EVALUATION OF CELLULAR CHANGES, BIOCHEMICAL CHANGES AND BACTERIAL CONTAMINATION IN BLOOD STORED FOR TRANSFUSION AT BUNGOMA COUNTY REFERRAL HOSPITAL

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DEDICATION

I dedicate this work to my beloved wife, Roselyne Aswan Wawire together with my entire family for their tireless support to see me up to this stage. God bless you.

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ABSTRACT

During the storage of transfusion blood, it may undergo a series of cellular and biochemical changes. Bacterial contamination may also occur, which together pose a risk of using prolonged stored blood. This study assessed the cellular changes, biochemical changes and bacterial contamination in whole blood stored for transfusion at Bungoma county referral hospital, Kenya. The study employed prospective design involving 20 randomly selected blood pints in citrate phosphate dextrose adenine (CPDA-1) anticoagulant that were assessed for 35 days. The cellular changes were tested using the Celtac F, MEK-8222 K analyzer. Potassium and sodium levels were tested using Humalyte plus^{5} analyzer while the pH level was estimated using Hanna pH meter. Bacterial contamination was established using Bactec instrument, culture and biochemical tests. Statistical Analysis of variance (ANOVA) was employed and was executed using statistical package for social sciences (SPSS V.23). Results were considered significant at $P \leq 0.05$. After 35 days blood storage at 2-6°C, White blood cells (WBC), Red Blood Cells (RBC), platelets counts and Mean Cell Haemoglobin Concentration (MCHC) decreased significantly *(P*= <0.0001, 0.0182, <0.0001, 0.0023). The Mean Cell Volume (MCV) and haematocrit (HCT) increased significantly (*P= <0.0001, 0.0003)* while haemoglobin (HGB) level had insignificant variance *(P=0.4185).* Potassium significantly increased while Sodium and pH levels significantly decreased *(P <0.0001, <0.0001).* Bacterial contamination had insignificant variance (*P*=0.3335). Findings from this study shed light on the need to carefully monitor the cellular change, biochemical changes and bacterial contamination during blood storage to secure the safety of transfused blood.In conclusion, Platelets, WBC and RBC counts and its indices are significantly altered in stored blood especially when stored over two weeks. Potassium significantly increases while Sodium and pH levels significantly decrease. Bacterial contamination insignificantly changes but *Staphylococcus aureus* is a possible contaminant of stored blood. The study, therefore, recommends the use of fresh blood and bacterial surveillance during blood storage to avoid the adverse outcome of cellular changes, biochemical changes and bacterial contamination.

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LIST OF ABBREVIATIONS

fL- Femtolitre

g- Gram

µg- Microgram

HCT- Haematocrit

HGB-Haemoglobin

Ho_1-Hypothesis

L- Litre

µL- Microlitre

mL- Millilitre

Pg- Picogram

PLT- Platelet

LIST OF ACRONYMS

ACD-Acid Citrate Dextrose

ANOVA- Analysis of Variance

ATCC-American Type Culture Collection

ATP- Adenosine triphosphate

BD- Becton Dickinson

BCRH- Bungoma County Referral Hospital

CFU- Colony Forming Unit

CDPA-1- Citrate Phosphate Dextrose-Adenine 1

DPG-DiphospoGlycerate

ECF- Extracellular Fluid

ERC- Ethical Review Committee

HIV- Human Immunodeficiency Virus

HBV- Hepatitis B virus

HCV- Hepatitis C virus

HTLV-Human T- lymphotrophic virus

ICF- Intracellular Fluid

JOOTRH- Jaramogi Oginga Odinga Teaching and Referral Hospital

LDH- Lactate Dehydrogenase

MCHC- Mean Cell Haemoglobin Concentration

MCV- Mean Cell Volume

NACOSTI- National Commission for Science, Technology& Innovation

NADH- Nicotinamide adenine dinucleotide (NAD) + hydrogen (H)

NADPH- Nicotinamide adenine dinucleotide phosphate

NIATRs- Non- infectious adverse transfusion reactions

NO- Nitric acid

PLA- Platelet Leucocyte Aggregate

RBC- Red Blood Cell

SARS- Severe Acute Respiratory Syndrome

SE- Standard Error

SEM- Standard Error Mean

SPSS- Statistical Package for Social Sciences

USA- United States of America

WBC- White Blood Cell

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background to the study

Blood transfusion is a life-saving process performed in the time of the care of scores of severely sick patients to substitute deficiency in haemoglobin to uphold oxygen transport to fundamental organs (Klein, Spahn, & Carson, 2007). Annually, about 117.4 million blood pints are collected globally (WHO, 2015). In numerous instances, patients are transfused in desperate conditions to rescue lives and the availability of prepared stored blood pints is cardinal. As a result of these, blood transfusion organizations ratified regulations that guarantee the preparing and storage of whole blood and blood products in advance of any medical crisis in need of a transfusion. Blood to be transfused is kept for up to 35 days at 2-6°C in preservatives such as citrate phosphate dextrose adenine (CPDA) (Wang, Sun, Solomon, Klein, & Natanson, 2012); (Oyet, Okongo, Onyuthi, & Muwanguzi, 2018).

An adverse reaction is an unpleasant outcome or consequence in a victim, temporarily connected with the dispensing of blood or blood component (Sahu, Hemlata, 2014). Presently, even in first world countries, the notable danger to the patient rests in non-infectious problems of transfusions that are responsible for notable morbidity and mortality (Sahu, Hemlata, 2014). The non-infectious unfavourable incidents linked to blood transfusions are interpreted as non-infectious adverse transfusion reactions (NIATRs).

During storage, blood experience a sequence of cellular and biochemical changes that may reduce its lifespan and purpose, and the most affected is whole blood, (Yalcin, Ortiz, Tsai, Johnson, & Cabrales, 2014); (Rafael Obrador, Sarah Musulin, 2014). These changes referred to unanimously

as red blood cell storage lesions are accelerating episodes that have an effect on blood and blood products stored for an extended duration than those stored for a short duration (Lagerberg, Korsten, Van Der Meer, 2017). These changes include raised lactate levels with decreased pH, increased cell disintegration, raised potassium concentration, decreased 2,3-diphosphoglycerate and adenosine triphosphate (ATP) concentration because of the continuous glycolysis that takes place when blood is reserved in a blood bag that occasions the production of adenosine deaminase leading to the disintegration of adenosine (Adams, Bellairs, Bird, 2015). An elevation in protons triggers the pH to drop and in due course modifies glycolytic metabolism. The drop in pH triggers the 2, 3-diphosphoglycerate amounts to reduce with a concurrent rise in adeno-triphosphate (ATP) creation. Glycolysis is retarded and, as acid piles up, the amounts of ATP drop, and the structure of the red blood cell is slowly modified from spherical to echinocytic forms (Orlov, 2015). Red blood cell storage lesion, hence, decreases the potency of the transfused blood and blood products by rising the speed of elimination of the transfused RBCs by macrophages and immunomodulation (Oyet et al., 2018). This may be linked to studies that transfusing older, stored blood might be correlated with raised fatality; severe infections, multiple organ breakdown, thrombosis, and lengthened hospital stay (Hod & Spitalnik, 2011).

It is thought-provoking whereby the prolonged storage and existence of White Blood Cells can manipulate the membrane surface of Red Blood Cells and then their functions. Comprehending the attributes and design of cellular and functional changes in stored blood might give approaches to advance the correspondence of value and danger of Red Blood Cell (RBC) transfusion (Bennett-Guerrero et al., 2007).

The word "storage lesion" has been used to portray the revolutionary degeneration of the RBC framework and activity which occur in the time of the routine red cell storage (Kim-Shapiro, Lee,

2011). It is convenient, despite, to also consider the accumulation of bio-reactive materials that take place in the time of storage beneath the umbrella of the storage lesion, as these materials might not be safe to the recipient when transfused. Subsequently, within fourteen days of reservation, by-products of glycolytic metabolism, lactic acid, and proteins accrue (Adams, Bellairs, Bird, 2015). These by-products, which *in vivo* are readily evacuated from the bloodstream, remain and give rise to structural and functional changes (Shevkoplyas, 2010). As the storage period lengthens to over 14 days, the RBCs become a little elastic and therefore not able to cross tiny receptacles of the microcirculation, conclusively leading to diminished oxygen transport since the aerated red cells can't cross the end-organ capillary beds (Adams, Bellairs, Bird, 2015).

The adjustment in architecture from basic biconcave rings to echinocytic red blood cells also, makes the cells easier to clump, increasing the possibility of blocking the microcirculation, leading to tissue ischemia (Diez-Silva, Dao, Han, Lim, 2010). Complications in recipients are brought about by the emission of the haemoglobin from transfused erythrocytes experiencing hemolysis (disintegration) in the circulation. Hemolysis is known to transpire in erythrocytes stored for extended durations when chemical changes that give rise to RBC “storage lesions” make alterations to the membranes of donated red cells that are stored for an extended duration (Shevkoplyas, 2010).

The problems occasioned by unbound haemoglobin comprise critical and persistent vascular disease, inflammatory condition, seizure, and renal failure (Schaer, Buehler, Alayash, Belcher, 2013). These physical changes may have major repercussions in recipients who are already critically sick and be given enormous transfusions of blood stored for the highest extent (Adams, Bellairs, Bird, 2015). Long stored blood for transfusion has a large potassium level due to haemolysis (Orlov, 2015). In patients coupled with a renal breakdown, the transfusion of the

aforementioned blood can lead to a demanding rise in plasma potassium levels (Knichwitz, Zahl, Van Aken, Semjonow, & Booke, 2002). There is increased testimony that the potassium shifts are responsible for the complications realized in transfused patients (Burger et al., 2013). Electrolyte disturbances may be associated with various circumstances comprising drug usage however the kidney is anticipated to control it (Opoku-Okrah, Acquah, 2015). Elevated potassium concentrations have been observed as a complication for many admitted patients with hyperkalaemia being suggested for problems caused by multiple blood transfusions (Christos Rizos, Haralampos Milionis, 2017). Hyperkalemia is a regular clinical condition that can cause life-threatening cardiac arrhythmias (Parham, Mehdirad, 2006). It has been noted that after transfusion of stored blood, disadvantages such as raised potassium concentration, hypernatremia, and citrate poisoning among other circumstances do take place (Opoku-Okrah, Acquah, 2015). The precipitate effect of transfusion of stored blood on the potassium and acid-base balance on the patient is extremely complex however, it is mainly influenced by the rate of transfusion, amount of blood transfused, the speed of citrate metabolism, and the varying condition of the peripheral perfusion of the patient (Murthy, Waiker, Neelakanthan, 1999); (Opoku-Okrah, Acquah, 2015). Negligence to confirm the clear electrolyte changes has been established to be dangerous on some occasions (Hall, Barnes, Miller, Bethencourt, 1993).

Sodium is the prime electrolyte in the extracellular fluid (ECF) with almost 98 percent of its aggregate being in the ECF and only two percent being in the intracellular fluid (ICF). A reduction in sodium amounts occasions cellular inflammation which affects the cerebrospinal nervous system (Opoku-Okrah, Acquah, 2015). The elevation in extracellular Na+ ions prompts intracellular to transfer out of the cells into the extracellular matrix that gives rise to cellular dry up. Cardiac performance is also lowered because of declined myocardial contraction giving rise to

cardiac failure (Opoku-Okrah, Acquah, 2015). Transfusion of stored blood might have a complex effect on some occasions due to reduced-sodium amounts.

Bacterial contamination of blood for transfusion can be explained as the existence of bacteria in the blood or blood products that have been collected, stored, and processed for transfusion (Agzie, Niguse, 2019). Bacterial contamination of blood and blood components is a renowned transfusion danger giving rise to the morbidity and death linked with blood transfusions. The danger of transmission of bacterially contaminated blood components is abundantly higher than the merged danger of routine transmissible viruses (Chavan, Bhat, Ojha, Kelkar, 2012). Attempts have been made to lower the contamination levels of blood for transfusion by bacteria using several practices such as upgraded skin disinfection before donation, continuous donor assessment, and culturing of the donated blood. Regardless of these initiatives having been established, many deaths attributable to septic responses connected with transfusion have been reported (Chelsea Sheppard, Cassandra Josephson, 2005). Understanding the frequency of blood for transfusion Contamination with bacteria and their origin such asymptomatic donor bacteremia, deficient blood bags or blood acquiring equipment, blood donation collection technique, transportation of donated pints, storage of donated pints and samples transfer technique is cardinal for the designing of halting and scaling down a course of action that minimizes death and fatality rate coming about from transfusion of blood and blood products contaminated by bacteria (Opoku-Okrah, Feglo, 2009). Most of the blood and blood product receivers are infants and females; minimizing the fatality rate in this bracket will be by acquiring safe blood (Makuni, Simango, & Rooyen Mavenyengwa, 2015). Surveillance of microbial contamination in the course of blood for transfusion storage is cardinal in assuring blood safety.

Transfusing older, stored blood could be correlated with increased death; severe infections, multiple-organ breakdown, thrombosis, and prolonged hospital span stay (Hod & Spitalnik, 2011). The establishment of blood storing arrangements allows donation and transfusion to be detached in duration. This detachment has allowed the decentralization of blood donation functions with successive savings and advancements in the standardization and accessibility of blood components.

Nonetheless, the accessibility of stored blood for transfusion increases the interrogation of at what extend blood components can and should be reserved and to what extent are they safe and potent (Zimrin & Hess, 2009). Blood and blood components could be exogenously contaminated due to deficient blood bags or blood acquiring apparatus, by contamination of the blood during bleeding or preparation or storage (Agzie, Niguse, 2019). Donated blood can also be endogenously contaminated as occasioned by symptomless sepsis in the donor or as a consequence of incompetent cleansing of the donor venepuncture area (Liumbruno, Catalano, Piccinini, 2009).

1.2 Statement of the problem

Elongated blood storage increases mortality, serious infections, and multi-organ failure after transfusion; however, the causes of these remain unknown (Chelsea, Sheppard, Cassandra Josephson, 2005). Many deaths attributable to septicemia reactions connected with transfusion have transpired and been outlined however the exact "residual risk" of microbial contamination is unknown (Chelsea Sheppard, Cassandra Josephson, 2005). Potassium leakage, a notable episode of extended erythrocyte storage, initiates vesicle development and may have a significant influence on the after-transfusion work and after effects of stored Red Blood Cells (Burger et al., 2013). According to records in Bungoma County Referral Hospital, a monthly average of 2-3% of patients reacts to transfused blood especially aged blood (>20 days) (BCRH, 2018). Blood toxicity has

been speculated to be a result of changes as blood ages which are not monitored during storage in the Kenyan context (Burger et al., 2013). Despite this, little is understood about the changes that take place in the course of storage of blood cells, blood biochemistry, and microbial contamination that occur during blood storage at Bungoma and Kenya at large. This study thus; determined the cellular, biochemical changes, and bacterial contamination in whole blood stored for transfusion so that transfusion safety is ensured.

1.3 Significance of the study

Assuring transfusion safety is a significant element of health care. At the moment, prohibiting transmission of infectious diseases is a cardinal safety concern with the laboratory testing for explicit infectious disease markers is a reputable strategy for forbidding contaminated donations. However, monitoring of cellular changes, biochemical changes, and bacterial contamination during storage may need to be considered to ensure blood safety. This study's findings shed light on the need to carefully monitor the cellular changes, biochemical changes, and bacterial contamination during blood storage to secure the safety of transfused blood.

1.4 Objectives of the study

1.4.1 Broad objective

To assess Cellular changes, Biochemical changes, and Bacterial contamination of whole blood during storage at 2-6°C for 35 days at Bungoma County Referral Hospital.

1.4.2 Specific Objectives

The specific objectives of the study were:

i. To assess the cellular changes in whole blood during storage at 2-6°C for 35 days at Bungoma county referral hospital, Kenya.

ii. To assess the biochemical changes in whole blood during storage at 2-6°C for 35 days at Bungoma county referral hospital, Kenya.

iii. To assess the bacterial contamination in whole blood during storage at 2-6°C for 35 days at Bungoma county referral hospital, Kenya.

