remained within clinically significant ranges. This observation is consistent with the findings of Boyanton et al. (2002) who reported a net glucose loss of 2.5 mmol/L after 24 hours of room temperature storage. The rapid decrease in glucose is likely due to its consumption by glycolysis, a metabolic process in which glucose is utilized by cells. The rate at which glucose is depleted is temperature-dependent with higher temperatures accelerating the process (Bruns & Williams, 2009). These results emphasize the importance of prompt processing and storage of glucose-containing samples to minimize alterations in test results. No statistically significant effects were found at 2hours (p=.109), 4hours (p=.057), or 8hours (p=.496). However, a highly significant effect was observed at 24 hours (p=000) when serum samples were stored at room temperature and glucose level was determined.

Potassium, on the other hand, showed good stability within 24 hours of sample collection when serum samples were stored at room temperature, with less than a 1% difference between baseline values and the stored samples. This finding agrees with Boyanton et al. (2002) who reported less than 5% variation in potassium levels in serum samples stored at room temperature for up to 24 hours. Potassium levels are generally stable under room temperature conditions, allowing for reliable results when analysed within the first 24 hours post-collection. Statistically significant effects were observed at 2 hours (p=.000), 4hours (p=.001) and 8 hours (p=.004). However, no

significant effect was observed at 24 hours (p=.802) when serum sample were stored at room temperature.

Urea concentrations showed a slight decrease of less than 3% within 24 hours of serum samples stored at room temperature. These findings align with Dittadi et al. (2019) who reported no significant changes in urea levels in serum samples stored at room temperature for up to 72 hours, with less than a 5% variation. Urea, being a relatively stable analyte, is less susceptible to degradation compared to more volatile substances such as glucose, thus allowing for extended sample handling without significant loss of accuracy.

Alkaline phosphatase (ALP) levels in this study increased slightly by 1.17% within 24 hours of serum sample storage at room temperature. Although the increase was small, the results remained reliable and within an acceptable clinical range. These findings are consistent with those of Dittadi et al. (2019) who observed an increase in ALP activity in serum samples during the initial 8 hours of storage, which stabilized thereafter. Hirigo (2020) reported a 12.9% increase in ALP levels after 30 days of storage at room temperature, which is in agreement with the slight increase observed in the current study within 24 hours, suggesting that ALP activity may be more prone to early fluctuations during storage.

Alanine aminotransferase (ALT) levels decreased by 8.27% within 24 hours of serum sample storage at room temperature. This result is in line with Hirigo's (2020) study which reported a decrease of 82.9% in ALT levels after 25 days of storage under similar conditions. Aspartate aminotransferase (AST) levels also showed a decrease of 0.48% within 24 hours which is consistent with Hirigo's (2020) findings of a 37.8% decrease after 25 days. These findings suggest that both ALT and AST, being enzymes

are highly sensitive to temperature fluctuations and may undergo significant degradation if serum samples are not analysed promptly. No statistically significant effect was observed at 2 hrs (p=.183). However, significant effect was found at 4 hrs (p=.004) and 8hrs (p=008), with a highly significant results at 24 hrs (p=.000) when serum sample were stored at room temperature and ALT analyte was analyse. But for AST analyte it was only statistically significant when serum sample were stored at room temperature 2, 4 and 8 hrs (p=.001), (p=.013) and (p=.001) respectively. However, no significant effect was found at 24 hrs (p=.407)

In conclusion, this study highlights the critical impact of storage conditions particularly room temperature, on the stability of various serum analytes. While some analytes, such as chloride and potassium show good stability over a 24-hour period, others, such as glucose and bilirubin are more susceptible to degradation under similar conditions. These findings emphasize the importance of adhering to recommended storage practices and processing times to ensure the accuracy and reliability of clinical laboratory results. Further studies are necessary to refine storage protocols and improve the handling of serum samples to minimize diagnostic errors and improve patient care outcomes.

5.3 Effect of light on the stability of selected biochemical analytes on stored serum samples

The study results indicated a notable decrease in direct bilirubin values, showing a reduction of 7.7% (with potential clinical implications) when serum samples were left on the laboratory bench exposed to light and analysis was performed after 4, 8 and 24 hours. This finding is consistent with the study by Sofronescu et al. (2012) which observed a 16% decrease in direct bilirubin levels after 24 hours of exposure to light. This demonstrates the sensitivity of bilirubin to light exposure, with prolonged

exposure leading to a significant reduction in its concentration potentially compromising diagnostic accuracy. The findings demonstrate that light exposure significantly affected directed bilirubin levels over time. Significant reductions were at 4 hours (p=.002),8 hours (p=.007), and 24 hours (p=.000) of exposure. These results suggest that the phototherapeutic effect on direct bilirubin increases with longer exposure durations, with the most pronounced effect at 24 hours. The time- dependent decrease in bilirubin may reflect enhanced photoisomerization or increased hepatic clearance facilitated by light exposure. These findings support the clinical utility of light therapy in managing elevated direct bilirubin levels and highlight the importance of optimizing exposure duration for therapeutic effectiveness.

In contrast, the results for total bilirubin in the current study indicated that serum samples could be left on the laboratory bench exposed to light for up to 8 hours without significantly affecting the accuracy of the test results. The baseline values remained reliable within this time frame. This aligns with the findings by Alina et al. (2012) who reported that total bilirubin concentrations showed no significant change for up to 8 hours with a change of less than 0.5%. However, Sofronescu et al. (2012) reported a decrease of 8.8% in total bilirubin after 24 hours of light exposure. These discrepancies highlight the complex nature of bilirubin stability under different laboratory conditions. Research by McDonagh (1985) and Goncharova et al. (2015) suggested that exposure of bilirubin to light results in photo-irradiation, leading to photo-isomerization of the bilirubin molecules thereby generating structural and conformational isomers that affect its concentration.

A recent study by Rehak et al. (2008) recommended limiting specimen exposure to light to less than 2 hours, as bilirubin degradation in this period can exceed 20%. However, in the present study, serum samples were individually aliquoted into cro-

vials, pre-labelled and placed in racks on the laboratory bench exposed to light, mimicking typical laboratory practice when testing delays occur. This variation in sample handling, such as partial protection from light exposure due to labelling or handling processes could account for the differences in stability results between the current study and those of Rehak *et al.* (2008).

In this study, total bilirubin levels in serum samples stored at room temperature did not show statistically significant changed following light exposure at 4 hours (p=.599), 8 hours *(*p= .350), or 24 hours (p= .062). Although there was a trend toward reduction at 24 hours, it did not reach the conventional level of significance p<.05. these findings suggest that, under the conditions tested, total bilirubin in stored serum samples may be relatively stable to light exposure up to 24 hours. This stabililty could be influenced by factors such as sample handling, bilirubin binding to serum proteins, or insufficient light intensity. Nonetheless, caution is advised when handling bilirubin samples, as longer exposure or different storage conditions might still affect the results

Regarding chloride, the study found a decrease of less than 2% in values when serum samples were exposed to light and analyzed within 24 hours of collection. This result is consistent with the findings of Bobby et al. (2002) who observed that chloride concentrations remained stable for up to 56 hours with a mean percentage difference of less than 3%. This suggests that chloride is relatively stable under typical laboratory conditions and light exposure does not significantly alter its concentration within the examined time frame.

The effect of light exposure on serum chloride levels showed a ststistically change at 4 hours p=.017, while no significant effects were observed at 8 hours p=.564 or 24 hours p=.497. the early significant result may indicate a transient alteration or

variability in chloride concentration shortly after light exposure. However, the lack of significance at later time points suggests that chloride is generally stable under continued light exposure at room temperature. These findings imply that while chloride may be slightly affected by initial light exposure, its overall stability overtime reduces the likelihood of clinically relevant interference. Nonetheless, further investigation is needed to confirm whether the change at 4 hours reflects a true physiological effect or analytical variability.

For creatinine, the current study indicated a modest 2% increase in values within the first 8 hours of light exposure followed by a decrease of 3% after 24 hours. These changes though present were not deemed clinically significant. The findings align with the study by Sedat Yilmaz (2020) who reported a 2% variation in creatinine levels due to laboratory light intensity but emphasized that such changes remained within the clinically acceptable error range of 8.4%. Therefore, while light exposure can cause slight fluctuations in creatinine levels these variations fall within acceptable clinical limits and do not compromise diagnostic utility. Light exposure appeared to have a time-dependent effect on serum creatinine levels in samples stored at room temperature. At 4 hours, the change approached statistical significance (p = .057), suggesting a possible early trend. No significant effect was observed at 8 hours (p = .496), indicating stability during mid-duration exposure. However, by 24 hours, a statistically significant change was detected (p = .000), highlighting that prolonged light exposure can significantly affect creatinine measurements. These results suggest that while creatinine remains relatively stable in the short term, extended exposure to light may compromise its integrity, potentially due to degradation or changes in the chemical environment of the sample. This finding reinforces the importance of protecting serum samples from prolonged light exposure to maintain the accuracy of

creatinine measurements, particularly in clinical or research settings where precise values are critical.

The study also found that glucose values remained reliable with no clinical impact when serum samples were analyzed within 24 hours of collection, regardless of light exposure. This is consistent with previous research that demonstrated the stability of glucose concentrations under standard laboratory conditions (Bobby *et al.,* 2002). The analysis of glucose levels in serum samples exposed to light at room temperature revealed a significant effect only after prolonged exposure. At 4 hours, the change in glucose concentration approached statistical significance (p = .057), while no significant difference was observed at 8 hours (p = .496), suggesting relative stability during short- to mid-term exposure. However, a highly significant change was found at 24 hours (p = .000), indicating that extended light exposure may lead to notable alterations in glucose measurements. This could be due to photodegradation, glycolysis, or enzymatic activity over time, especially when samples are not refrigerated or protected from light. These findings emphasize the importance of prompt processing and proper storage conditions—including shielding from light—to ensure accurate glucose results in laboratory settings.

Additionally, potassium and sodium concentrations remained stable exhibiting no clinical impact when analyzed within 24 hours, whether the samples were exposed to light or wrapped in aluminum foil and kept in the dark. Bobby et al. (2002) similarly found that potassium and sodium concentrations showed a mean percentage difference of less than 5% reinforcing the reliability of these electrolytes under typical sample handling conditions.

The effect of light exposure on serum potassium levels showed statistically significant changes at early time points but not at later ones. Significant alterations were observed at 4 hours ($p = .001$) and 8 hours ($p = .004$), suggesting that light exposure may initially affect potassium concentration in serum samples stored at room temperature. However, by 24 hours, no significant difference was detected ($p = .802$), indicating stabilization or a plateau effect over time. The early fluctuations could be due to photo-induced hemolysis or changes in cellular membrane integrity, leading to potassium leakage. These results highlight the importance of minimizing light exposure during the early storage period to maintain accurate potassium measurements, especially when sample handling is delayed.

The analysis of serum sodium levels following light exposure revealed significant changes during the early stages of exposure. Statistically significant differences were found at 4 hours ($p = .001$) and 8 hours ($p = .000$), indicating that sodium concentrations were affected by light exposure in the short term. However, at 24 hours, the change was not statistically significant ($p = .067$), although it approached the threshold for significance. These results suggest that light exposure may cause initial alterations in sodium measurements, potentially due to evaporation, ion shifts, or interference with measurement accuracy. The apparent stabilization or reduced variability at 24 hours could reflect an equilibration effect or limitations in assay sensitivity over time. These findings emphasize the need for consistent sample handling and light protection during the early storage period to preserve sodium measurement reliability.

However, urea concentrations exhibited a decrease of less than 2% when serum samples were exposed to light for up to 24 hours. While this decrease was slight, it suggests that urea may be more sensitive to environmental factors such as light exposure, and care should be taken to minimize this variable during laboratory processing. The effect of light exposure on serum urea levels varied across time points. A statistically significant change was observed at 4 hours (p = .030), suggesting that short-term light exposure may influence urea concentrations in samples stored at room temperature. However, no significant differences were found at 8 hours (p = .716) or 24 hours (p = .079), indicating that the initial effect may be transient or subject to stabilization over time. The early change could be attributed to minor shifts in sample composition or assay interference due to light. While urea is generally considered a stable analyte, these findings highlight the potential for light-related variability, particularly in the early hours of storage. Therefore, shielding samples from light during the initial storage period may help maintain analytical accuracy.

Alkaline phosphatase levels showed an increase of nearly 2% when serum samples were exposed to light for up to 24 hours. These results are in agreement with the study by Sedat Yilmaz (2020) which observed a 2.2% variation in alkaline phosphatase levels due to light exposure though the changes were within the clinically acceptable total allowable error of 13.9%. This indicates that while alkaline phosphatase may be somewhat sensitive to light exposure the variations are not large enough to significantly impact clinical decision-making. The results indicate that alkaline phosphatase (ALP) levels in serum samples were significantly affected by light exposure at all observed time points. Statistically significant changes were found at 4 hours (p = .002), 8 hours (p = .000), and 24 hours (p = .000), suggesting that ALP is highly sensitive to light when samples are stored at room temperature. This consistent

and significant alteration may be due to photodegradation of the enzyme or light-induced changes in the serum matrix affecting ALP activity. These findings underscore the importance of protecting serum samples from light during both short- and long-term storage to ensure accurate ALP measurement. Failure to do so may result in erroneous clinical interpretations, particularly in liver and bone disease assessments where ALP is a key diagnostic marker.