1.5 The hypotheses of the study

The null hypotheses of the study were:

i. **Ho_1:** There are no cellular changes detected in transfusion blood during 35 days storage at 2-6°C.

ii. **Ho_1:** There are no biochemical changes detected in transfusion blood during 35 days storage at 2-6°C.

iii. **Ho_1:** There is no bacterial contamination in transfusion blood during collection and 35 days storage at 2-6°C.

1.6 Assumption of the study

Additive solutions containing saline, adenine, and dextrose in the anticoagulant meant to preserve stored blood did not interfere with the cells morphology or, biochemical -composition, and there is no bacterial contamination of blood during the 35 days storage period at 2-6°C.

1.7 Scope of the study

The scope of this study was to assess changes in the blood for transfusion stored at 2-6°C for 35 days at Bungoma County Referral Hospital. The scope of the research was directed by the specific objectives of the research and by the testing scope of Bungoma County Referral Hospital Laboratory.

1.8 Limitations of the study

The current study assessed biochemical, cellular changes, and bacterial contamination in blood for transfusion stored at 2-6℃ at a single facility, which may not be representative of the whole country.

1.9 Operational Definitions of terms

Bacterial contamination: The presence of bacteria in the blood for transfusion stored at 2°C to 6°C for 35 days.

Biochemical changes: The biochemical (Sodium, Potassium, and pH levels) changes during 35days of blood for transfusion storage interval at 2°C to 6°C.

Blood transfusion: The exercise of transplanting blood or blood components into a patient's/recipient's circulation intravenously.

Blood bank condition: The refrigerator temperature range of 2°C to 6°C.

Cellular change: The cells and their related indices count (WBC, RBC, HGB, MCV, MCHC, HCT, and platelet) changes during 35days of blood for transfusion storage at 2°C to 6°C.

Haematocrit: The proportion (percentage) that is made up of RBCs in stored blood at 2°C to 6°C.

Haemoglobin level: The haemoglobin concentration in g/dL in stored blood at 2°C to 6°C.

Mean Cell volume: The mean size of RBCs in fL in stored blood for transfusion at 2°C to 6°C.

Mean Cell Haemoglobin concentration: The average amount of haemoglobin inside a single red blood cell in g/dL in stored blood for transfusion at 2°C to 6°C.

pH level: A measure of the acidity or alkalinity of stored blood at 2°C to 6°C.

Platelet Count: The number of platelets x 10^3/µL in stored blood for transfusion at 2°C to 6°C.

Red Blood Cell Count: The number of RBCs x 10^6/µL in stored blood for transfusion at 2°C to 6°C.

Storage period: The period in which blood is reserved at 2°C to 6°C.

White blood cell count: The number of WBCs x 10^3/µL in stored blood for transfusion at 2°C to 6°C.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Concept of Blood Transfusion

Blood transfusion is a prime part of daily clinical practice (Goodnough, 2003). This procedure is applied all over the management of scores of severely sick patients to substitute decrease in haemoglobin to uphold oxygen transport to fundamental parts of the body (Klein et al., 2007). The rational use of whole blood or its components; vigilant donor assortment and inflexible testing by the blood centres is mandatory to guarantee safe blood accessibility. Blood transfusion might be significant for many medical treatments where it could be lifesaving. Nonetheless, donated blood is a rare resource and hospital blood transfusion exercise must focus on guarantying safe and opportune use (Allard, 2013).

From the time of the First World War (1914–1918), technical knowledge has been at hand to reserve blood for transfusion under cool conditions for brief intervals of time employing sodium citrate as an anticoagulant. The start of the II World War (1939– 1945) and the inauguration of a preservative carrying an acid-citrate-dextrose (ACD) solution which notably reduced the required amount of preservative, resulted in cold blood to be stored for twenty one days and blood storage turning into an reality (Adams, Bellairs, Bird, 2015). This permitted large amounts of blood to be transfused, prolonged storage interval, and decreased recipients encountering citrate injury. At a subsequent time, further improvements in the storage of donated blood were enabled by the initiation of phosphates and adenine that permitted prolonged storage period of blood pints (Adams, Bellairs, Bird, 2015). Abundant benefits have been realised in blood safety throughout the past twenty years, predominantly for transfusion-acquired viral diseases. Presently, the greatest severe popular dangers from a blood transfusion are clerical inaccuracy (causing ABO-

incompatible blood transfusion), transfusion-associated critical lung distress, and contamination of whole blood and blood products by bacteria (Goodnough, 2003).

Although advancement in appreciating the responsibility of blood transfusion dangers has been made, numerous significant knowledge gaps recur. Investigations are desired to further illustrate the effect of blood storage and transfusion plans in those patients in need of enormous transfusion and with severe confined or comprehensive tissue chlorosis (Kor & Gajic, 2010). There are indications that restrictive blood transfusion with a transfusion spark of 7-8 g/dl haemoglobin level or the existence of manifestations of anaemia is safe and not correlated with increased mortality in comparison with liberal transfusion. Therefore, the restrictive plan is sturdily endorsed in surgical and severely ill-patients (Yaddanapudi, 2014).

The transfusions of RBCs are significant in the remedy of anaemia activated by diverse conditions or because of haemorrhage generated by physical injury or incision. Worldwide, roughly 117.4 million pints of blood are collected annually of which 52% of blood transfusions in resource-limited nations are dispensed to children less than 5 years while cases aged over 65 years constituted about 75% of transfusions in developed nations (WHO, 2015). The blood for transfusion collection annual rate is 32.6 million pints in developed countries, 15.1million pints in upper-middle-developed countries, 8.1million pints in lower-middle-developed countries and 4.4 million pints in resource limited countries (WHO, 2015)**.** There has been persistent discussion in the scientific ring between persons pursuing to expand the period between blood collection and transfusion and those who are concerned with the stored blood potency and safety. Although the majority of clinical personnel concur that deterioration in blood and cellular components happen immediately, it is removed from the donor's arm, recipients in need of transfusions rely on the blood and blood components safety and effectiveness (Isbister, 2003). Blood is altered from the

time of collection and gradually escalate up to the expiry time. The level of these changes is governed by collection expertise, a particular component, the storage mechanism, the devices, and the storage period and temperature. The threshold storage period for blood components has mainly been casually set on by *in vitro* surveys and evaluation of *in vivo* survival. Storage might lead to commensurate and/or dependent deficit in blood components, which in turn decrease the effectiveness of a transfusion. Concurrently to these storage changes, there is an aggregation of degraded substances such as micro aggregates and Procoagulant matter, the unleashing of vasoactive substances, cytokine production, and hemolysis (Isbister, 2003). Significantly, adjustments that take place during this storage procedure are believed responsible for plenty of progressively recognized hostile effects connected with blood transfusion (Oyet et al., 2018; Burger et al., 2013). In most African nations, the dispensing of white blood cells depleted red blood cells for transfusion is now routine exercise, as a consequence of advanced red blood cells performance and persistence (Bosman, Lasonder, Groenen-Döpp, Willekens, 2012).

During storage, blood undergoes a sequence of cellular and biochemical changes that reduce its endurance and function. Some investigations have proposed that the threat following transfusion is elevated when blood for transfusion is stored for extended intervals. In the course of storage, blood cells suffer continuous mechanical and performance changes that might decrease RBCs and survival post- transfusion (Colleen, Liang, Daniel, Priscilla, Gerald, Tomislav, 2008). Storage has an adverse consequence on red blood cells oxygen transport (Bennett-Guerrero et al., 2007) and evolving evidence proposes that allergenic RBCs infusion might injure recipients. Substantial attestation advocates that transfusion augments the danger of severe problems and mortality in severely sick patients. Present investigations have shown that the red blood cell hypothermic storage lesion is accountable for the correlation of transfusion with an increase of staying in the

hospital, reduced tissue oxygen use, pro-inflammatory and immunomodulatory consequences, elevated diseases, diversified organ system collapse, and eventually elevated morbidity and death (Bennett-Guerrero et al., 2007).

Over the past years, a data-driven perspective to the danger/benefit equivalence in the verdict to give blood components has been given exceptional recognition. The clinically significant pitfalls of blood components therapy have been categorized as immunological or infectious. Most studies paying particular attention to the processing, storage and preservation of blood products has been *in vitro*, with studying of biochemical variables and occasionally morphological and rheological features (Isbister, 2003). *In vitro* statistics and animal research have long raised argument that the changes emerging from the storage of blood components might have unfavourable repercussions (Anniss & Sparrow, 2006). Despite that, blood supply organizations and hospital transfusion utilities are engrossed in the supply of compatible and disease-unbound blood components. Even though continual surveillance in the vicinity of these blood safety concerns is cardinal, the immunologic and infections dangers of transfusion have substantially been handled. Further surveillance can now be pointed at queries regarding the suitability of transfusion, the standard of blood components and the repercussions of storage changes (Isbister, 2003).

2.2 Cellular Changes in Stored Blood for transfusion

Blood is a composite tissue constituting cell and non-cell elements which perform multiple roles (Nuaimy, 2008). The non-cell elements comprise of the plasma and its derivatives. The cell components are made up of WBCs, PLTs and RBCs (Nuaimy, 2008). The deterioration in blood and cellular constituents happen almost immediately it is removed from the donor and recipients in need of transfusions rely on the blood and blood components safety and potency (Isbister, 2003). To minimize the dangers linked with blood transfusion, advanced anticoagulants, additive

solutions, red blood cell membrane stabilizers, preservatives, and bags were manufactured (Diana Noguira, Susana Rocha, Estela Abreu, 2015). Even with these developments, several changes in blood stored for transfusion have been encountered and referred to as 'red blood cell storage lesions'. These comprise of changes in red blood cells structure and metabolism, in losing of carbohydrates, lipids and proteins, in excretion and cell fastening together with in the oxygen transportation (Diana Noguira, Susana Rocha, Estela Abreu, 2015).

After transfusion integral hemodynamic is similar for current and stored cells; nonetheless, micro-vascular hemodynamic are acutely effected by stored cells which minimizes blood movement and oxygen transport. Furthermore, the existence of stored cells in the bloodstream alter cell-cell and cell-wall interchanges and modify the cell (Yalcin et al., 2014). The oxidative injury manifests red cells extra vulnerable to stress as indicated by increased osmotic fragility in the course of the storage and resultant discharge of haemoglobin (HGB) and intracellular enzymes such as lactate dehydrogenase (LDH) into the floating plasma (Orlov, 2015).

Even though RBCs might be stored at 2-6°C for up to forty two days prior to transfusion, less is understood of how changes to RBCs in the course of storage might alter their attachment properties (Anniss & Sparrow, 2006). Some studies have shown that stored RBCs show radical deformability changes in the course blood storage at 2-6°C. Studies have denoted that the distortion index of RBCs does not vary substantially during blood storage at 2-6°C. Nonetheless, radical differences prevail in time constants and circularity distribution widths, that can be utilized to estimate stored red blood cells attribute or age (Zheng et al., 2014). During blood storage at 2-6°C, glycolysis is retarded and, as acid pile up, the amount of ATP reduce and the structure of the RBC is bit-by-bit changed from disc- shaped to echinocytic shapes (Yoshida, 2019). This reshaping of red blood cell shape vanishes when stored blood is warmed (Adams, Bellairs, Bird, 2015).

The discharge of unbound haemoglobin following red blood cells lysis during blood storage and its effect on the intravascular nitric oxide metabolism after transfusion has been considered a predominant role (Vermeulen, de Wit, Sertorio, 2012). Studies have denoted that transfusion of long stored blood is linked with a rise in plasma unbound haemoglobin and hunting of nitric oxide *in vitro* (Finney, 2012). In line with this discovery, elevated unbound haemoglobin levels in patients with persistent and severe hemolysis have been connected with reduced nitric oxide bioavailability within the micro-capillary bed, reduced organ perfusion and raised organ injury (Reiter, Wang, Tanus-Santos, Hogg, Cannon, Schechter, 2002). Similarly, transfusion of stored blood pints may increase unbound haemoglobin levels in recipients after transfusion - for example, as a result of pre-mature intravascular burst of the transfused RBCs, or because of the transfusion of the unbound haemoglobin -containing storage medium (Gladwin, 2009). Consistent to this hypothesis, studies have shown that transfusing of unbound haemoglobin containing stored blood cause a significant rise of blood pressure in rats that is correlated with the unbound haemoglobin levels in the stored blood (Donadee, Raat, Kanias, Tejero, Lee, Kelley, Zhao, Liu, Reynolds, Azarov, Frizzell, Meyer, Donnenberg, Triulzi, Kim-Shapiro, 2011).

With prolonged storage, there is a shortage of ATP, then the pumps may not be able to maintain the ionic homeostasis of the red blood cell, leading to changes in shape and mean cell volume (MCV), haematocrit (HCT), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC). The water influx to the cytosol give rise to the swelling of erythrocytes during storage and non-existence of selective channels of performance as done in the spleen are associated with the change in shape and volume of the red blood cell during storage (Bosman, Lasonder, Groenen-Döpp, Willekens, 2012).

Studies have revealed that white blood cells have a short life span in stored blood (only few hours) and transfusion after 24 hours storage have been proved to be ineffective in raising the WBC count of patients (Nuaimy, 2008). Many studies have revealed that during storage the total WBC count decreases, and they associate this count reduction to degeneration of the granulocytes (Nuaimy, 2008). Studies have denoted that WBC depletion in the course of blood storage has been connected with ATP depletion and white blood cells being used in the development of micro-aggregates, which are a mixture of white blood cells, platelets, fibrin, cold insoluble globulin and cellular debris formed during storage (Bhargava, Gupta, Vivek, & Khare, 2016).

Studies show that in the course of storage, changes take place in both platelet and storage device, which may lead to triggering of platelet and malfunction (Aubron, Flint, 2018). The stimulation of Platelets in the course of storage of blood lead to Platelet-white blood cells aggregates (PLAs) accumulation that introduces WBC apoptosis. Pro-coagulant action, likely correlated with micro-particles from apoptotic white blood cells, might lead to harmful properties of stored blood (Keating, Butenas, Fung, & Schneider, 2011). Results from some studies indicate that canine platelets survive when stored at room temperature for up to eight hours in CPDA-1 treated whole blood. However, a gap still exists as to whether there are changes after 8 hours of storage (Crowther, Ford, Jeffrey, Urbaniak, & Greaves, 2000).

Studies indicate that transfusing older, reserved blood could be correlated with an escalation in death; severe infections, multiple-organ breakdown, thrombosis, and prolonged hospital stay (Hod & Spitalnik, 2011). The establishment of blood storing arrangements allows time between collection of blood and transfusion to be increased. This time increase has allowed the decentralization of blood donation utilities with successive savings and advancements in the accessibility of blood components. However, the accessibility of blood storage increases the

question of at what extend blood components can and should be stored and what extend are they safe and potent (Zimrin & Hess, 2009).

Studies in Europe show that there is sufficient proof that red blood cells experience harmful changes during storage (Roback, 2016).In sub-Saharan Africa, where the uptake of transfusion is high, little research has been carried out on the attribute and safety of transfusion blood (Sophie Uyoga, 2019).Despite the high levels of post-reaction after transfusion in Bungoma County Referral Hospital (BCRH, 2018) and the possibility of the risk to patients due to the cellular alterations, the cellular changes in blood stored for transfusion has not been studied and remains unknown.

2.3 Biochemical Changes in Stored Blood for transfusion

Biochemical changes take place during transfusion blood reservations, affecting their function (Yalcin et al., 2014). For a great number of years, researchers have investigated the biochemical changes that take place in blood stored before transfusions. More lately, clinical investigations have proposed that blood pints stored for extended periods (frequently detailed as 14–21 days) might be detrimental to the recipients, causing morbidity and mortality (Roback, 2011). Potassium is the prominent intracellular cation with sodium being the prominent extracellular cation (Terry, 1994). Maintenance of the circulation of potassium and sodium between the intracellular and the extracellular environments depends on a handful of homeostatic mechanisms (Orlov, 2015).