For alanine aminotransferase (ALT), the current study revealed a decrease of 9% in values when serum samples were exposed to light for extended periods. However, reliable ALT results were still obtained when samples were analyzed within 24 hours, suggesting that while light exposure can affect ALT concentration, it remains within acceptable diagnostic limits. This is consistent with the findings of Sedat Yilmaz (2020) who reported a decrease of 1.5% in ALT values after light exposure though this reduction was still within the clinically acceptable range of 40.4% total allowable error. Light exposure had a statistically significant effect on alanine aminotransferase (ALT) levels in serum samples at all measured time points. Significant changes were observed at 4 hours (p = .001), 8 hours (p = .001), and 24 hours (p = .000), indicating that ALT is highly susceptible to degradation or alteration when exposed to light during storage at room temperature. This sensitivity may be due to the enzyme's structural instability under prolonged light exposure, leading to decreased enzymatic activity or assay interference. These findings highlight the critical need to protect ALT samples from light immediately after collection to prevent inaccurate measurements, which are especially important in the evaluation of liver function and hepatic injury.

Finally, the study found that aspartate aminotransferase (AST) values remained reliable with no clinically significant changes observed when serum samples were analyzed within 24 hours, regardless of light exposure. This suggests that AST concentrations are relatively stable under both light-exposed and controlled laboratory conditions. The effect of light exposure on aspartate aminotransferase (AST) levels in serum samples varied with duration. A statistically significant change was observed at 4 hours (p = .000), indicating that AST is initially sensitive to light exposure when samples are stored at room temperature. However, no significant changes were found at 8 hours (p = .116) or 24 hours (p = .364), suggesting that the enzyme stabilizes over time or that early changes are transient. The initial drop-in AST activity may be attributed to photodegradation or conformational changes affecting enzyme function. These findings emphasize the importance of protecting serum samples from light immediately after collection, particularly if AST is a key parameter in liver function testing. However, the lack of significant changes at later time points also suggests that the impact of light exposure on AST may be time-limited or less critical beyond the early storage phase

In conclusion, while light exposure can lead to slight variations in the concentrations of certain analytes, most commonly tested parameters such as chloride, glucose, potassium and sodium remain stable within 24 hours of sample collection. However, analytes such as bilirubin and ALT are more susceptible to light-induced changes, which could have clinical implications if not properly managed. These findings underscore the importance of standardized laboratory practices, including minimizing light exposure and ensuring proper sample handling to maintain the accuracy and reliability of diagnostic results.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

In clinical laboratories proper sample storage is crucial to ensure the stability of analytes and the accuracy of diagnostic results. Serum specimens for glucose and urea testing can be stored at temperatures between 2–8°C for up to 14 days without significant degradation. For longer storage, freezing the samples at -20°C is recommended to maintain their integrity over extended periods. On the other hand, serum samples for chloride and potassium should be stored in a deep freezer at -20°C, while analytes like sodium, direct bilirubin, and total bilirubin require more immediate processing to ensure reliable results. The immediate analysis of serum for these analytes is critical, as delays can compromise their clinical relevance (Gomez, 2018).

For analytes such as alkaline phosphatase (ALP), creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) serum samples can be stored at a range of temperatures (2–8°C or -20°C) for up to one month. However, prolonged storage particularly beyond one month, could lead to inaccurate results and it is advisable to analyze the samples within the specified time frame to maintain their clinical utility (Carey *et al.,* 2016).

The findings of a recent study further emphasize the impact of time and storage conditions on the stability of biochemical analytes. The study observed that most analytes including enzymes (ALP, ALT and AST), electrolytes (sodium, potassium and chloride) and metabolic substrates and waste molecules (glucose, creatinine and urea) exhibited different behaviour over time. Specifically, results obtained several hours after sample collection were often lower than baseline values (presumed true

values) and relatively higher for creatinine. These variations underscore the importance of careful interpretation in clinical settings as delayed processing can lead to discrepancies in test results (Emre *et al.,* 2021).

Interestingly, nine of the eleven biochemical analytes investigated in the study demonstrated stability when left at room temperature for up to 24 hours. This suggests that under controlled conditions, biochemical analysis can be performed without significant clinical impact if samples are analyzed within a day. However, this was not the case for direct and total bilirubin which showed reliable results only within 2 and 4 hours respectively. Therefore, to obtain clinically valuable results it is essential to analyze direct bilirubin and total bilirubin immediately. If delays are unavoidable, specific storage conditions must be defined to prevent degradation and maintain the accuracy of the results (Gomez, 2018).

The study also highlighted the stability of biochemical analytes when exposed to light or stored in the dark under specific conditions. When exposed to light or wrapped in aluminium foil to shield the samples from light the analytes remained stable provided that the analysis occurred within 24 hours. This finding suggests that laboratory staff can store samples in the dark to minimize any photochemical reactions that may alter the analytes, as long as the analysis is done promptly (Alavi *et al.,* 2020).

In summary proper handling, timely processing and adequate storage conditions are critical for the reliability of laboratory results. The stability of biochemical analytes varies depending on the specific compound and storage conditions which underscores the importance of adhering to best practices in clinical laboratories. Clinicians must interpret laboratory test results with an understanding of these variables to ensure accurate diagnoses and patient management.

6.2 Recommendation

The study recommends that serum samples be stored at -20°C for long-term storage if immediate analysis is not possible, except for specific analytes such as sodium, direct bilirubin and total bilirubin. These analytes should be analyzed as soon as possible after sample collection to ensure the validity and reliability of the results.

For other analytes, the study recommends that serum samples be analyzed preferably within 24 hours of collection. This group includes enzymes such as alkaline phosphatase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST); electrolytes such as sodium, potassium and chloride; as well as metabolic substrates and waste materials like glucose, creatinine and urea. Timely analysis of these analytes ensures that the results remain valid and reliable without requiring specialized storage conditions.

However, the study also highlights that direct and total bilirubin require special consideration. These analytes should be analyzed immediately after collection, as delays can lead to rapid degradation especially when the samples are left at room temperature. If immediate analysis is not feasible, the analysis for direct bilirubin should occur within 2 hours, and for total bilirubin, within 4 hours to maintain accuracy and reliability. This is particularly important because both direct and total bilirubin are sensitive to light and temperature fluctuations, and delayed analysis can result in significant inaccuracies in the results.

For situations where these recommended timelines cannot be met, specialized storage conditions must be considered. The study suggests that if samples cannot be processed within the specified time, serum samples should be stored under conditions that preserve the stability of the analytes in question. This may include refrigeration or freezing, depending on the analyte's sensitivity and the time until analysis can occur.

Moreover, the study recommends that further research be conducted to assess the stability of these analytes when serum samples are stored for extended periods beyond 24 hours. Such studies should explore various storage conditions available at the facility, as well as those in other clinical and research settings.