Literature discloses that potassium efflux, a renowned effect of prolonged red blood cell reservation, causes vesicle creation and may have a significant effect on the transfusion outcome (Burger et al., 2013). Studies have also shown a steady decrease in pH throughout the blood storage

period which renders the cell membrane very inflexible and predisposes the cells to disintegration (D'Alessandro, Gevi, 2013) .

Blood storage improves access to blood for transfusions. However, questions arise regarding the toxicity and value of such blood stored for extended periods. In various medical retrospective investigations, writers propose that transfusion of stored blood may cause harm, particularly in severely ill patients such as patients with severe myocardial infarction conditions (Roback, 2011). They detected elevated incidence of death, morbidity, infections, renal and lung breakdown, swelling, and thrombosis in patients who were transfused with extensively stored blood compared to those who were transfused with fresh blood (Hod & Spitalnik, 2011); however, the causes of these remain unknown. These adverse effects may be attributed to the inability of red blood cells in reserved blood to transport oxygen as a result of the reduced concentration of adenosine triphosphate and 2, 3-diphosphoglycerate (D'Alessandro, Gevi, 2013). Also, cytokines, enzymes, and ions such as potassium and calcium from white blood cells in stored blood may modify red blood cells and adversely affect transfused recipients. These modifications affect an assortment of membrane molecules involved with adhesion, oxygen delivery and complement modulation (Adams, Bellairs, Bird, 2015). It is interesting how the storage of blood for extended periods can result in the manipulation of the membrane exterior of red blood cells, and consequently their biological utilities (Adams, Bellairs, Bird, 2015).

According to records of Bungoma County Referral Hospital, the transfusion rate averages 300 transfusions monthly (BCRH, 2018) and there has been an increase in blood reactions in the patients that receive a blood transfusion. A monthly average of 2% – 3% of patients react to transfused blood, especially aged blood, which is blood that has been stored for over 20 days (BCRH, 2018).

It has been well documented all over the world that various biochemical changes take place during the 35 to 42 days storage of blood at temperatures between 2°C and 6°C (Adams, Bellairs, Bird, 2015). In Africa few studies have shown that in the course of storage, a number of biochemical changes take place, which can have an effect the effectiveness of blood transfusion (Oyet et al., 2018). No study in Kenya has been carried out to assess biochemical changes during storage of transfusion blood.

Despite the high levels of post-reaction after transfusions in Bungoma County Referral Hospital (BCRH, 2018) and the risk of toxicity to patients due to leakage of blood chemicals, the biochemical changes in blood stored for transfusions have not been studied and remain unknown. Therefore, this study aimed to assess the biochemical changes in whole blood stored for transfusions at Bungoma County Referral Hospital.

2.4 Bacterial Contamination of Blood for transfusion

A ready to be transfused blood should be free from microbial contaminants among them bacteria (Walther-Wenke, 2008). However bacterial contamination of blood for transfusion may take place as a a consequence of endogenous or exogenous routes (Cawley, McDonald, Ancliff, Roy, MacLennan, Brant, Pichon, 2011). Bacterial contamination of blood for transfusion have been observed as severe danger over years and is second to ABO mismatch in causing transfusion – related deaths(Agzie, Niguse, 2019) .

Infectious agents including bacterial, parasitic and viral micro-organisms spread via blood and blood products has long been documented (Bolarinwa, Aboderin, Odetoyin, & Adegunloye, 2011). Blood recipients have been known to get infected with human immunodeficiency virus (HIV), Hepatitis B virus (HBV), Hepatitis C (HCV), human T- lymphotrophic virus (HTLV), Cytomegalovirus and West Nile virus, severe acute respiratory syndrome (SARS), malaria, Chagas

disease, babesiosis, leishmaniasis, Jacob disease, Zika, Dengue, Chikungunya, anaplasmosis, *Treponema pallidum and Mycobacterium tuberculosis* through blood transfusion (Justiz Vaillant, 2020).The bacteria that can be transmitted in blood and blood components are Gram negative bacteria including *Klebsiella pneumonia, Escherichia coli, Yersinia entocolitica, Pseudomonas fluorescen, Pseudomonas aeruginosa* and Gram positive bacteria including Listeria, *Bacillus species, Staphylococcus aureus, Staphylococcus epidermidis* and other coagulase-negative *Staphylococci species* (Opoku-Okrah, Feglo, 2009*;*Andrew Anthony Adjei et al., 2009). Possibly , bacterial contamination of blood and blood products can happen at various stages including production of blood bags, donor venipuncture, blood donor bacteraemia, blood component separation, transportation, storage, preparation (cross-matching) or at the time of transfusion (Bolarinwa et al., 2011).

Transfusion blood Contamination with bacteria is a chronic setback which has been incompletely contained as a result of current phlebotomy applications, preservation of blood, plasma freezing and better equipment for blood collection and storage. In the USA, Contamination with bacteria is regarded as the second universal cause of death in general following transfusion (behind administrative errors) with death rates spanning from 1:20000 to 1:85000 donor contacts. Approximations of severe disease and death span from 100 to 150 transfused persons annually (Hillyer et al., 2003). Transfusion blood Contamination with bacteria was first recognised over sixty years ago; yet, plans to resolve this setback have proven difficult despite on-going appreciation and rising concern especially in the last few years (Brecher, Hay, Palavecino, Jacobs, & Yomtovian, 2005).

Occult donor sepsis and contamination of the blood in the time of collection have been the principal routes for introduction of micro-organisms. Gram-positive skin flora, introduced to the pint in the

course of the collection, are the most common contaminants isolated in a retrospective analysis of blood pints (Chelsea Sheppard, Cassandra Josephson, 2005). Three studies have shown that seventy percent of contaminants isolated were Gram-positive micro-organisms, while eighty percent of deaths on account of transfusion-linked sepsis were caused by Gram-negative bacteria (Perez, Salmi, Folléa, Schmit, de Barbeyrac, Sudre, 2001);(Roth, Kuehnert, Haley, Gregory, Schreiber, Arduino, Holt, Carson, Elder, 2001).

Symptomless donor sepsis is the cause where healthy individuals may be permitted to donate when infectious, bringing about blood Contamination with bacteria. In this instance, even rigorous donor assessment will not detect this likely root of contamination. There are several cases of donors who innocently donate blood while septic. By way of illustration, an instance of transfusion linked *Staphylococcus aureus* blood contamination has been documented in a blood donor who went for a tooth removal three hours ahead of donating blood and this was the possible cause of temporary sepsis (Chelsea Sheppard, Cassandra Josephson, 2005).

Categorically at the time of collection, the amount of contamination is comparatively small (1 to 10 colony forming units (CFU)/mL (Brecher et al., 2005). Nevertheless, once the blood is reserved, the bacteria can multiply to amounts of 10^6 CFU/mL or more (Chelsea Sheppard, Cassandra Josephson, 2005). This level of bacteria, when transfused within a short interval of time, can lead to sepsis, high body temperature, chills, hypotension, nausea, vomiting, diarrhoea, and oliguria, which might advance to septicemia and eventually multi-system organ collapse and death. On the other hand, the transfusion of contaminated cellular blood components may lead to asymptomatic or long-lasting clinical repercussions (Brecher et al., 2005). The intensity of the response is influenced by the bacteria species present, the inoculum, the rate of bacterial growth, and cardinal disease, white blood cells count and the strength of the immune system (Chelsea Sheppard,

Cassandra Josephson, 2005). Gram-negative bacteria appear to bring about increased acute reactions owing to the existence of endotoxin released by these bacteria. While giving rise to only one-fifth of the instances, Gram-negative bacteria have been shown to be responsible for forty percent of fatalities on account of septic transfusion reactions (Hillyer et al., 2003) (Chelsea Sheppard, Cassandra Josephson, 2005). Transfusion related bacterial infection has stood more recurrent than viral infection and is connected with high mortality due to rapid development of septic shock (Hailegebriel Wonndimu)

Worldwide, the precise prevalence of blood for transfusion Contamination with bacteria is unknown (Agzie, Niguse, 2019). However various studies have shown donated blood Contamination with bacteria as 0.2 percent, 0.15 percent and 0.1percent in United States of America, United Kingdom and France respectively (Kuehnert, Roth, Haley, Gregory, Elder, Schreiber, Arduino, Holt, Carson, Banerjee, 2001).

Studies in sub-Saharan African nations indicate that the prevalence of contamination of blood for transfusion is higher than that of first class countries (Bolarinwa et al., 2011).While a study in Ethiopia has shown prevalence of blood for transfusion as 9.2 percent (Agzie, Niguse, 2019).

A study done in Kenya some thirteen years ago documented a blood for transfusion bacterial contamination prevalence as 8.8 percent (Hassall, Maitland, Pole, Mwarumba, Denje, Wambua, Lowe, Parry, 2009).

Steady monitoring of bacterial contamination in the course blood storage and consciousness among clinical personnel on the hypothetical problems can minimize disease infection to patients. Modern methods of unmasking bacterial contamination, pathogen minimization in stored blood will increase blood safety (Mathai, 2009).

Despite the high levels of post-reaction after transfusion in Bungoma County Referral Hospital (BCRH, 2018), and the possibility of the risk of toxicity to patients due to production of endotoxins by contaminating bacterial, the bacterial contamination in stored blood for transfusion at Bungoma County Referral Hospital have not been studied and remain unknown.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

The study was carried out between February and August 2019 at Bungoma County Referral Hospital that is located in Bungoma County (coordinates 0.4213°N to 1.1477° N along the latitude and 34.3627° E to 35.0677° E along the longitude) in Western Kenya as shown in *figure 3.1.* The capital is Bungoma Town and the estimated population is 1,670,570, comprising 812,146 males and 858,389 females according to the 2019 census report and an area of 2,069 km^2 (KNBS, 2019). The major economic occupation includes farming, industry, and general trade. Bungoma County lies at 1421m above sea level and the climate is tropical and the temperature averages 21.1 °C | 69.9 °F. The County encounters two rainy episodes, the long rains from March to July and short rains from August to October. The yearly rainfall in the County varies from 400mm (lowest) to 1,800mm (highest). The yearly temperature in the County fluctuates between 0°C and 32°C because of different levels of altitude, with the peak of Mt. Elgon experiencing slightly lower than 0°C. The average wind velocity is 6.1 Kilometers/hour. In the past ten years, the County encountered rising instability in rainfall and temperature patterns that have led to changes in agricultural seasons (Https://en.climate-data.org/). The County is split into 9 sub-counties specifically Bumula, Kanduyi, Sirisia, Kabuchai, Kimilili, Tongaren, Webuye East, Webuye West, and Mt. Elgon. The sub-counties are split further into 45 county assembly wards, with the county headquarters being located in Bungoma town, Kanduyi sub-county. The county borders Uganda, Kakamega county, Busia county, and Trans-Nzoia county. A Map showing Bungoma County is shown in *figure 3.2.*

Bungoma County Referral Hospital was a good study area because the problem of the study was common (BCRH, 2018). The hospital has a 250 bed capacity with an average of 1200-1500

monthly admissions and with an average of 300 monthly transfusions (BCRH, 2018). The hospital has a blood transfusion satellite that collects an average of 600 blood pints monthly (averagely 20 pints per day) (Bungoma County Blood Transfusion satellite records, 2018). The hospital also has an accredited laboratory that is well-equipped with haematology, biochemistry, and microbiology equipment required for the study.

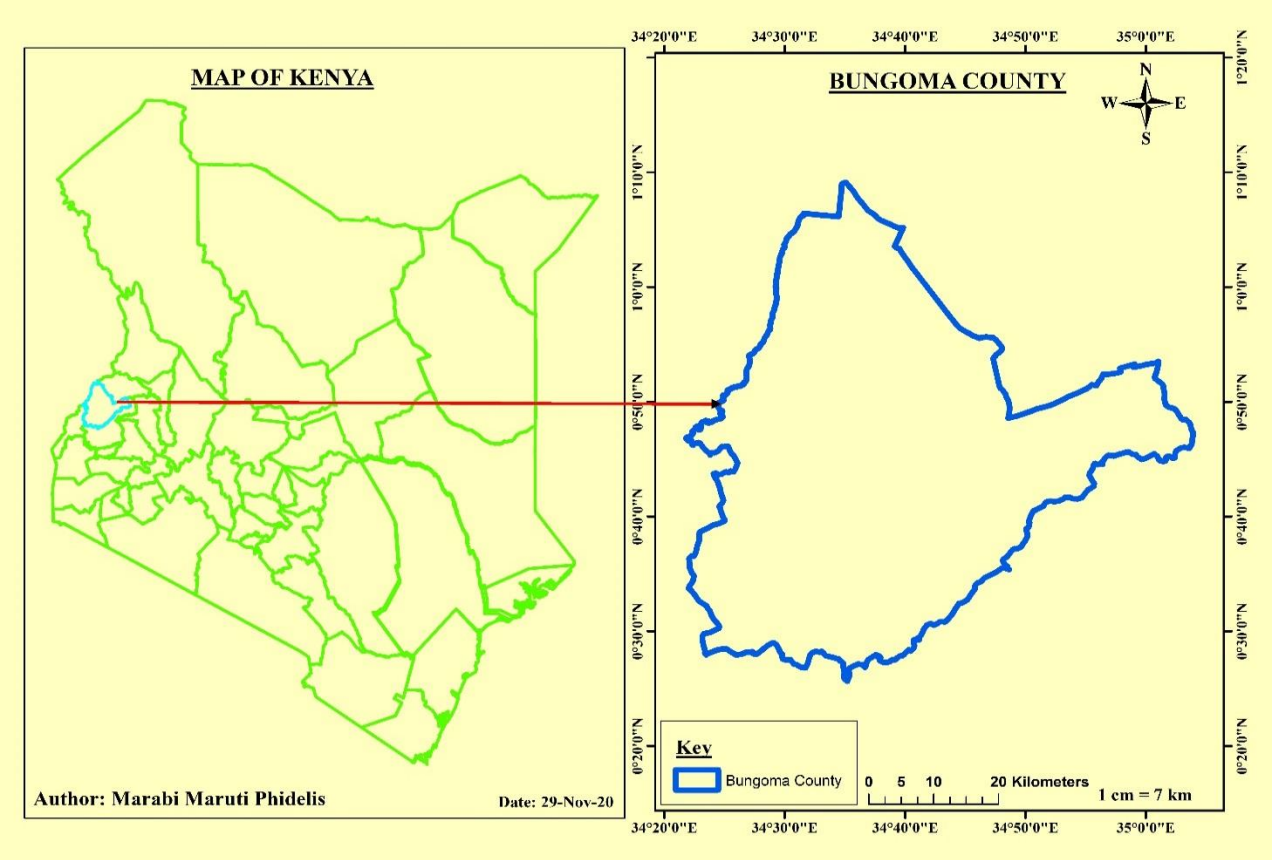

Figure 3. 3*: Map showing the location of Bungoma County in Kenya.*

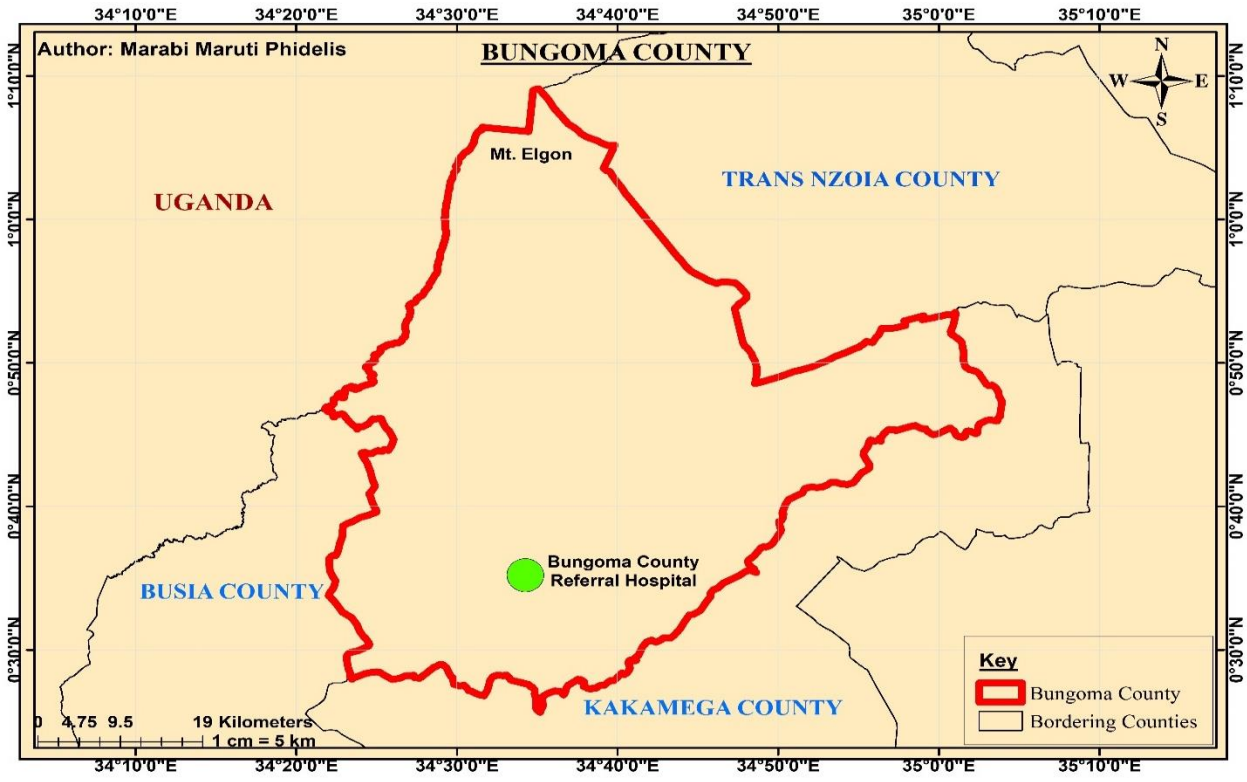

Figure 3. 4: *Bungoma County map with Location of Bungoma County Referral Hospital.*

3.2 Research design

The research employed prospective study design and involved collection of blood pints in blood bags carrying citrate phosphate dextrose adenine (CPDA-1) anticoagulant from normal volunteers and stored under blood bank conditions of 2-6°C, then tested for baseline and then serially at day seven, fourteen, twenty-one, twenty-eight and thirty-five. The whole pints of blood were used in the study to ensure that all storage condition for blood meant for transfusions was observed. The baseline result was regarded as a control in this study.

3.3 Population of the study

3.3.1 Target population

The study targeted blood donors at the Bungoma County Blood transfusion satellite. The transfusion satellite collects averagely 600 blood pints monthly (averagely 20 pints per day) (Bungoma county, 2018).

3.3.2 Inclusion and Exclusion Criteria

3.3.2.1 Inclusion Criteria

Blood pints from donors who met the donor suitability criteria following the World Health Organization guidelines on assessing donor suitability for blood donation (WHO, 2012) were included in the study.

3.3.2.2 Exclusion Criteria

Blood pints that did not meet the volume criteria following the World Health Organization guidelines on assessing donor suitability for blood donation (WHO, 2012) were excluded from the study.

3.4 Sample size determination

The sample size of the research was determined by Yamane Taro's formulae for a finite population (Israel, 1992).

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

n = required sample size

N = population size (20 blood pints collected per day)

e = standard error in this case 0.05

On substitution

n=20/ (1+20(0.0025)

=19.05

Therefore a sample size of 20 blood pints was adopted for the study.Our sample size was also similar to those used in previous studies (Vaibhav Mane, Vinayak Mane, Pawar, 2015)

3.5 Sampling Technique

A simple random sampling technique was used in this study where every 10^{th} sample was selected into the study sample. This gave equal chances to all the blood pints from the donors to be used in the study and eliminated biases.

3.6 Data collection procedures

3.3.2 Blood Sample collection and preparation

Blood was collected in blood bags carrying CPDA-1 as an anti-coagulant preservative. All blood pints were collected according to blood transfusion donor guidelines as described by the World Health Organization (WHO, 2012). At baseline, samples were immediately separated from blood pints collected from the volunteer donors to test for WBC count, RBC count, HGB level, MCV, MCHC, Platelet count, Potassium, Sodium, PH level and cultured for bacterial detection. Blood pints were then stored at blood bank conditions of 2-6°C for 35 days. Samples were then serially separated from the stored blood pints after every 7 days for 35 days and tested immediately for White blood cell count, Red blood cell count, and Haemoglobin level, Mean cell volume, Mean Cell Haemoglobin Concentration, platelet count, Potassium, Sodium, pH level and cultured for bacterial detection. The samples for testing were aseptically transferred from blood pints to plain test tubes during separation while samples for blood culture were transferred directly to the BD Bactec plus Aerobic/F and Anaerobic/F Culture vials using a sterile syringe. No, any other anticoagulant or additives were added to the separated samples. The separated samples were brought to the right temperature as per the manufacturer's instructions before analysis. The pH estimation and blood culture were done using the whole blood at the study points. The laboratory results were recorded on a standard data collection form developed for this study (Appendix IX).

3.6.2 Laboratory analysis

3.6.2.1 Blood cell analysis for cellular changes

Cellular changes (RBC count, WBC count, and platelet count, HCT, MCV, MCH, and MCHC) and haemoglobin changes were tested using the Celtac F, MEK-8222 K automatic haematology analyser (NIHON KOHDEN Corporation, 1-31-4 Nishiochiai, Shinjuku-ku, Tokyo 161-8560, Japan) as used before (Kondo, Akiyama, Tatsumi, 2004). This analyzer measures white blood cells, red blood cells, and platelets by electrical resistance sensing technique while it measures haemoglobin using a spectrophotometric method. The analyzer derives haematocrit (HCT) by multiplying together the red blood cell count and the mean cell volume, then dividing the results by 1000. The mean cell haemoglobin (MCH) is derived by dividing the haemoglobin result by red blood cell count. While mean cell haemoglobin concentration (MCHC) is derived by dividing haemoglobin result by haematocrit, then multiplying by 100.

For the electrical resistance technique, the analyzer employs the fact that cells constituting a blood sample have assorted sizes with different diameters and does not transmit direct current. When the cells stream through the slit, the conductivity cross-section of the slit filled with conductor fluid reduces and resistance from that of the empty slit is registered over the fluid. The variance of resistance is relative to the dimensions of the non-conductive molecules partly obstructing the slit. The larger the change, the larger the particle passing through the slit was.

For the spectrophotometric technique, the analyzer consists of a photodiode, a cuvette with a wavelength of 15nm, and a filter at a wavelength of 535nm. It employs the fact that the sample haemoglobin estimation absorbs or transmits light over a certain range of wavelengths. The amount of emitted light is d commensurable to the amount of haemoglobin in the sample.

Briefly, the procedure involved pressing numerical on the screen to enter samples identification details; placing well-mixed samples on the autoloader; pressing the start icon on the screen to start sample aspiration and analysis; the results were shown on the equipment visual display unit and automatically send to print immediately the instrument completed the analysis.

The reference ranges are: White blood cells count: 3.5-10.0 x $10^{9/}$/L; Red blood cells: 3.50-5.50 x 10^{12}/L; Haemoglobin: 11.5-16.5g/dL; Haematocrit: 35.0- 55.0%; Mean cell volume: 75.0 – 100.0 fL; Mean cell haemoglobin: 26-33 pg; Mean cell haemoglobin concentration: 31.0- 38.0 g/dL; and platelets: 130-400 x10^{9}/L. Low, normal, and high levels of control materials were analyzed before the samples were analyzed.

3.6.2.2 Blood cell biochemistry analysis for biochemical changes

Biochemical changes (and PH) were tested using Humalyte plus5 analyzer (Human Diagnostics Worldwide, Max-Planck-Ring 21 • 65205 Wiesbaden, Germany) for Potassium and sodium as used before (HumaLyte, 2010) while pH was estimated using Hanna pH meter (Scientific Instrumentation, Woonsocket, Rhode Island, USA) as used before (Hanna edge Multi-parameter Bench-top Meter instructions manual, 2015). Briefly, the aliquots were centrifuged to get plasma that was later fed on the equipment for automated analysis of potassium and sodium. The instrument measures the electrode potentials, and the data is processed by the microprocessor to obtain the concentration of a given ion. The reference range of Potassium is 3.5 to 5.5mmol/L while Sodium is 145 to 155mmol/L. Hanna pH meter is automated and samples were analyzed for pH by inserting the electrodes in the samples and results displayed on the screen. The expected range for blood pH is 7.35 to 7.45.

3.6.2.3 Testing for bacterial contamination

This study used BD Bactec FX 40 instrument (Becton Dickinson 26-28 Decoy Road, Worthing, West Sussex, BN14 8ND, United Kingdom), Gram staining, culture using blood and MacConkey medium, and biochemical tests to establish if the blood pints were contaminated with bacteria

3.6.2.3.1 Culture

3.6.2.3.1.1 Media preparation

Blood agar, an enriched nutritious medium that aids the growth of fastidious micro-organisms by supplementing it with sheep blood was prepared following the manufacturer's instruction. Prepared plates were stored at 2-8°C in fastened plastic containers to avert loss of moisture. The life-span of prepared blood agar is up to 4 weeks (BD, 2009) ; (Rebecca, 2005)

MacConkey agar used for the differentiation of lactose fermenting from lactose non-fermenting bacteria was prepared as per the manufacturer's instructions. The plates were stored at 2-8°C in fastened plastic containers to avert loss of moisture. The life-span of prepared blood agar is up to 4 weeks (BD, 2009) ; (Allen, 2005).

Mueller Hinton Agar used for antibiotic susceptibility testing was prepared as per the manufacturer's instructions. The plates were stored at 2-8°C in fastened plastic containers to avert loss of moisture. The life-span of prepared blood agar is up to four weeks (BD, 2009) ; (Leber, 2016).

3.6.2.3.1.2 Bacterial isolation, identification, and susceptibility testing

i. Culturing

Bacterial contamination was tested using the BD Bactec FX 40 instrument (Becton Dickinson 26-28 Decoy Road, Worthing, West Sussex, BN14 8ND, United Kingdom) as used previously (BD,

2015). The samples to be tested were aseptically injected into ready to use BD Bactec plus Aerobic/ F and Anaerobic/F culture bottles which contained soybean-casein digest broth, yeast, amino acids, sugar, vitamins, and sodium polyanetholsulfonate. The bottles were then placed in the BD Bactec FX 40 device for incubation and systematic analysis. Each bottle carries a chemical detector that can detect an elevation in carbon dioxide (CO_2) generated by the sprouting of bacteria. The detector is checked by the device every 10 minutes for an elevation in its fluorescence, which is commensurable to the level of CO_2 available. A positive result designates the availability of viable bacteria in the bottle. Bottles placed into the equipment were automatically analyzed every 10 minutes for 24 hours.

ii. Gram staining

The positive bottles were removed from the device mixed well and aliquot aseptically obtained for Gram staining. Smears were prepared from the obtained aliquots, dried by air, and heat-fixed. The smears were then streamed with crystal violet staining reagent for one minute. The slides were then washed in a gentle running tap water for 2 seconds and flooded with Gram's iodine for 1 minute. The slides were then gently washed in running tap water for two seconds. The slides were then flooded with the decolorizing agent (Acetone-alcohol decolorizer) for 10-15seconds and counterstained with neutral red for 30 seconds to 1 minute. The slides were then gently washed in running tap water until no color appeared in the outflow and then air-dried. The results of the Gram staining procedure were examined under oil immersion (100x) using a Bright field microscope and the results were interpreted as Gram-positive: Blue/Purple Color and Gram-Negative: Red/Pink Color (Ann Smith, 2005).

iii. Biochemical tests

The vials were sub-cultured on blood and MacConkey agar according to the Gram stain results. The bottles were well mixed and samples were obtained with a sterile disposable loop (10µL) and sub-cultured on respective solid agar. The growth of bacteria was interpreted following the Difco & BBL Manual of Microbiological Culture Media, Second Edition ((BD, 2009).

Catalase test as used before (Reiner, 2010) was used to identify the isolated bacteria. This test rests on the fact that bacteria able to synthesize the enzyme catalase, hydrolyzes hydrogen peroxide into water and oxygen, which leads to the release of gas bubbles was performed as one of the biochemical tests to identify the isolated bacteria. About four to five drops of 3% H_2O_2 were added to a test tube and using a wooden applicator stick, a small number of organisms from a well-isolated colony were collected and placed into the test tube. The tube was observed for immediate bubbles. The positive control was 18-24 hours *Staphylococcus aureus* ATCC25923 broth culture and the Negative control was the sterile broth. The positive test was indicated by the immediate appearance of bubbles while the negative test was indicated by no bubbles (no gas formation).

A coagulase test as used before (Sue, 2010) was performed to identify the isolated bacteria. This test rests on the fact that coagulase is an enzyme-like protein and makes plasma clot by transforming fibrinogen to fibrin was performed to identify isolated bacteria. Plasma was diluted 1 in 10 in normal saline by mixing 0.2 ml of plasma with 1.8 ml of saline. Test tubes were labelled as T (Test), P (+ve Control), and N (-ve Control). The positive control was 18-24 hours *Staphylococcus aureus* ATCC25923 broth culture and the Negative control was the sterile broth. The diluted plasma of 0.5 ml was pipetted into each tube and 5 drops (0.1 ml) of the test organisms were added to the tube labelled "T", 5 drops of Staphylococcus aureus ATCC25923 culture to the tube labelled “P” and 5 drops of sterile broth to the tube labelled “N”. After mixing, the three tubes were incubated at 35-37°C and examined for clotting after 1 hour. If no clotting had taken place,

examined at 30 minutes spans for up to 6 hours. The positive test was indicated by the presence of clumps while the negative test was indicated by the absence of clumps.

iv. Susceptibility tests

After identification of bacteria, pure isolates were sub-cultured on Mueller Hinton agar for susceptibility test by use of Kirby-Bauer Disk Diffusion as used before (Leber, 2016) was also performed to check on the susceptibility pattern of the isolated and identified bacteria. The organisms to be tested were selected from a pure culture plate and aseptically emulsified in the sterile saline solution and mixed rigorously to make sure that no solid matter from the colony was seen in the saline solution. The density of the inoculum which was expected to be equal to the density of 0.5 McFarland standard was checked using densiCHECK plus instrument (BIOMERIEUX SA, 376 Chemin de I'Oreme, 69280 Marcy-I'Etoile-France). If the density of the inoculum was found to be high or low, it was adjusted accordingly by either addition of more saline or more colonies until the density equaled that of the standard. The inoculum was then sub-cultured on Mueller Hinton agar by using the swab with the test organism to streak for a lawn of growth. After the streaking was complete, the plates were allowed to dry for five minutes and sterile forceps were used to place the antibiotic discs on the surface of the agar. The discs were then gently pressed onto the surface of the agar using flame sterilized forceps. The inoculated plates were carefully inverted and incubated for twenty-four hours at 37° C. After incubation, a ruler was used to measure the circumference of the zone of inhibition to the nearest millimeter and indexed as sensitive, intermediate, or resistant for each antibiotic used. The measurement obtained from the individual antibiotics was compared with the Clinical and Laboratory Standards Institute (CLSI) antibiotics zone size interpretive chart (Weinstein, Patel, Bobenchik, Campeau, Cullen, Galas, Gold, 20019). The antibiotic discs used were penicillin 10 units, oxacillin 30µg,

clindamycin 2 µg, and cefazolin 30 µg following the CLSI selection of antibiotics by organism and specimen type criteria (Weinstein, Patel, Bobenchik, Campeau, Cullen, Galas, Gold, 20019).

3.6.3 Quality Assurance of the data

To ensure that quality data was collected, only blood pints that meet donation criteria were used. A qualified phlebotomist was used to collect the blood pints ensuring the right quantity of 450 to 500ml was collected. The pints were transported and stored as per blood transfusion guidelines at 2-8 °C. The separated samples were brought to a temperature consistent with the manufacturer's directions before testing. Samples were analyzed independent duplicates for each sample and an average did to ensure accuracy. Both external and internal quality controls were verified and ensured during the study, ATCC25922 micro-organisms were used as control strains. Recorded results were verified by the second personnel to ensure accuracy.