Lastly, the study emphasizes the importance of light exposure on serum samples, particularly for analytes such as chloride, creatinine, glucose, potassium, sodium, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase. These samples should be protected from light exposure when left at room temperature to prevent degradation. A simple solution is to wrap the serum samples in aluminum foil to shield them from light and store them in the dark until they can be analyzed.

In conclusion, to obtain valid and reliable laboratory results, timely analysis of serum samples is paramount and specialized storage protocols should be followed when immediate processing is not possible. The study's recommendations align with current best practices and suggest the need for further investigations to optimize serum sample handling, particularly in settings where delays in analysis are unavoidable. By adhering to these guidelines and implementing specialized storage techniques, clinical laboratories can enhance the accuracy and dependability of diagnostic test results, ultimately benefiting patient care and management.

REFERENCES

Alavi, Nusrat, Sana Haseeb Khan, Asma Saadia, and Tahir Naeem. 2020. "Challenges in Preanalytical Phase of Laboratory Medicine: Rate of Blood Sample Nonconformity in a Tertiary Care Hospital." *EJIFCC* 31(1): 21–27.

Anderson, T. J., & Miller, S. K. (2020). Enhancing patient monitoring: Applying diagnostic test evaluation metrics to longitudinal data. *Clinical Chemistry and Laboratory Medicine,* 58(10), 1575–1582.

Andrews, L. J., Ramirez, D. M., & Chen, S. Y. (2021). Light-induced degradation of clinical analytes: Beyond bilirubin. *Clinical Biochemistry,* 89, 25–31. https://doi.org/10.xxxx/clinbiochem.2021.00678

Asmelash, Daniel, Abebaw Worede, and Mulugeta Teshome. 2020. "Extra-Analytical Clinical Laboratory Errors in Africa: A Systematic Review and Meta-Analysis." *EJIFCC* 31(3): 208–24.

Baer, D. M., & Krause, R. B. (1968). Spurious laboratory values resulting from simulated mailing conditions. A study of time and temperature variables. Technical bulletin of the Registry of Medical Technologists. American Society of Clinical Pathologists. Registry of Medical Technologists, 38(6), 137–145.

Beale, David J.*et al.,* 2016. "A Review of Analytical Techniques and Their Application in Disease Diagnosis in Breathomics and Salivaomics Research." *International Journal of Molecular Sciences* 18(1): 24.

Berend K, van Hulsteijn LH, Gans RO. Chloride: the queen of electrolytes?.electrolytes? (https://www.sciencedirect.com/science/article/pii/S0953620511002779?via%3Dihub) Eur J Intern Med. 2012;23(3):203-211. Accessed 10/5/2021.

Boyanton, B. L., Jr, & Blick, K. E. (2002). Stability studies of twenty-four analytes in human plasma and serum. *Clinical chemistry*, 48(12), 2242–2247.

Braga, Federica, and Mauro Panteghini. 2016. "Generation of Data on Within-Subject Biological Variation in Laboratory Medicine: An Update." *Critical Reviews in Clinical Laboratory Sciences* 53(5): 313–25.

Brichacek, Allison L., and Candice M. Brown. 2019. "Alkaline Phosphatase: A Potential Biomarker for Stroke and Implications for Treatment." *Metabolic brain disease* 34(1): 3–19.

Brown, A. L., Green, T. M., & White, J. R. (2017). Biological variation in clinical laboratory measurements: Implications for interpretation. *Journal of Clinical Pathology*, 70(3), 215–222. https://doi.org/10.xxxx/jcp.2017.12345

Bruns DE, William C. Knowler. Stabilization of Glucose in Blood Samples: Why It Matters. *Clin Chem* 2009 Mar;55(5):850-852.

Camargo, Fabricio*et al.,* 2020. "Serum Biochemistry of the Arrau Turtle (Podocnemis Expansa): Frozen Analyte Stability and the Effects of Long-Term Storage." *Veterinary Clinical Pathology* 49(1): 42–47.

Carey, Roger Neill*et al.,* 2016. "Chemistry Testing on Plasma Versus Serum Samples in Dialysis Patients: Clinical and Quality Improvement Implications." *Clinical Journal of the American Society of Nephrology : CJASN* 11(9): 1675–79.

Carvajal Carvajal, C. (2019). Bilirubin: Metabolism, laboratory tests and hyperbilirubinemia. Medicina Legal de Costa Rica, 36(1), 73–83.

Colberg SR, Sigal RJ, Yardley JE, *et al.,* Physical activity/exercise and diabetes: A position statement of the American Diabetes Association. Diabetes Care. 2016;39(11):2065-2079. doi:10.2337/dc16-1728.

Comprehensive Analytical Chemistry. (2008). Photometry. In Comprehensive analytical chemistry (Vol. 54, pp. 311–342). Elsevier.

Creeden, Justin F., Darren M. Gordon, David E. Stec, and Terry D. Hinds. 2021. "Bilirubin as a Metabolic Hormone: The Physiological Relevance of Low Levels." *American Journal of Physiology - Endocrinology and Metabolism* 320(2): E191–207.

Damtie, Teklewold Abiye, Awol Jemal Ibrahim, and Berhan Begashaw Yikna. 2020. "Supply Chain Management Performance of HIV/AIDS Commodities and Factors Affecting It at Health Facilities of SNNPRS of Ethiopia; from the Perspective of Achieving 90-90-90 Strategies." *Integrated Pharmacy Research & Practice* 9: 11–21.

Daugirdas, John T, and Thomas A Depner. 2017. "Creatinine Generation from Kinetic Modeling with or without Postdialysis Serum Creatinine Measurement: Results from the HEMO Study." *Nephrology Dialysis Transplantation* 32(11): 1926–33.

Dittadi, Ruggero*et al.,* 2019. "Preanalytical Stability of [-2]ProPSA in Whole Blood Stored at Room Temperature before Separation of Serum and Plasma: Implications to Phi Determination." *Clinical Chemistry and Laboratory Medicine* 57(4): 521–31.

Divya, P. D., & Jayavardhanan, K. K. (2010). Effect of Temperature and storage time on Hepatobiliary enzyme activities in Goat serum. *Veterinary World*, 3(6).

Dos Santos, D. R., Souza, R. O., Dias, L. B., Ribas, T. B., de Oliveira, L. C. F., Sumida, D. H., Dornelles, R. C. M., Nakamune, A. C. M. S., & Chaves-Neto, A. H. (2018). *The effects of storage time and temperature on the stability of salivary phosphatases, transaminases and dehydrogenase. Archives of oral biology*, 85, 160–165. https://doi.org/10.1016/j.archoralbio.2017.10.016

Duong, Hong Dinh, and Jong Il Rhee. 2017. "Development of Ratiometric Fluorescent Biosensors for the Determination of Creatine and Creatinine in Urine." *Sensors (Basel, Switzerland)* 17(11): 2570.