The laboratory is enrolled in the Human Quality assessment services (HUQAS) External Quality Assurance Scheme for haematology, chemistry, and microbiology scope including Complete Blood Count, pH, and Sodium, Potassium, and blood culture tests. The haematology, chemistry, and microbiology scope is also accredited by the Kenya Accreditation Services (KENAS) which further assured the quality of data collected.

3.7 Data analysis

The data was stored in Microsoft spreadsheets. The interrogation was done using the Statistical Package for Social Science (SPSS V.23) (IBM Corporation, Chicago, Illinois, United States). Descriptive statistics (frequencies mean and standard deviation) were used to describe the data. The trends of the cellular- biochemical changes and bacterial contamination were shown using line plots. Analysis of variance (ANOVA) was used to establish if there were significant cellular-biochemical changes and bacterial contamination in transfusion blood at baseline and every 7 days

of study points compared to the baseline for 35 days of storage. Findings were considered significant at $\alpha \leq 0.05$. Tukey's Honest Significant Difference test was used to collate all feasible pairs of means. Results were presented in form of tables and charts.

3.8 Ethical Considerations

The permission to carry out the study was granted by Kisii University (Appendix I); Kenya National blood Transfusion services (KNBTS) (Appendix II), the Ministry of Education (Appendix III), and Bungoma County (Appendix IV).

The study was cleared by the Ethical committee of Jaramogi Oginga Teaching and Referral Hospital (#ERC.IB/VOL.1/454) (Appendix V) and authority to carry out the research was issued by the National Commission for Science, Technology, and Innovation (#NACOSTI/P/19/32125/27143), (Appendices VI and VII). Written informed consent was obtained from each donor after a brief explanation of what the study is about (Appendix VIII). Participation in the study was voluntary. Donor details were kept confidential by not including any form of identification on the data collection tool, keeping filled data tools at a lockable place, and by use of a password on the computer. The donors were explained that their blood units if selected into the study was not transfused to patients.

CHAPTER FOUR

4.0 RESULTS

The study investigated cellular and biochemical changes in transfusion blood at baseline and every 7 days for 35 days of storage at 2-6°C. It also shows the findings of bacterial contamination in transfusion blood at baseline and every 7 days for 35 days at 2-6°C.

4.1 Cellular changes in stored blood for transfusion

4.1.1 Summary of the cellular findings in stored blood for transfusion

To show the summary of the cellular findings in the progressing storage period of blood, the minimum and maximum range of white blood cell (WBC) counts, red blood cell (RBC) counts, haemoglobin (HGB) levels, mean cell volume (MCV), hematocrit (HCT), Mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and platelet (PLT) counts were tabulated at baseline (Day0), First week (Day7), second week (Day 14), third week (Day21), fourth week(Day 28) and firth week (Day 35) of storage as shown in *table 4.1*.

Table 4.1 Summary (range) of the cellular findings in stored blood at Bungoma County Referral Hospital, Kenya, February to August 2019.

Parameter	Day0	Day7	Day14	Day21	Day28	Day35
WBC	3.5-10.8×10^9/L	2.3-6.9×10^9/L	1.4-5.7×10^9/L	1.6-5.3×10^9/L	1.1-4.3×10^9/L	1.3-3.8×10^9/L
RBC	4.30-7.13×10^{12}/L	4.25-6.88×10^{12}/L	4.12-6.16×10^{12}/L	4.11-5.96×10^{12}/L	4.09-5.77×10^{12}/L	3.98-5.63×10^{12}/L
HGB	12.9-20.2g/dL	13.2-19.7g/dL	13.4-18.9g/dL	12.1-19.4g/dL	13.6-18.4g/dL	13.5-17.8g/dL
MCV	57.2-83-8fL	63.7-86.0fL	66.0-86.3fL	68.6-86.3fL	68.7-87.3fL	68.9-91.3fL
HCT	21.5-64.9%	23.2-61.1%	28.2-53.7%	20.0-81.2%	28.5-65-6%	24.1-72.0%
MCH	17.5-32.3pg	18.6-32.0pg	25.0-55.3pg	23.7-63.0pg	23.7-65.0pg	22.8-70.0pg
MCHC	33.1-55.0g/L	30.9-75.6g/L	33.0-73.0g/L	30.5-56.8g/L	30.2-49.8g/L	31.7-46.2g/L
PLT	66-434×10^9/L	69-312×10^9/L	54-269×10^9/L	50-211×10^9/L	48-201×10^9/L	40-188×10^9/L

Baseline=Day0, week1=Day7, week2=Day14, week3=Day21, week4=Day28, week5=Day35

4.1.2 Trends of cellular changes in stored blood for transfusion

To show the trends of the cellular changes in the progressing storage period of blood, the mean of WBC counts, RBC counts, HGB levels, MCV, HCT, and PLT counts were measured at baseline (Day0), First week (Day7), second week (Day 14), third week (Day21), fourth week (Day 28) and firth week (Day 35) of storage for the samples and were illustrated using a line graph. The white blood cell count demonstrated a decreasing trend from $5.85x10^9/L \pm 0.41$ to $2.12 \pm 0.14x10^9/L$, RBC count demonstrated a slightly decreasing trend from $5.36x10^{12}//L \pm 0.41$ to $4.91x10^{12}/L \pm 0.40$, HGB level demonstrated a slightly increasing trend from $15.47g/dL \pm 0.45$ to $15.89g/dL \pm 0.25$ while platelet count demonstrated a decreasing trend from $188.90x10^9/L \pm 19.67$ to$93.80x10^9/L \pm 8.26$ respectively as shown in *figure 4.1*.

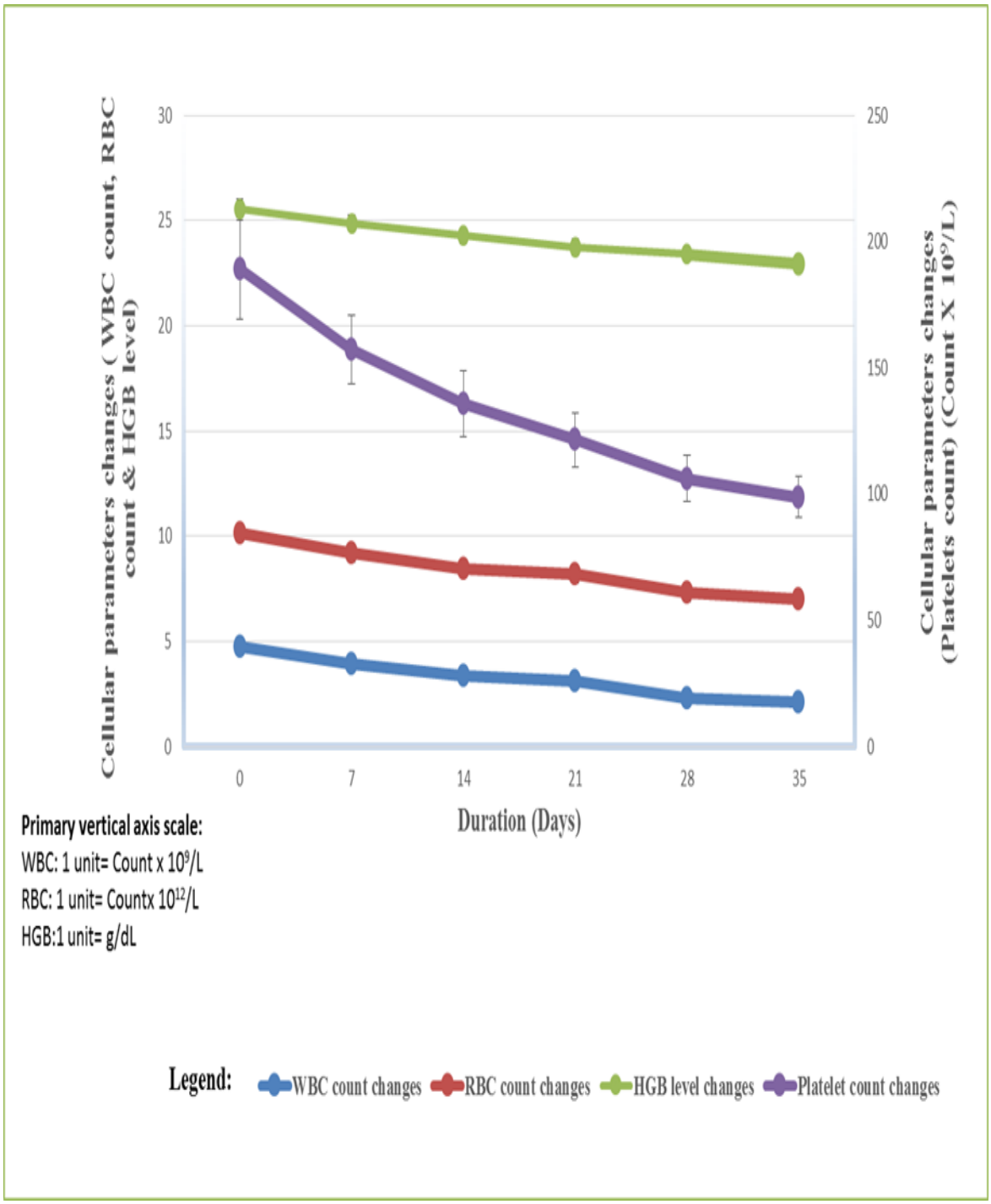

Figure 4.9: *The trend of WBC, RBC, and HGB changes in the progressing storage period of transfusion blood at Bungoma County Referral Hospital, Kenya. (**Key**: WBC=White Blood Cell (Count×10^9/L), RBC=Red Blood Cell (Count×10^{12}/L) and HGB= Haemoglobin (g/dL).*

Trends of Red blood indices were also evaluated and the findings are presented in figure 4.2 below. The MCV demonstrated an increasing trend of 71.14.25fL ± 1 to 83.21.10fL ± 1; HCT demonstrated a slightly increasing trend of 38.08% ± 2.73 to 44.33% ± 2.49; MCH demonstrated a slightly increasing trend of 27.42pg ± 0.71 to 33.49pg ± 2.17 while MCHC demonstrated a slightly decreasing trend of 41.14g/L ± 1.27 to 36.08g/L ± 1.01 as shown in *figure 4.2.*

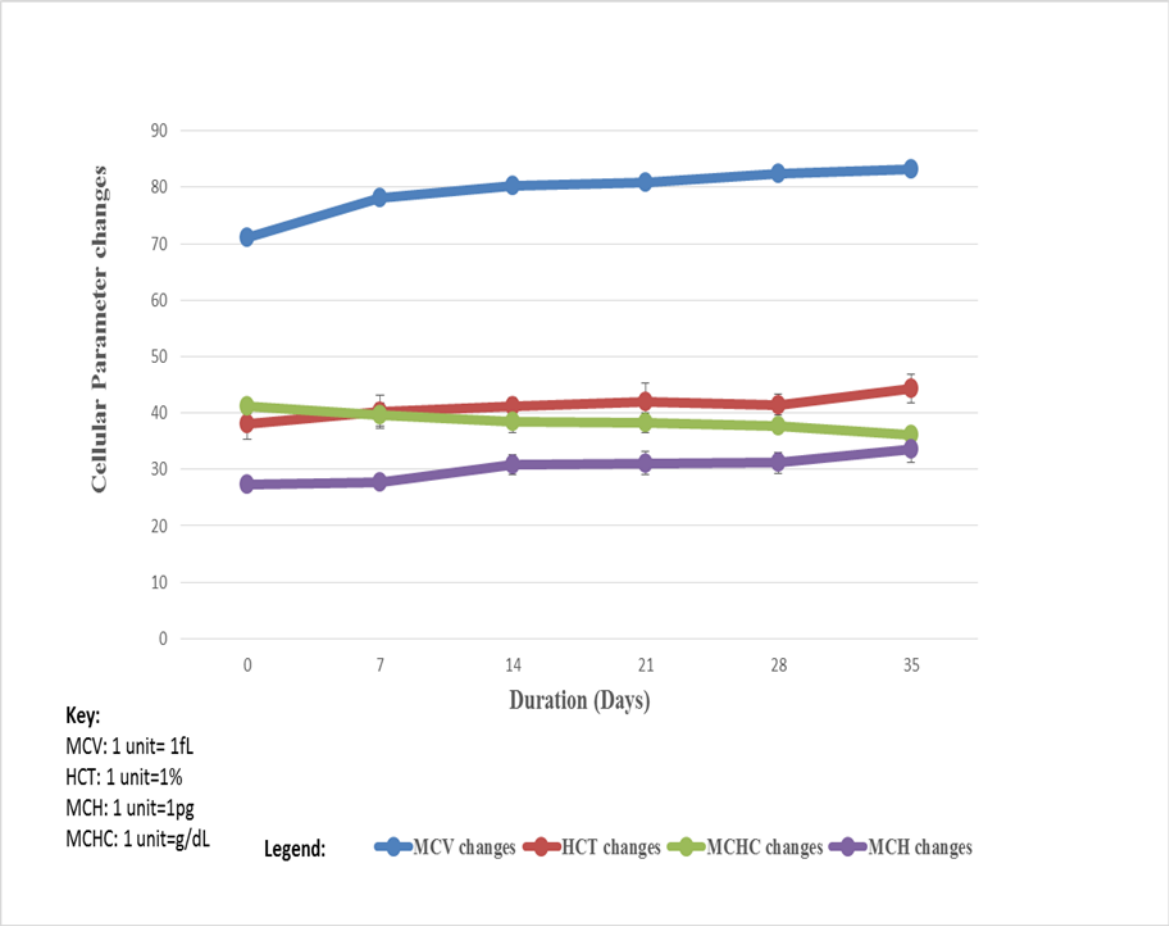

Figure 4.10: *The trend of red blood indices (MCV, HCT, and MCH &MCHC) changes in the progressing storage period of transfusion blood at Bungoma County Referral Hospital, Kenya. (**Key**: MCV= Mean Cell Volume (fL), HCT= Haematocrit) (%), MCH=Mean Cell Haemoglobin (pg) and MCHC= Mean Cell Haemoglobin Concentration (g/dL).*

4.1.3 Cellular changes in progressing blood transfusion storage period

White blood cell count is affected by storage at 2-6°C right from the 14th day of storage as shown in *table 4.2.* Red blood cell count is affected by storage from the 35th day of storage as shown in *table 4.2*. There were no significant changes in haemoglobin level at each testing time point as shown in *table 4.2.* Mean cell volume is affected by storage at 2-6°C from the 7th day of storage as shown in *table 4.2*. Haematocrit is affected by storage at 2-6°C right from the 28th day of storage as shown in *table 4.2*. Mean cell haemoglobin is affected by storage from the 35th day of storage as shown in *table 4.2.* Mean cell haemoglobin concentration is affected by storage from the 35th day of storage as shown in *table 4.2.* While platelets count is affected by storage right from the 14th day as shown in *table 4.2.*

Table 4.2: The Cellular variance (change) throughout the blood storage period at Bungoma County Referral Hospital, Kenya, February to August 2019.