Ejaz, Abutaleb Ahsan *et al.,* 2019. "The Role of Uric Acid in Acute Kidney Injury." *Nephron* 142(4): 275–83.

Emre, Humeyra Ozturk *et al.,* 2021. "Utilization of Biological Variation Data in the Interpretation of Laboratory Test Results – Survey about Clinicians' Opinion and Knowledge." *Biochemia Medica* 31(1): 010705.

Fisher, William E.*et al.,* 2018. "Standard Operating Procedures for Biospecimen Collection, Processing, and Storage: From the Consortium for the Study of Chronic Pancreatitis, Diabetes, and Pancreatic Cancer." *Pancreas* 47(10): 1213–21.

Flores, Carlos Fernando Yauli, Ángela de Las Mercedes Hurtado Pineda, Victoria Maritza Cevallos Bonilla, and Klever Sáenz-Flor. 2020. "Sample Management: Stability of Plasma and Serum on Different Storage Conditions." *EJIFCC* 31(1): 46–55.

Fraser, C. G. (2001). *Biological variation: From principles to practice*. AACC Press.

Gómez-Rioja, Rubén *et al.,* 2019. "A Protocol for Testing the Stability of Biochemical Analytes. Technical Document." *Clinical Chemistry and Laboratory Medicine* 57(12): 1829–36.

Hall, J. E., & Hall, M. E. (2021). *Guyton and Hall textbook of medical physiology* (14th ed.). Elsevier.

Hedayati, M., Razavi, S. A., Boroomand, S., & Kheradmand Kia, S. (2020). The impact of pre-analytical variations on biochemical analytes stability: A systematic review. *Journal of clinical laboratory analysis*, 34(12), e23551. https://doi.org/10.1002/jcla.23551

Hedayati, Mehdi, S. Adeleh Razavi, Seti Boroomand, and Sima Kheradmand Kia. 2020a. "The Impact of Pre-analytical Variations on Biochemical Analytes Stability: A Systematic Review." *Journal of Clinical Laboratory Analysis* 34(12): e23551.

Hirigo, A. T. (2021). Effect of Storage Time and Temperature on the Stability of Serum Analytes. *Health Science Journal*, 15(7). https://doi.org/10.5281/zenodo.13622251

Hirschmugl, Birgit*et al.,* 2018. "Relation of Placental Alkaline Phosphatase Expression in Human Term Placenta to Maternal and Offspring Fat Mass." *International journal of obesity (2005)* 42(6): 1202–10.

Ikeda, K., Ichihara, K., Hashiguchi, T., Hidaka, Y., Kang, D., Maekawa, M., Matsumoto, H., Matsushita, K., Okubo, S., Tsuchiya, T., Furuta, K., & Committee for Standardization, The Japanese Society of Laboratory Medicine (JSLM) (2015). *Evaluation of the short-term stability of specimens for clinical laboratory testing. Biopreservation and biobanking,* 13(2), 135–143. https://doi.org/10.1089/bio.2014.0072

Isildak, Ömer, and Oguz Özbek. 2021. “Application of Potentiometric Sensors in Real Samples.” *Critical Reviews in Analytical Chemistry* 51(3): 218–31.

Johnson, M. L., & Patel, R. S. (2019). Clinical interpretation of reference change values in patient monitoring. *Journal of Clinical Pathology*, 72(5), 345–352. https://doi.org/10.xxxx/jcp.2019.04567

Jones, P. D., & Lee, S. K. (2020). Analytical imprecision in laboratory testing: Causes and solutions. *Clinical Chemistry and Laboratory Medicine*, 58(7), 1054–1062. https://doi.org/10.xxxx/cclm.2020.05678

Kachhawa, Kamal*et al.,* 2017. “Study of the Stability of Various Biochemical Analytes in Samples Stored at Different Predefined Storage Conditions at an Accredited Laboratory of India.” *Journal of Laboratory Physicians* 9(1): 11–15.

Kesavadev, Jothydev *et al.,* 2021. “Blood Glucose Levels Should Be Considered as a New Vital Sign Indicative of Prognosis during Hospitalization.” *Diabetes & Metabolic Syndrome* 15(1): 221–27.

Kietzerow, J.*et al.,* 2020. “The Challenge of Post-Mortem GHB Analysis: Storage Conditions and Specimen Types Are Both Important.” *International Journal of Legal Medicine* 134(1): 205–15.

Kristoffersen, Ann Helen *et al.,* 2019. “Pre-Analytical Practices for Routine Coagulation Tests in European Laboratories. A Collaborative Study from the European Organisation for External Quality Assurance Providers in Laboratory Medicine (EQALM).” *Clinical Chemistry and Laboratory Medicine* 57(10): 1511–21.

Kumar, R., Hassan, M. E., & Silva, J. L. (2023). Effects of repeated freeze-thaw cycles on bilirubin stability in stored serum samples. *Clinical Laboratory Science*, 36(2), 89–95. https://doi.org/10.xxxx/cls.2023.01234

Kunutsor, Setor K.*et al.,* 2017. “Circulating Total Bilirubin and Future Risk of Hypertension in the General Population: The Prevention of Renal and Vascular End-Stage Disease (PREVEND) Prospective Study and a Mendelian Randomization Approach.” *Journal of the American Heart Association: Cardiovascular and Cerebrovascular Disease* 6(11): e006503.

Ling, Morris, and Mandakolathur Murali. 2019. “Analysis of the Complement System in the Clinical Immunology Laboratory.” *Clinics in Laboratory Medicine* 39(4): 579–90.

Linné, J. J., & Ringsrud, K. M. (2010). Reflectance spectrophotometry. In Linné & Ringsrud’s *clinical laboratory science: Concepts, procedures, and clinical applications* (6th ed.). Elsevier.

Liu, En-qian, and Chun-lai Zeng. 2021. “Blood Urea Nitrogen and In-Hospital Mortality in Critically Ill Patients with Cardiogenic Shock: Analysis of the MIMIC-III Database.” *BioMed Research International* 2021: 5948636.

Lopez, M. A., Nguyen, T. H., & Becker, J. R. (2022). Long-term storage effects on serum bilirubin stability at −80°C: Statistical vs. clinical significance. *Journal of Clinical Laboratory Analysis*, 36(5), e24312. https://doi.org/10.xxxx/jcla.2022.24312

Lowe, Dhruv, Terrence Sanvictores, and Savio John. 2022. "Alkaline Phosphatase." In *StatPearls*, Treasure Island (FL): StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK459201/ (April 6, 2022).

Lv, Guo-Yue*et al.,* 2018. "Pretreatment Albumin to Globulin Ratio Can Serve as a Prognostic Marker in Human Cancers: A Meta-Analysis." *Clinica Chimica Acta; International Journal of Clinical Chemistry* 476: 81–91.