		Day0 compared to the normal reference interval median	Day7 compared to baseline	Day14 compared to baseline	Day21 compared to baseline	Day28 compared to baseline	Day35 compared to baseline
WBC	Mean±SEM	5.85×10^9/L±0.41	3.91×10^9/L±0.27	3.35×10^9/L±0.26	3.14×10^9/L±0.23	2.28×10^9/L±0.18	2.12×10^9/L±0.14
	F	3.90	2.90	2.33	11.89	30.53	32.63
	P-value	**0.0777**	**0.965**	**0.0062**	**0.0014**	**<0.0001**	**<0.0001**
RBC	Mean±SEM	5.36×10^{12}/L±0.41	5.26×10^{12}/L±0.41	5.10×10^{12}/L±0.40	5.03×10^{12}/L±0.40	5.01×10^{12}/L±0.40	4.91×10^{12}/L±0.40
	F	0.20	0.21	1.85	3.20	3.77	6.09
	P-value	**0.3222**	**0.6473**	**0.81814**	**0.0816**	**0.0597**	**0.0182**
HGB	Mean±SEM	15.47g/dL±0.45	15.72g/dL±0.39	15.82g/dL±0.36	15.57g/dL±0.41	16.10g/dL±0.31	15.89g/dL±0.25
	F	1.10	0.18	0.37	1.32	1.32	0.67
	P-value	**0.6765**	**0.5456**	**0.2578**	**0.2578**	**0.2578**	**0.4185**
MCV	Mean±SEM	71.14fL±1.0	78.14fL±0.97	80.26fL±1.00	80.94fL±1.04	82.33fL±1.14	83.21fL±1.25
	F	0.83	17.12	31.31	37.39	50.15	52.53
	P-value	**0.3676**	**0.0002**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
HCT	Mean±SEM	38.08%±2.73	40.21%±2.94	41.16%±1.58	40.92%±3.37	41.48%±1.78	44.33%±4.49
	F	1.89	1.86	4.00	3.36	5.15	16.30
	P-value	**0.1768**	**0.1810**	**0.0528**	**0.0707**	**0.0289**	**0.0003**
MCH	Mean±SEM	27.42pg±0.71	27.73pg±0.65	30.83pg±1.71	31.10pg±2.07	31.15pg±1.92	33.49pg±2.17
	F	8.49	3.36	3.36	2.82	3.30	7.05
	P-value	**0.0060**	**0.07544**	**0.0745**	**0.1013**	**0.0772**	**0.0115**
MCHC	Mean±SEM	41.14g/L±1.27	39.73g/L±2.11	38.50g/L±2.00	38.28g/L±1.72	37.74g/L±1.38	36.08g/L±1.01
	F	27.25	0.33	1.24	1.79	3.41	9.70
	P-value	**<0.0001**	**0.5716**	**0.2720**	**0.1885**	**0.0724**	**0.0035**
PLT	Mean±SEM	188.90×10^9/L±19.67	157.25×10^9/L±13.78	135.70×10^9/L±12.91	121.40×10^9/L±10.60	10.00×10^9/L±9.10	93.80×10^9/L±8.26
	F	14.97	1.74	5.11	9.12	14.63	19.87
	P-value	**0.0004**	**0.1955**	**0.0296**	**0.0045**	**0.0005**	**0.0001**

Baseline=Day0, week1=Day7, week2=Day14, week3=Day21, week4=Day28, week5=Day35

4.2 Biochemical changes in stored blood for transfusion

4.2.1 Summary of the biochemical findings in stored blood for transfusion

To show the range of the biochemical in progressing storage period of transfusion blood, the Potassium (K+) level, Sodium (Na+) level, and pH were measured at baseline (Day0), First week (Day7), second week (Day 14), third week (Day21), fourth week(Day 28) and fifth week (Day 35) of storage as in *table 4.3*.

Table 4.3: Summary (range) of the biochemical findings in stored blood at Bungoma County Referral Hospital, Kenya, February to August 2019.

Parameter	Day0	Day7	Day14	Day21	Day28	Day35
Potassium	5.64-9.75 mmol/L	7.36-12.6 mmol/L	10.09-17.54 mmol/L	11.78-22.01 mmol/L	11.39-22.96 mmol/L	13.6-25.1 mmol/L
Sodium	147.0-159.2 mmol/L	143.8-148.4 mmol/L	131.6-141.0 mmol/L	123.5-139.5 mmol/L	113.4-136.0 mmol/L	108.3-130.1 mmol/L
pH	7.27-7.81	7.11-7.74	7.00-7.63	6.04-6.74	6.00-6.70	5.90-6.56

Baseline=Day0, week1=Day7, week2=Day14, week3=Day21, week4=Day28, week5=Day35

4.2.2 Trends of biochemical changes in stored blood for transfusion

To show the trends of the biochemical changes in the progressing storage period of transfusion blood, the Potassium (K+) level, Sodium (Na+) level, and pH were measured at baseline (Day0), First week (Day7), second week (Day 14), third week (Day21), fourth week (Day 28) and fifth week (Day 35) of storage. The trend of changes was illustrated using a line graph as shown in figure 4.3. Potassium (K+) levels demonstrated an increasing trend from 7.31mmol/L±0.25 to 20.14mmol/L±0.70, while sodium (Na+) levels and pH demonstrated a decreasing trend from150.72mmol/L±0.56 to 121.56mmol/L±1.35) and from 7.48±0.04 to 6.15±0.04) respectively as the storage period progressed as shown in *figure 4.3.*

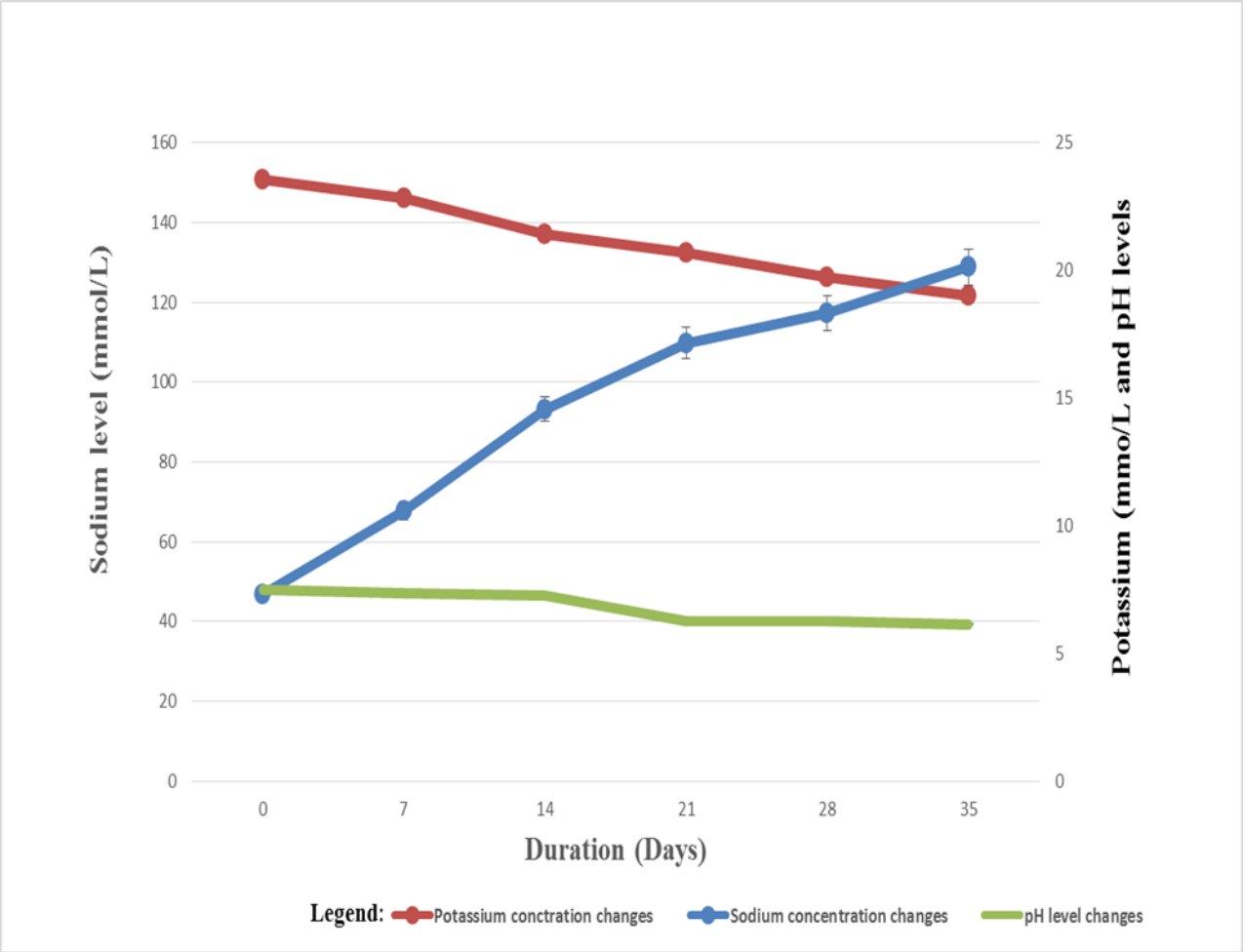

Figure 4.11: *The trend of Potassium ions (mmol/L), Sodium (mmol/L), and pH changes in progressing storage period of transfusion blood at Bungoma County Referral Hospital, Kenya.*

This study observed significant Potassium (K+) change at the baseline compared to the normal reference interval median of 4.5mmol/L±0.32. Potassium also demonstrated a significant variance with an increase throughout the blood storage period compared to the baseline mean of 7.31mmol/L±0.25 as shown in *table 4.4.* Sodium (Na+) concentration demonstrated insignificant variance at the baseline compared to the normal reference interval median of 150mmol/L±1.58, it, however, had a significant decrease throughout the blood storage period from Day7 compared to the baseline as shown in *table 4.4*. The pH level demonstrated insignificant variance at baseline compared to the normal reference interval median of 7.4±0.02 and with significant variance from

Day14 which decreased significantly to Day 35 of the storage period compared to the baseline 7.31 ±0.04 as shown in *table 4.4.*

Table 4.4: The biochemical variance (change) throughout the blood storage period at Bungoma County Referral Hospital, Kenya, February to August 2019.

		Day0 compared to the normal reference interval median	Day7 compared to baseline	Day14 compared to baseline	Day21 compared to baseline	Day28 compared to baseline	Day35 compared to baseline
Potassium (K+)	Mean± SEM	7.31mmol/L ±0.25	10.59mmol/ L±0.37	14.57mmol/ L±0.49	17.15mmol/ L±0.62	18.33mmol/ L±0.69	20.14mmol/ L±0.70
	F	128.62	54.06	176.82	219.81	22290	296.40
	P-value	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
Sodium (Na+)	Mean± SEM	150.72mmol/ L±0.56	146.16mmol/ L±0.33	137.21mmol/ L±0.54	132.53mmol/ L±1.07	126.26mmol/ L±1.35	121.56mmol/ L±1.35
	F	1.62	49.04	300.75	225.12	278.72	400.18
	P-value	***0.2102***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***	***<0.0001***
pH	Mean± SEM	7.48±0.04	7.38±0.04	7.26±0.04	6.45±0.05	6.27±0.04	6.15±0.04
	F	4.41	33	15.51	279.82	446.44	570.09
	P-value	***0.4826***	***0.0757***	***0.0003***	***<0.0001***	***<0.0001***	***<0.0001***

Baseline=Day0, week1=Day7, week2=Day14, week3=Day21, week4=Day28, week5=Day35

4.3 Bacterial contamination in stored blood for transfusion

Two out of twenty studied blood pints grew *bacteria* at day zero, which equivalent to 10% while one unit grew bacteria at day 28 and day 35 respectively, which was equivalent to 5%.

4.3.1 Colonies morphologies findings

To show colonies morphologies inoculum from blood pints with theoretical availability of viable bacteria detected by Bactec Fx40 instrument blood culture were sub-cultured on blood and

MacConkey solid agar. The colonies on solid blood agar media had a golden appearance with some haemolysis while there was no growth on solid MacConkey agar as shown in *figures 4.4.*

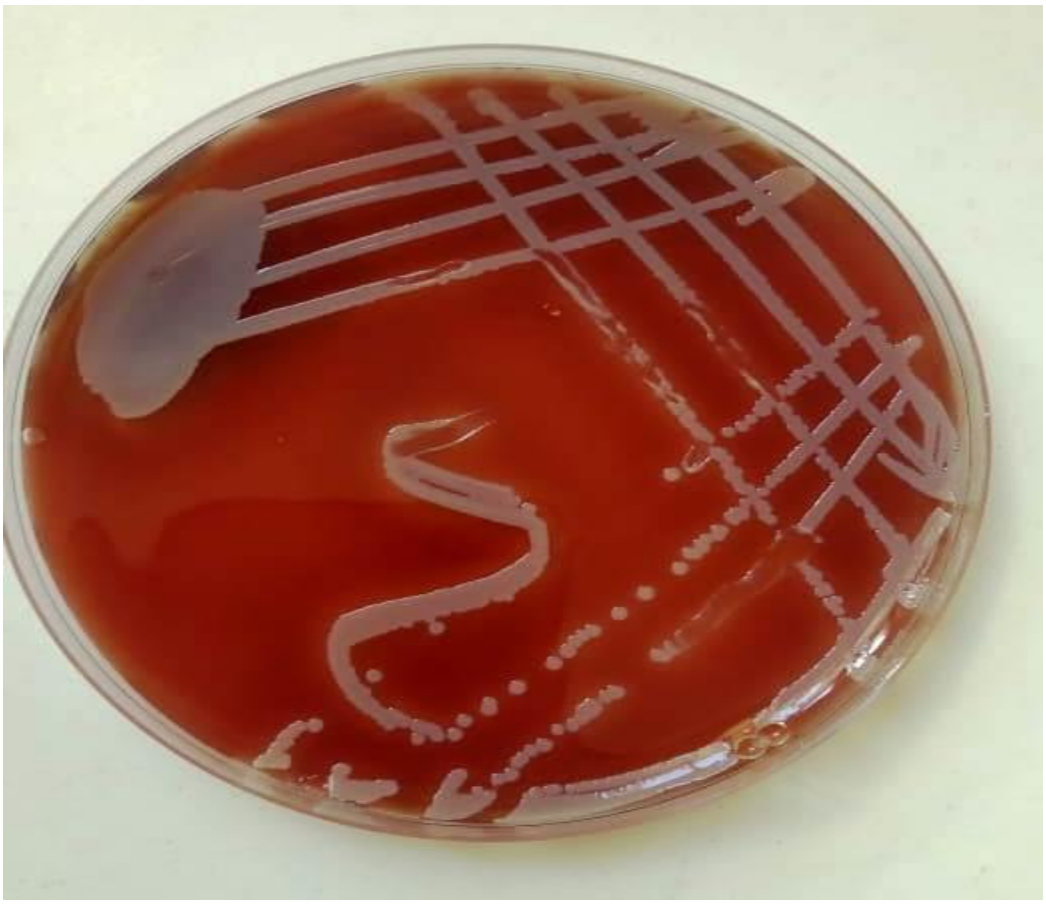

Figure 4.12: *Staphylococcus aureus growth and colony appearance on solid blood agar at Bungoma County Referral Hospital, Kenya. (Photo courtesy of Bungoma County Referral Hospital Laboratory).*

4.3.2 Gram staining findings

To show the Gram stain reaction of the isolated micro-organisms, the smears were stained with Gram stain reagents. The stained slides demonstrated cocci in clusters with purple appearance a characteristic of *S. aureus* as shown in *figures 4.5*

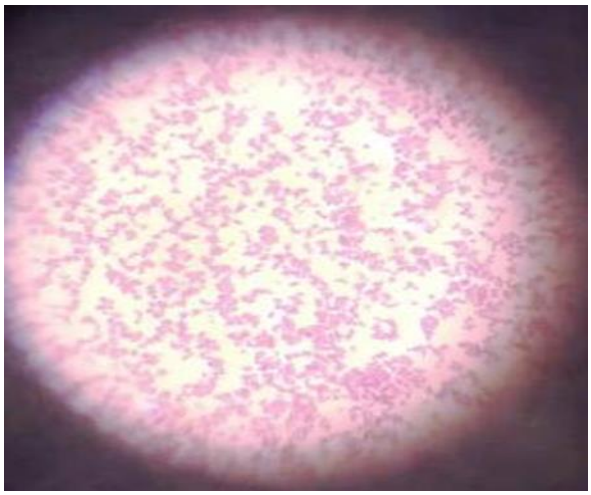

Figure 4.13: *Gram stain film showing Gram Positive cocci in clusters at Bungoma County Referral Hospital, Kenya. (Photo courtesy of Bungoma County Referral Hospital Laboratory).*

4.3.3 Biochemical tests reactions findings

To show the isolates' reaction to various biochemical tests, the isolates were subjected to various biochemical tests and the outcomes were characteristics of *Staphylococcus aureus*. This included positive catalase and coagulase reaction as shown in *table 4.5 and figures 4.6 and 4.7*

Table 4.5: Biochemical test reaction outcomes at Bungoma County Referral Hospital, Kenya, February to August 2019.