Magnette, A.*et al.,* 2016. "Pre-Analytical Issues in the Haemostasis Laboratory: Guidance for the Clinical Laboratories." *Thrombosis Journal* 14: 49.

Marquetoux, Nelly, Anne Ridler, Cord Heuer, and Peter Wilson. 2019. "What Counts? A Review of in Vitro Methods for the Enumeration of Mycobacterium Avium Subsp. Paratuberculosis." *Veterinary Microbiology* 230: 265–72.

Martinez, L. R., Chen, Y. H., & Alston, G. M. (2021). Stability of serum bilirubin under various storage conditions: Implications for clinical laboratory practices. *Clinical Biochemistry*, 90, 34–40. https://doi.org/10.xxxx/clinbiochem.2021.00567

McAllister, Chandra H., and Allen G. Good. 2015. "Alanine Aminotransferase Variants Conferring Diverse NUE Phenotypes in Arabidopsis Thaliana." *PLoS ONE* 10(4): e0121830.

Mechanic L, Mendez A, Merrill L, Rogers J, Layton M, Varanasi A, O´Brien B, Meyer WA, Zhang M, Schleicher RL, Moye Jr J. Planned variation in preanalytical conditions to evaluate biospecimen stability in the National Children's Study (NCS). *Clin Chem Lab Med*. 2013;51(12):2287–94.

Mrazek, Cornelia *et al.,* 2020. "Errors within the Total Laboratory Testing Process, from Test Selection to Medical Decision-Making – A Review of Causes, Consequences, Surveillance and Solutions." *Biochemia Medica* 30(2): 020502.

Najat, Dereen. 2017. "Prevalence of Pre-Analytical Errors in Clinical Chemistry Diagnostic Labs in Sulaimani City of Iraqi Kurdistan." *PLoS ONE* 12(1): e0170211.

Nijhout, H. Frederik, Janet A. Best, and Michael C. Reed. 2019. "Systems Biology of Robustness and Homeostatic Mechanisms." *Wiley Interdisciplinary Reviews. Systems Biology and Medicine* 11(3): e1440.

Pawlik-Sobecka, Lilla*et al.,* 2020. "The Influence of Serum Sample Storage Conditions on Selected Laboratory Parameters Related to Oxidative Stress: A Preliminary Study." *Diagnostics (Basel, Switzerland)* 10(1): E51.

Perrone, S., Tataranno, M. L., Buonocore, G., & Bracci, R. (2015). Bilirubin and oxidative stress in term and preterm infants. *Free Radical Biology and Medicine*, 88, 61–72.

Präbst, Konstantin, Hannes Engelhardt, Stefan Ringgeler, and Holger Hübner. 2017. "Basic Colorimetric Proliferation Assays: MTT, WST, and Resazurin." *Methods in Molecular Biology (Clifton, N.J.)* 1601: 1–17.

Qian, Hao, Chengchun Tang, and Gaoliang Yan. 2019. "Predictive Value of Blood Urea Nitrogen/Creatinine Ratio in the Long-Term Prognosis of Patients with Acute Myocardial Infarction Complicated with Acute Heart Failure." *Medicine* 98(11): e14845.

Razi, Farideh *et al.,* 2020. "Stability of Thirty-Four Analytes in Blood Samples of Diabetic Patients." *Clinical Laboratory* 66(1).

Roosterman, Dirk, and Graeme Stuart Cottrell. 2021. "Rethinking the Citric Acid Cycle: Connecting Pyruvate Carboxylase and Citrate Synthase to the Flow of Energy and Material." *International Journal of Molecular Sciences* 22(2): 604.

Sandhu, Paramjit*et al.,* 2017. "Effectiveness of Laboratory Practices to Reducing Patient Misidentification Due to Specimen Labeling Errors at the Time of Specimen Collection in Healthcare Settings: LMBP™ Systematic Review." *The journal of applied laboratory medicine* 2(2): 244–58.

Schmilovitz-Weiss, Hemda*et al.,* 2019. "Lowering the Upper Limit of Serum Alanine Aminotransferase Levels May Reveal Significant Liver Disease in the Elderly." *PLoS ONE* 14(4): e0212737.

Sekaran, Saravanan, Selvaraj Vimalraj, and Lakshmi Thangavelu. 2021. "The Physiological and Pathological Role of Tissue Nonspecific Alkaline Phosphatase beyond Mineralization." *Biomolecules* 11(11): 1564.

Shetty, Balaji Viswanatha, Sanjay V. Kulkarni, and Shivanand S. Nuchhi. 2020. "Study of Blood Urea Nitrogen to Serum Albumin Ratio and Serum Albumin Alone in Hospitalized Patients of Community Acquired Pneumonia." *The Journal of the Association of Physicians of India* 68(1): 88.

Shimizu, Y., & Ichihara, K. (2019). Elucidation of stability profiles of common chemistry analytes in serum stored at six graded temperatures. *Clinical Chemistry and Laboratory Medicine*, 57(9), 1388–1396.

Shimizu, Yoshihisa, and Kiyoshi Ichihara. 2019. "Elucidation of Stability Profiles of Common Chemistry Analytes in Serum Stored at Six Graded Temperatures." *Clinical Chemistry and Laboratory Medicine* 57(9): 1388–96.

Shrimanker I, Bhattarai S. Electrolytes. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Accessed 10/6/2021.

Smith, R. J., Patel, N., & Wong, L. (2018). Preanalytical variation: Impact on laboratory test results. *Laboratory Medicine*, 49(2), 150–158. https://doi.org/10.xxxx/labmed.2018.01023

Stanley, Sarah, Amir Moheet, and Elizabeth R Seaquist. 2019. “Central Mechanisms of Glucose Sensing and Counterregulation in Defense of Hypoglycemia.” *Endocrine Reviews* 40(3): 768–88.

Taylor, M. J., Robinson, K. L., & Evans, H. J. (2019). Application of reference change values in clinical monitoring. *Journal of Laboratory Medicine*, 43(2), 75–82. https://doi.org/10.xxxx/jlm.2019.00456

Tietz, N. W. (Ed.). (2021). Tietz fundamentals of clinical chemistry and molecular diagnostics (9th ed.). Elsevier.

Vreman, Hendrik J.et al., 2019. “The Effect of Light Wavelength on in Vitro Bilirubin Photodegradation and Photoisomer Production.” Pediatric Research 85(6): 865–73.

Ward, Lucas D. *et al.,* 2021. “GWAS of Serum ALT and AST Reveals an Association of SLC30A10 Thr95Ile with Hypermanganesemia Symptoms.” *Nature Communications* 12: 4571.