Isolate	**Catalase test**	**Coagulase test**
Day 0 isolate 1	+	+
Day 0 isolate 2	+	+
Day 28 isolate	+	+
Day 35 isolate	+	+

Key: += Positive reaction

Figure 4.14: *Catalase biochemical test showing a positive reaction at Bungoma County Referral Hospital, Kenya. (Photo courtesy of Bungoma County Referral Hospital Laboratory).*

Figure 4. 15: *Coagulase biochemical test showing a positive reaction at Bungoma County Referral Hospital, Kenya. (Photo courtesy of Bungoma County Referral Hospital Laboratory).*

The study did not identify any other bacteria. All the bacterial species isolated were Gram-positive *Staphylococcus aureus* on day zero, day 28, and day 35 as shown in *table 4.6.*

Table 4.6: Pathogens isolated during the blood storage period at Bungoma County Referral Hospital, Kenya, February to August 2019.

Probable bacteria (N=20)	Day 0	Day 7	Day 14	Day 21	Day 28	Day 35
Klebsiella pneumonia	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-
Yersinia entocolitica	-	-	-	-	-	-
Pseudomonas fluorescens	-	-	-	-	-	-
Staphylococcus aureus	+,+	-	-	-	+	+
coagulase-negative *Staphylococci species*	-	-	-	-	-	-

KEY: *+= Bacteria isolated -= Bacteria not isolated*

This study also determined the antibiotics sensitivity and resistance pattern of isolated bacteria, the antibiotic susceptibility test was performed and the findings were as shown in *table 4.7*

Table 4.7: The antibiotic sensitivity and resistance pattern for isolated bacteria in blood for transfusion during storage at Bungoma County Referral Hospital, Kenya.

Isolate	**Penicillin 10 units**				
	Isolate measurement in mm ± SD	Sensitive measurement range in mm	Intermediate measurement range in mm	Resistant measurement range in mm	Interpretation (S, I, R)
Day 0 isolate 1	8mm ± 1.4	≥29mm	-	≤28mm	R
Day 0 isolate 2	14mm ± 0.56	≥29mm	-	≤28mm	R
Day 28 isolate	7mm ± 0.85	≥29mm	-	≤28mm	R
Day 35 isolate	9mm ± 0.14	≥29mm	-	≤28mm	R
Isolate	**Cefazolin 30µg**				
	Isolate measurement in mm	Sensitive measurement range in mm	Intermediate measurement range in mm	Resistant measurement range in mm	Interpretation (S, I, R)
Day 0 isolate 1	23mm ± 1.4	≥15mm	-	≤14mm	S
Day 0 isolate 2	10mm ± 1.13	≥15mm	-	≤14mm	S
Day 28 isolate	26mm ± 0.42	≥15mm	-	≤14mm	S
Day 35 isolate	20mm ± 0.28	≥15mm	-	≤14mm	S
Isolate	**Clindamycin 2µg**				
	Isolate measurement in mm	Sensitive measurement range in mm	Intermediate measurement range in mm	Resistant measurement range in mm	Interpretation (S, I, R)
Day 0 isolate 1	24mm ± 0.14	≥21mm	15-20mm	≤14mm	S
Day 0 isolate 2	12mm ± 1.4	≥21mm	15-20mm	≤14mm	S
Day 28 isolate	28mm ± 0.71	≥21mm	15-20mm	≤14mm	S
Day 35 isolate	24mm ± 0.84	≥21mm	15-20mm	≤14mm	S
Isolate	**Oxacillin 30µg**				
	Isolate measurement in mm	Sensitive measurement range in mm	Intermediate measurement range in mm	Resistant measurement range in mm	Interpretation (S, I, R)
Day 0 isolate 1	10mm ± 1.3	≥22mm	-	≤24mm	R
Day 0 isolate 2	7mm ± 0.28	≥22mm	-	≤24mm	R
Day 28 isolate	9mm ± 0.99	≥22mm	-	≤24mm	R
Day 35 isolate	12mm ± 0.85	≥22mm	-	≤24mm	R

Key: I=Intermediate, mm=Millimeters, R=Resistance, S=Sensitive

4.3.4 Rate of bacterial contamination in progressing storage interval of transfusion blood

To show the rate of the bacterial contamination in progressing storage interval of transfusion blood, bacterial contamination percentage in stored blood was calculated at Day 0, Day 7, Day 14, Day 21, Day 28, and Day 35 of storage. The changes were illustrated using a bar graph (*Figures 4.8*).

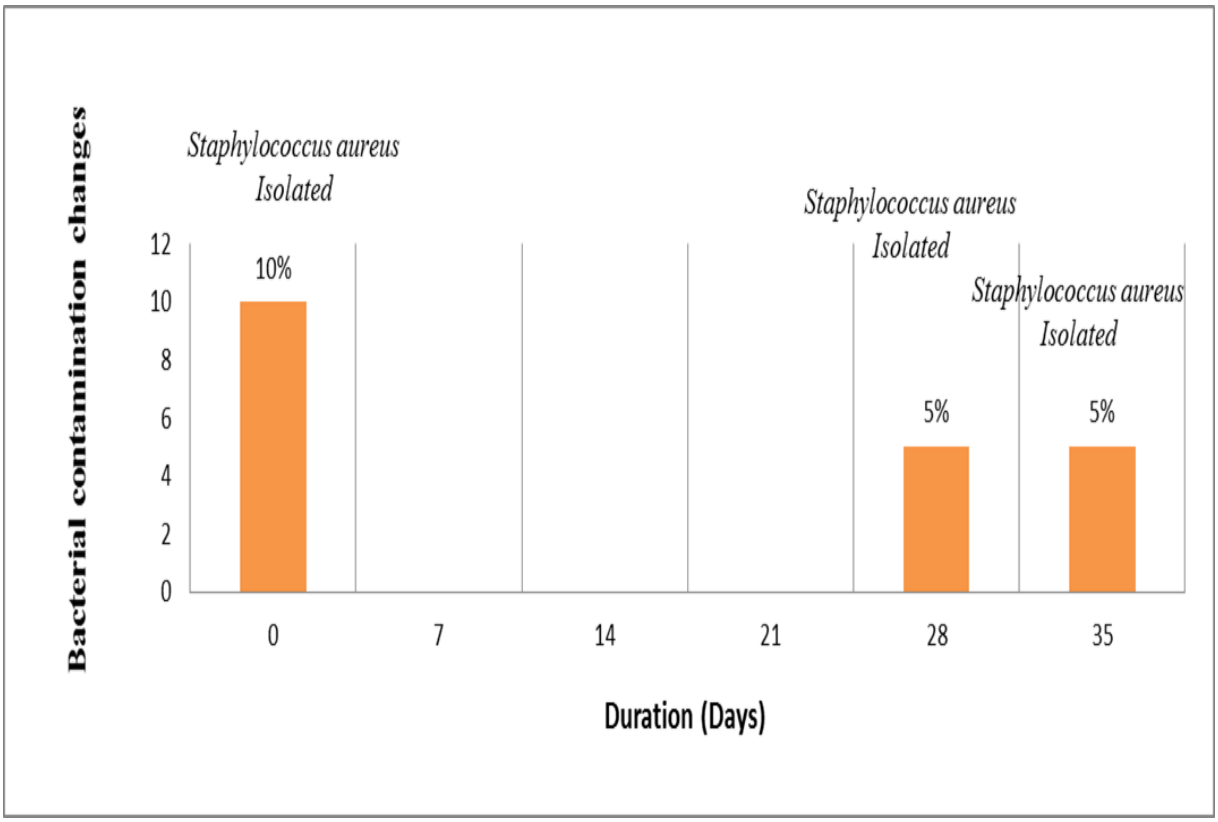

Figure 4.16: *The percentage rate of bacterial contamination in progressing storage period of transfusion blood at Bungoma County Referral Hospital, Kenya.*

CHAPTER FIVE

5.0 DISCUSSION

This study sought to understand the cellular changes, biochemical changes, and bacterial contamination in whole blood for transfusion stored for 35 days at 2-6°C. Regardless of the advantages of transfusion (a potent step to stop anaemia, prepare patients for incision procedures, and minimize manifestations of blood loss), it may manifest danger to patients (Oyet et al., 2018). There are assorted troublesome repercussions of transfusion in disregard of careful laboratory applications in processing and cross-matching the donors and the recipients. The unintended consequences might be a result of changes in the usual micro-environment of the cells in the course of blood storage.

The present study has shown that there are cellular changes during the storage period. The white blood cells count changes were non-significant after one week of storage. However significant reduction was observed after 14 days which further decreased significantly through to 35 days of storage. These results, therefore, indicate that; White blood cells during storage are significantly altered by the 7^{th} day. Factors that contribute to the white blood cells reduction during blood for transfusion storage could be loss viability because of ATP depletion. More so, leukocytes are used in the formation of white blood cell- platelet micro-aggregates, which are a mixture of white blood cells, platelets, fibrin, cold globulin, and cellular debris formed during storage (Ahmed, 2008). The clinical significance of this finding is that stored blood for transfusion could be particularly ineffective as a clinical tool in the management of aplastic anaemia and other leucopenic patients since the most critical establishment in these conditions is almost always neutropenia (Batham, 2018). These findings do compare with findings of a study done in Braithwaite Memorial Specialist Hospital (BMSH), Port Harcourt, Rivers State, Nigeria which demonstrated that at 28

days, there were significant changes in white blood cell differential and absolute counts (Teddy Adias, 2012). These results also concurred with the findings of another study carried out in Aminu Kano Teaching Hospital, Kano, Nigeria which illustrated that the percentage fall from day-zero to day-thirty five was 97% for white blood cells (Ahmed, 2008). These findings also concurred with findings of another study done in the Veterinary Transfusion Research Laboratory, 85 University of Milan, Italy which showed that there was a statistically significant drop in WBC count after storage for 35 days of transfusion blood (Eva Spada, Roberta Perego, Luciana Baggiani, 2018). Another study done in L. N. Medical College and J. K. Hospital, Bhopal, India which demonstrated that WBC count constantly decreased throughout the 28 days storage period also concurred with these findings (Bhargava, Gupta, Vivek, & Khare, 2016). To the strength of the findings from the present study, white blood cells count monitoring during blood for transfusion storage intending to improve blood transfusion efficacy and safety and is recommended.

In the current study, the red blood cells count changes were insignificant up to three weeks (21 days) of storage. However significant reduction was observed at day 28 and further significantly decreased through to 35 days of storage. These results, therefore, indicate that; Red blood cells during storage are significantly altered by the 28^{th} day. The current findings can be explained by the fact that the systemic and biochemical changes that red blood cells go through in the course of storage are anticipated to be instrumental to the drop in red blood cell count as storage span increase (García-Roa, Del Carmen Vicente-Ayuso, Bobes, 2017). During blood storage at 2-6°C, glycolysis is reduced and as acid level increase, the amount of ATP reduce, and the structure of the red cell is slowly changed from discoid to echinocytic shape (Mustafa, Marwani, Mamdouh Nasr, Abdulla Kano, 2016). If there is a shortage of ATP, then the pumps (co-transporters) may be unable to maintain the ionic homeostasis of the cell, leading to changes in red blood shape and

volume (Orlov, 2015). The number of undamaged red blood cells that remain in a prolonged-stored blood unit before transfusion is not known and warrants additional research. A human red blood cell has a lifecycle of about one hundred and twenty days (Arias, 2017). In normal conditions, about 2.4 million new red blood cells are generated per second with the concomitant eviction of an equivalent quantity of senescent red blood cells from the bloodstream. Hence, human blood constitutes reds blood cells that vary from zero to one hundred and twenty days of age, which is identical to a pint of freshly collected blood. (Wei-Wei Tuo, Di Wang, 2014). This experience may likely be suggestive of some level of cell selection where older and more labile cells die initially rapidly, thus leaving a cohort of younger and more stable cells that die later at a much slower rate (Ahmed, 2008). The clinical significance of these findings is that adjustment in architecture from basic bio-concave rings to echinocytic red blood cells makes the cells easier to clump, increasing the possibility of blocking the microcirculation, leading to tissue ischemia (Adam, 2015). These less elastic red blood cells are not able to cross tiny micro-vessels of the micro-circulation, leading to reduced oxygen transport since the aerated red cells can't cross the end organ capillary beds (Yalcin, 2014). Transfused stored RBCs can provoke a pro-inflammatory response by the cytokines and eicosanoids. Storage lesions also promote adhesion to endothelial cells, complement system activation, and changes in coagulability. These effects also damage the endothelial lining to cause capillary leakage (Yoshida, 2019). The pro-inflammatory nature of stored RBCs has been correlated with an increased fatality, multiple organ failure, thrombosis, and protracted hospital stay (Hod & Spitalnik, 2011).

These findings compare with findings from the previous study done in Port Harcourt, Rivers State, Nigeria which showed no statistically significant changes in red blood cell count in the course of the 28 days storage period (Teddy Adias, 2012). These findings also compare with the findings of

a study conducted in Bhopal, India which demonstrated no significant changes in red blood cell count during the 28 days storage period (Bhargava et al., 2016). These findings also compare with the findings of a study conducted in Rohtak, India which demonstrated no significant changes in red blood cell count during the 28 days storage period (Sonia Chhabra, Saurav Chaudhary, Sehgal, Sunita Singh & Sen, 2017). However, the finding of this study contradicts the findings of a study done in L. N. Medical College and J. K. Hospital, Bhopal, India which showed that RBC count increased during the 28 day storage period (Bhargava et al., 2016). These findings also differ with the findings of a study done in São João Hospital, Porto, Portugal which showed that the RBC count kept unchanged throughout the 42 days of storage (Diana Noguira, Susana Rocha, Estela Abreu, 2015). These findings also differ from the findings of a study done in Sanjay Gandhi Memorial Hospital, Rewa, India which demonstrated that red blood cell count showed no significant change during the 35 days storage period (Batham, 2018). In light of the findings from the present study, red blood cells count monitoring during blood for transfusion storage intending to improve blood transfusion efficacy and safety is recommended.

In the current study, haemoglobin level estimation demonstrates an insignificant increase throughout the blood storage period. The slight increase in Haemoglogin level can be explained by the fact that during storage, the byproducts of glycolytic metabolism, lactic acid, and proteins accrue, which in *vivo* are readily removed from the bloodstream, remain and give rise to physical changes and cell lysis releasing unbound haemoglobin into plasma (Arif, Yadav, Rehman, 2017). The clinical significance of these findings is that transfusing older pints with unbound haemoglobin has transfusion-related harm especially for patients who have a history of unbound haemoglobin in their circulation (Vermeulen, de Wit, Sertorio, 2012). Unbound haemoglobin may trigger vasoconstrictive, pro-oxidative, and pro-inflammatory events that have transfusion-related

harm to the transfused patient (Yoshida, 2019). Our findings do compare with a previous study in São João Hospital, Porto, Portugal which showed that the haemoglobin amount remained un-varied in the course of the 42 days of reservation (Diana Noguira, Susana Rocha, Estela Abreu, 2015). However, our findings contrast a previous study done in the Department of Pathology, S.S. Medical College Rewa, and India which demonstrated that haemoglobin concentration gradually decreased during the 35 day storage period (Batham, 2018). In light of the findings from the present study, haemoglobin monitoring during blood for transfusion storage and conditions of patients that might lead to the release of unbound haemoglobin in their circulation need be considered before indicating a transfusion to improve blood transfusion efficacy and safety and is recommended.