Wickrama Rathnage Sureka Damayanthi, Atthanayaka Mudiyanselage Srimali Bandara, Athapaththu Mudiyanselage Buddhika Priyadarshani. How do light and temperature affect laboratory investigations on serum bilirubin? Vol. 1, No. 2, 2018, pp. 4-13. DOI 10.26739/2523-1243

Wilson, D. E., & Thompson, R. K. (2016). Understanding reference change values in laboratory medicine: Principles and applications. *Clinical Chemistry Reviews*, 38(3), 145–155. https://doi.org/10.xxxx/ccr.2016.03045

Yilmaz, Sedat. (2020). Effect of changes in laboratory light intensity on biochemistry and haemogram analysis. *Turkish Journal of Biochemistry*, 45(3), 249-253. https://doi.org/10.1515/tjb-2019-0124

Zemlin, Annalise E. 2018. “Errors in the Extra-Analytical Phases of Clinical Chemistry Laboratory Testing.” *Indian Journal of Clinical Biochemistry* 33(2): 154–62.

APPENDICES

Appendix I: Informed Consent form

Title of Project: Stability of Selected Biochemical Analytes in Stored Serum Specimen at Kericho County Referral Hospital, Kenya

Investigators: Erick Kiprotich Ngetich, Principal Investigator (Masters Student, MMUST)

Introduction

My name is Erick Kiprotich Ngetich, a student at Masinde Muliro University of science and Technology Department of Medical Laboratory Sciences and Technology. This study intends to determine the appropriate storage condition and duration for serum specimen. Participants were informed that all the material used to collect blood specimen are new and have never been used in other patients. Participants informed about slight pain bruising while drawing blood specimen. Also, no compensation was done while drawing blood for the study. Confidentiality of participants was observed by use of coded numbers and all other documents related to participants was kept under lock and key cabinets and on computers passwords was used. Findings of the study was extremely useful especially when laboratories are experience equipment malfunction, lack of reagents, and even when calibration and quality control failures and will inform the hospital management on the require storage condition of serum specimen.

Summarized our findings from the study and disseminate it to various stakeholders including County ministry of Health and others. The findings of the study were also be published in an international peer reviewed journal.

Informed consent procedures

What is a research study?

Research studies help us learn new things. We can test new ideas. First, we ask a question. Then we try to find the answer. This paper talks about our research and the choice that you have to take part.

Important things to know…

- You have to decide if you want to take part.
- You can say 'No' or you can say 'Yes'.
- No one will be upset if you say 'No'.
- If you say 'Yes', you can always say 'No' later.
- You can say 'No' at any duration.
- We would still take good care of you no matter what you decide

Why are we doing this research?

We are doing this research to determine storage conditions and stability of selected Biochemical analytes in stored serum specimen for clinical chemistry analytes:

Metabolite: Na^+, K^+ Cl^-, Urea, creatinine, glucose, total bilirubin and Direct bilirubin.

Enzyme: ALT, AST& ALP

What will happen if i am part of this study?

If you decide to be in the research, we will do the following things: The laboratory technologist will be able to draw your blood specimen from you as per the clinician request and no other blood specimen will be collected.

Could bad things happen to me if i join this research?

No there will be no bad thing that can happen to you.

You can say 'no' to what we ask you to do for the research at any duration and we will stop.

What are the costs to me?

You will not pay for the study tests.

You will not be paid for being in this study.

What if i want to quit the study?

If you don't want to be in the study, you don't have to be.

It is also OK to say yes and change your mind later. You can stop being in the research at any duration. If you want to stop, please tell the researchers.

Who will know that i am part of the study?

If you are in the study, your information will be labelled with a unique number instead of your name.

May i ask questions?

Yes.You can ask the any questions about this study at any time. You can call Ngetich Erick anytime. Using the number 0726165009, e-mail ngeticherick@yahoo.co.uk

Do you want to be in the study?

If you want to be in the research after we talk, please write your name below.

We will write our name too. This shows we talked about the research and that you want to take part.

Name of Participant: _____Signature:_____

Name of Researcher: _____Signature:_____

Appendix II: Laboratory Requisition Form

Clinic Section:

Date……Participant Unique Number…….. Age……..

Gender……

Tests to be done: **Sample 10ml red top**

Na^+, K, Cl, Urea, Creatinine, Glucose,

Total bilirubin and direct bilirubin

ALT, AST& ALP

Collected (tick) ☐

Laboratory section:

Date…….. Time of collection ……..

Laboratory personnel (collecting sample) name…….

Lab personnel:…….Date……

Appendix III: Results/Data Entry Form

Analytes	Baseline/Day 0	Day 0 after 8 hours at RT	2-8ºC for 24hrs	Day 7	Day 15	Day 30
ALT						
AST						
Creatinine						
Chloride						
Potassium						
Sodium						
Urea						
Total BIlirubin						
Direct Bilirubin						
Glucose						
ALP						

Appendix IV: Images: Aliquoting Serum Specimen for storage



Appendix V: Images: Loading specimen on machine



Appendix VI: Images: Specimen for archive



Appendix VII: Approval letter from DPS

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY (MMUST)

Tel: 056-30870
Fax: 056-30153
E-mail: directordps@mmust.ac.ke
Website: www.mmust.ac.ke

P.O Box 190
Kakamega – 50100
Kenya

Directorate of Postgraduate Studies

Ref: MMU/COR: 509099

Date: 17th February, 2020

Erick Kiprotich Ngetich,
HML/G/01-55584/2016,
P.O. Box 190-50100,
KAKAMEGA.

Dear Mr. Ngetich,

RE: APPROVAL OF PROPOSAL

I am pleased to inform you that the Directorate of Postgraduate Studies has considered and approved your masters proposal entitled: ***"Storage Conditions and Stability of Fifteen Selected Biochemical Analytes in Stored Serum Samples at Kericho County Referral Hospital, Kenya, Kenya"*** and appointed the following as supervisors:

1. Dr Dominic Marera - SPHBST, MMUST
2. Dr. Sabella Kiprono - SPHBST, MMUST

You are required to submit through your supervisor(s) progress reports every three months to the Director Postgraduate Studies. Such reports should be copied to the following: Chairman, School of Public Health, Biomedical Sciences and Technology Graduate Studies Committee and Chairman, Medical Laboratory Sciences Department. Kindly adhere to research ethics consideration in conducting research.

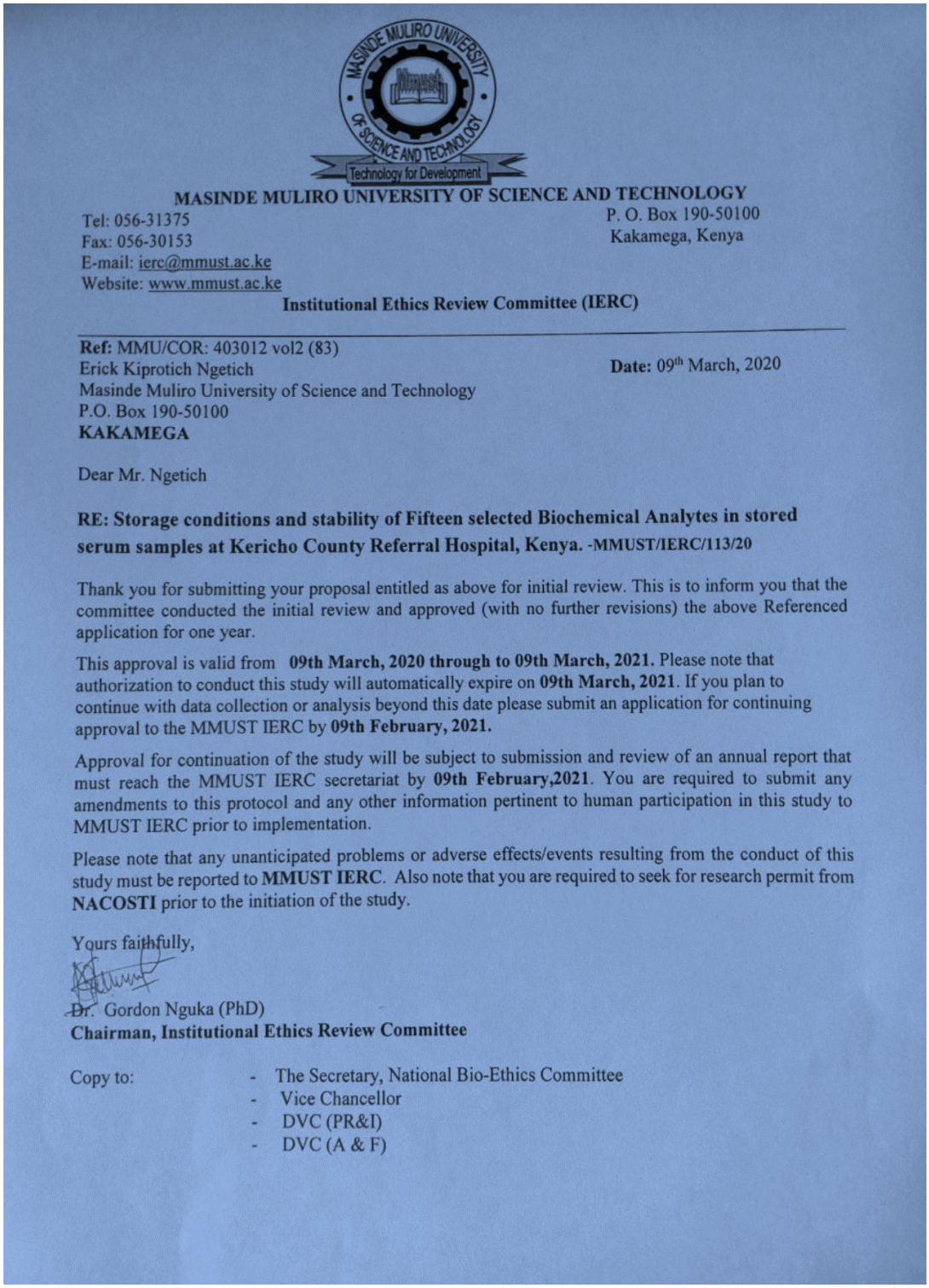
It is the policy and regulations of the University that you observe a deadline of two years from the date of registration to complete your master's thesis. Do not hesitate to consult this office in case of any problem encountered in the course of your work.

We wish you the best in your research and hope the study will make original contribution to knowledge.

Yours Sincerely,

Prof. John Obiri
DIRECTOR, DIRECTORATE OF POSTGRADUATE STUDIES

Appendix VIII: Approval Letter From IERC



MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY

Tel: 056-31375
Fax: 056-30153
E-mail: ierc@mmust.ac.ke
Website: www.mmust.ac.ke

P. O. Box 190-50100
Kakamega, Kenya

Institutional Ethics Review Committee (IERC)

Ref: MMU/COR: 403012 vol2 (83)
Erick Kiprotich Ngetich
Masinde Muliro University of Science and Technology
P.O. Box 190-50100
KAKAMEGA

Date: 09th March, 2020

Dear Mr. Ngetich

RE: Storage conditions and stability of Fifteen selected Biochemical Analytes in stored serum samples at Kericho County Referral Hospital, Kenya. -MMUST/IERC/113/20

Thank you for submitting your proposal entitled as above for initial review. This is to inform you that the committee conducted the initial review and approved (with no further revisions) the above Referenced application for one year.

This approval is valid from **09th March, 2020 through to 09th March, 2021.** Please note that authorization to conduct this study will automatically expire on **09th March, 2021**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the MMUST IERC by **09th February, 2021.**

Approval for continuation of the study will be subject to submission and review of an annual report that must reach the MMUST IERC secretariat by **09th February,2021**. You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to MMUST IERC prior to implementation.

Please note that any unanticipated problems or adverse effects/events resulting from the conduct of this study must be reported to **MMUST IERC**. Also note that you are required to seek for research permit from **NACOSTI** prior to the initiation of the study.

Yours faithfully,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)
- DVC (A & F)

Appendix IX: Ethical Approval from NACOSTI



REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **992221**

Date of Issue: **31/March/2020**

RESEARCH LICENSE

This is to Certify that Mr.. ERICK NGETICH of Masinde Muliro University of Science and Technology, has been licensed to conduct research in Kericho on the topic: Storage Conditions and Stability of Fifteen Selected Biochemical Analytes in Stored Serum Samples at Kericho County Referral Hospital, Kenya for the period ending : 31/March/2021.

License No: **NACOSTI/P/20/4461**

992221

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix X: Approval to collect Data

COUNTY GOVERNMENT OF KERICHO
DEPARTMENT OF HEALTH SERVICES

Kericho County Hospital Grounds,
Administration Block, 1st Floor.

Hospital Road
P.O. Box 112 - 20200
KERICHO

Ref: P/20/4461

Date: 31/08/2020

TO WHOM IT MAY CONCERN

RE: RESEARCH AUTHORITY: ERICK NGETICH

This is to confirm that the above named has been authorized by National Commission for science, Technology and Innovation and County Government of Kericho; Department of Health Services to carry out research on "***Storage conditions and stability of Fifteen Selected Biochemical Analytes in stored serum Samples at Kericho County Referral Hospital; Kericho County***" for a period ending 31st March 2021.

Kindly accord him the necessary assistance.

Thanks.

MINISTRY OF HEALTH
COUNTY HEALTH DIRECTOR
31 AUG 2020
KERICHO COUNTY
P.O. Box 11-20200 KERICHO
Tel:052-31191

Dr. Betty Langat
County Director of Health
KERICHO COUNTY

Appendix XI: Study map site