In the current study, MCV demonstrates a significant increasing trend from day0 to day 35 storage period. These findings, therefore, indicate that; MCV is significantly increased during storage. The current findings can be explained by the fact that the water influx to the cytosol giving rise to the swelling of erythrocytes during reservation and the non-existence of selective channels of performance, as done in the spleen, could describe the elevation of the mean cell volume in vitro and successive structural red blood cells changes(Shohag, Mohammad Raguib Munif, Nargis Jahan & Alam, 2020). The clinical significance of the present findings is that the cell mechanical properties and blood rheology are affected compromising blood hemodynamics, O_2 delivery, and the interaction between flowing blood and the vasculature (Yalcin et al., 2014). These findings compare with previously documented findings in São João Hospital, Porto, Portugal which demonstrated that MCV elevated notably from day 0 to day 21 and kept steady to the end of the storage period (Diana Noguira, Susana Rocha, Estela Abreu, 2015). Our findings also compared with another study done in Iran which demonstrated that MCV increased during storage of blood for transfusion (Ghezelbash, Azarkeivan, Pourfathollah, Deyhim, Hajati, 2018). However, these

results are in contrast with the result documented in a study done in Braithwaite Memorial Specialist Hospital (BMSH), city of Port Harcourt, Rivers State, Nigeria. which demonstrated that MCV had insignificant change throughout the 28 days storage period (Teddy Adias, 2012). In consideration of the findings from the present study, MCV monitoring during blood for transfusion storage to improve blood transfusion effectiveness and safety is advocated for.

In the present study, HCT demonstrates a slightly increasing trend with the significant change being noted from day 14 of the storage period and continues throughout the remaining period of storage time. The current findings can be explained by the fact that the increase in haematocrit reflects the morphological alterations that take place during blood storage (Bosman et al., 2008). The clinical significance of these findings is that increased morphological alteration minimizes the potency of transfused blood by increasing the speed of elimination of transfused cells by the macrophage (Oyet et al., 2018). These results compare with previously documented findings São João Hospital, Porto, Portugal which demonstrated that HCT increased from day 0 to day 14 and remained stable afterward (Diana Noguira, Susana Rocha, Estela Abreu, 2015). Our findings also compare with findings from another study done in Doha, Qatar that demonstrated a significant HCT increase after 35 days of blood storage (Mustafa, Marwani, Mamdouh Nasr, Abdulla Kano, 2016). However, the current study contrasts the findings of a study done in Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria. which showed a significant fall of haematocrit (Oluyombo, Oluyombo, Uchegbwu, Adegbamigbe, 2013). Our findings also contrast findings of a study done conducted in Braithwaite Memorial Specialist Hospital (BMSH), city of Port Harcourt, Rivers State, Nigeria which demonstrated an insignificant change of HCT throughout the 28 days storage period (Teddy Adias, 2012). To the strength of the findings from

the present study, HCT monitoring during blood for transfusion storage and use of blood less than 14 days to improve blood transfusion efficacy is advocated for.

In the current study, MCH and MCHC demonstrate insignificant variance from day 0 to day 28; it, however, demonstrates significant variance (increase and decrease respectively) at day 35 of storage time. The changes in MCH and MCHC appear to be the result of a deregulated mechanism of cell volume, which expounds the increase in the volume of the RBCs, the increasing hypocromia, and the anisocytosis. Hence, the decrease in MCHC does not result from a reduction in hemoglobin concentration but an increase in cell volume (Diana Noguira, Susana Rocha, Estela Abreu, 2015). These results compare with those findings documented in a study done in Braithwaite Memorial Specialist Hospital (BMSH), city of Port Harcourt, Rivers State, Nigeria which showed that the MCH and MCHC changes were insignificant during the 28 days of storage(Teddy Adias, 2012). However, the results contrast findings of a study done in Iran which demonstrated that MCH decreased during the storage period (Ghezelbash, Azarkeivan, Pourfathollah, Deyhim, Hajati, 2018). In light of the findings from the present study, MCH and MCHC monitoring during blood for transfusion storage and use of fresh blood less than 28 days with a focus on improving blood transfusion efficacy is recommended.

In the present study, Platelets demonstrates a significant decrease from day 14 and continues throughout the 35days storage period. The current findings can be explained by the fact that the cell loose viability owing to ATP depletion in addition to platelet utilization due to micro-aggregates development (Ahmed, 2008). The clinical significance of these findings is that this may expose patients to possible decreases in platelets effectiveness as well as likely increases in adverse incidences in addition to transfusion-related sepsis, such as inflammation and/or immune-mediated incidences. Seriously sick patients, including post-cardiac surgery patients and

haematology/oncology patients, may be specifically vulnerable to platelets' adverse incidences because of their pre-transfusion inflammatory state (Aubron, Flint, 2018). These results are in comparison with those obtained in a study done in Aminu Kano Teaching Hospital, Kano, Nigeria which demonstrated a constant decrease of platelets throughout the 35 days storage period (Ahmed, 2008). Our findings also compare with those results abstained in a study done in L. N. Medical College and J. K. Hospital, Bhopal, India which demonstrated that platelet decreased significantly during the storage period (Bhargava et al., 2016). Our findings also compare with the findings documented in a study done in Nigeria which demonstrated 86.2% platelet count fall from day 0 to day 28 storage times (Ahmed, 2008). Our findings, however, contrast the findings of a study done in Nigeria which demonstrated insignificant platelet count variance throughout the 28 days storage period (Teddy Adias, 2012)**.** In light of the findings from the present study, platelet count monitoring during blood for transfusion storage to improve blood transfusion efficacy and safety is recommended.

Overall, together with other parameters, cellular changes in stored blood for transfusion should be keenly monitored putting into consideration the patient to be transfused, and the clinical indication of the blood.

The current study sought to evaluate the biochemical changes in whole blood for transfusion stored at blood bank conditions. At the end of the 35 days of blood storage at blood bank conditions of 2-6°C, the Potassium level significantly increased from 7.31mmol/L±0.25 to 20.14mmol/L±0.70 while sodium level notably reduced from 150.72mmol/L±0.56 to 121.56 mmol/L±1.35 during the 35 days storage period. On the other hand, the pH level notably reduced from 7.48±0.04 to 6.15±0.04 during the 35 days storage period, however, the change was insignificant at week two of storage. Potassium level had a significant change at the baseline while sodium concentration

and pH had insignificant change. Regardless of the advantages of blood transfusion (a potent initiative to prevent anaemia, prepare patients for a surgical process, and limit manifestations of blood depletion), it may manifest danger to patients (Oyet et al., 2018). There are assorted troublesome after-effects of blood transfusion despite cautious laboratory methods in handling and cross-matching the donors and the recipient. The un-wished for consequences might be attributed to changes in the usual micro-environment of the cells in the course of blood conservation which includes the biochemistry of the blood.

The present study has denoted that there are biochemical changes during the 35 days storage period. Potassium (K+) ions increase was significant after one week and further increased significantly through to 35 days of storage. The current findings can be explained by the fact that cold condition of 2-8°C reduces the speed of cellular metabolism and energy need. This causes the $Na+/K^+$ ATPase pump not to function and in consequence allows K^+ ions to leave the cell and Na^+ ions to get into the cell through the semi-permeable membrane (Orlov, 2015). The clinical significance of these findings is that increased Potassium ions amounts in red blood cells might bring about a result of arrhythmia when babies or young children are transfused with a large amount of stored blood (Ahmed, 2008). It has also been documented that potassium efflux causes vesicle creation that may have a significant effect on the post-transfusion utility and side effects of stored blood (Burger e.tal, 2013). This result compares with a previous study in São João Hospital, Porto, Portugal which demonstrated that Potassium ions increased significantly during blood storage. (Diana Noguira, Susana Rocha, Estela Abreu, 2015). The findings of the present study also agree with the findings of a previous study done in Uganda which demonstrated that Potassium ions increased significantly during the 28 days storage period (Oyet et al., 2018). These findings also compare with the findings of a study done in Rohtak, India which demonstrated a

significant increase of Potassium ions during the 21 days storage period. The findings of this study also agree with a study done in Braithwaite Memorial Specialist Hospital (BMSH), city of Port Harcourt, Rivers State, Nigeria which demonstrated that potassium significantly increased throughout the 28 days storage period (Teddy Adias, 2012). These findings also compare with the findings of a study done in Bangalore, India which demonstrated Potassium ions increased during the 21 days of storage (Ujwal Upadya, Seema, 2018).

The current study observed an increase of plasma Potassium level to 10.59mmol/L±0.37 within the first week with an increased variance at the baseline compared to the normal reference values of potassium. The increase continued to 20.14mmol/L±0.070 at the end of the fifth week which was equivalent to a 12.83mmol/L rise after 35 days of storage. In the course of blood storage, there is a steady but continuous efflux of K^+ ions from cells into the encircling plasma (Teddy Adias, 2012). During critical kidney dis-order, even minute quantity of K^+ ions changes are potentially threatening, and proportionately recent or washed red cells are recommended. Potassium (K+) ions efflux is sanctioned to be resultant of the adjustments in the metabolic process with cooling (Teddy Adias, 2012). The depletion of DPG and lessened cellular respiration action are also correlated to dropping pH (Hess, 2006). The rapid effusion of K^+ ions from cells into encircling plasma is possibly accountable for the radical advancement in K^+ ions elevation in this study. To the strength of the findings from the present study, Potassium (K^+) ions concentration monitoring during blood for transfusion storage to improve blood safety is advocated for.

The current study observed that Sodium (Na^+) decrease was significant after two weeks (14 days) and further decreased significantly through to 35 days of storage. The present findings may be attributed to the fact that blood stored at blood bank conditions of 2-6° reduces the speed of cellular metabolism and energy demand which permits blood to be reserved for thirty-five to forty-two

days. This makes the sodium-potassium pump defective and as a result, permits potassium ions to depart the cell and sodium ions to move into the cells through the semipermeable membrane (Opoku-Okrah, Acquah, 2015). These findings do agree with previous studies in Rohtak, India which demonstrated that Sodium ions decreased significantly in the course of blood storage(Monica Verma et al., 2015). These findings also concur with the findings done in São João Hospital, Porto, Portugal which demonstrated that Sodium ions decreased significantly throughout the storage period (Diana Noguira, Susana Rocha, Estela Abreu, 2015). The findings of the study also compared with the findings of a study done in Komfo Anokye Teaching Hospital, Kumasi, Ghana which demonstrated that sodium levels decreased throughout the storage period (Opoku-Okrah, Acquah, 2015). The findings of the present study, however, contrasts with a study done in the Oxford Medical College Hospital and Research Centre, Bangalore, India which demonstrated that sodium did not show any significant changes due to storage time (Ujwal Upadya, Seema, 2018). The findings of the present study also contrast another study done in Braithwaite Memorial Specialist Hospital (BMSH), city of Port Harcourt, Rivers State, Nigeria in which storage period was not established to influence Sodium concentration (Teddy Adias, 2012). The difference in the findings can be attributed to the different rates of haemolysis and the hydration state of red blood cells during blood storage.

The Plasma Sodium level is anticipated to have an expected range of 145 to 155mmol/L, this study realized a decrease of plasma Sodium level from 150.72mmol/L±0.56 to 121.56mmol/L±1.35 which was equivalent to a 29.6mmol/L drop after 35 days of storage. These findings indicate that the decrease within the first week could fall below the normal range of sodium. Therefore, the significant changes may affect patients that may be affected by low levels of sodium e.g. increased chance of such recipient being prone to oedema especially in patients with low sodium intake or

those experiencing diarrhoea (Metheny, 2012). In light of the findings from the present study, Sodium (Na^+) ions concentration monitoring during blood for transfusion storage to improve blood safety is recommended.

This study reported a significant pH decrease after 14 days of storage and further decreased significantly through to 35 days of storage. This result compares with previous studies in Uganda, and Komfo Anokye Teaching Hospital, Kumasi, Ghana which demonstrated that the pH of stored blood reduced during the storage period (Oyet et al., 2018; Opoku-Okrah, Acquah, 2015). Blood pH is anticipated to have a reference range of 7.35 to 7.45, this study reported a decrease of blood pH from 7.48± 0.04 to 6.15± 0.04 which was equivalent to a 1.33 drop after 35 days of storage. This indicates that the pH decreased far below the normal range by the end of the second week. According to Oyet et al., 2018, the elevated lactate level with the reciprocal decrease in pH exerts ravaging consequences on blood recipients principally those who may be given several pints of blood in a short period. This diminishes blood potency and inclines blood recipients to undesired transfusion-associated morbidity and mortality (Oyet et al., 2018). The depletion of DPG and diminished glycolytic action are also linked to lessening pH (Teddy Adias, 2012). The study recommends that transfusion of blood stored for more than one week should be exercised with caution. Taking into account the findings from the present study, pH level monitoring during blood storage especially after the first week of storage with the focus of improving blood safety is suggested.

Overall, together with other parameters, biochemical changes in stored blood for transfusion should be skilfully monitored especially in consideration of the patient to be transfused and the clinical indication of the blood. Because of the elevation in potassium level with storage, caution is required in ascertaining the lifetime of blood, capacity transfused at a time, and rate of

transfusion to decrease hyperkalemia linked blood transfusion problems. Transfusing hyperkalaemic blood, nonetheless, maybe a short-life factor if the recipient's kidneys are performing properly and the Na+/K+ ATPase is also serving efficiently. However, this is a significant worry in recipients with renal failure.

The current study sought to evaluate the bacterial contamination in whole blood for transfusion stored at blood bank conditions of 2-6°C. Although the danger of transfusion linked with the spreading of viral conditions such as human immunodeficiency virus (HIV) and hepatitis has constantly reduced over the last four decades, the danger of the spread of bacteria has continued to be about the same. In consequence, on account of a favuorable outcome with viral pathogens, bacterial contamination now has the unresolved significance of being the major recurrent infectious danger from transfusion and has become a situation of rising agitation and curiosity. The present study has exhibited that there is negligible bacterial contamination with the isolation of *Staphylococcus aureus* sensitive to cefazolin 30µg & clindamycin30µg and resistant to penicillin 10 units & oxacillin 30µg in two pints at baseline (10%) and in one pint (5%) at week 4 and week 5 during the 35 days storage period. The danger of transfusing bacterially contaminated donor blood is high and transfusing blood with drug-resistant strains of bacteria may worsen the plight of the already sick and the immunocompromised (Opoku-Okrah, Feglo, 2009). Sepsis-related to the transfusion of RBCs contaminated with bacteria is generally acute and rapid in onset. Patients regularly develop a high fever (temperatures as high as 109°F have been noted) and chill in the course or immediately following transfusion (Brecher, 2005). A possible explanation for the isolation of penicillin and oxacillin resistant strains in donor blood might be associated with a high level of self-medication in Kenya (Mukokinya, Opanga, Oluka, 2018). *Staphylococcus aureus* toxins destroy biological membranes, causing cell death. Specifically, *Staphylococcus*

aureus generates potent hemolysins and leukotoxins. Amidst the latter, some were lately recognized to disintegrate neutrophils after ingestion, representing a specifically powerful weapon against bacterial removal by innate host defense. Furthermore, *Staphylococcus aureus* emits many factors that hinder the complement cascade or avert recognition by host defenses (Otto, 2014).

This study compares with the finding of a previous study done in Owolowo University, southwest Nigeria that demonstrated the isolation of Staphylococcus *aureus* at day 0 (Bolarinwa et al., 2011). Our findings also compare with findings of a study done in North Command Army and Tigray Region Banks, Ethiopia which expressed that bacterial contamination was seen in 9.2% of the blood and blood products, of which 77.8% and 22.2% were Gram-positive and Gram-negative bacteria, respectively (Agzie, Niguse, 2019). Our findings also concur with findings of another study done in the University of Gondar hospital blood bank, Northwest Ethiopia that isolated *Staphylococcus aureus* among many other isolates (Hailegebriel Wondimu, Zelalem Addis, Feleke Moges, 2013). The findings of the current study are almost at the same rate as those demonstrated in finding of studies in African nations inclusive of Ghana (9%), Nigeria (8.8%), and Kenya (8.8%) (Hassall, Maitland, Pole, Mwarumba, Denje, Wambua, Lowe, Parry, 2009; Andrew Anthony Adjei et al., 2009). The findings of the current study are however lower than findings of a study done at the University of Gondar hospital blood bank, Northwest Ethiopia that denoted the bacterial isolation rate of 15.3% (Hailegebriel Wondimu, Zelalem Addis, Feleke Moges, 2013). Our findings are also lower than the findings of a study done in Cairo, Egypt that demonstrated a bacterial detection rate of 17.9% (Samia Girgis, Bahgat, Ali, Ibrahim Rashad, & Ahmed, 2014). Nonetheless, the findings of the present study are comparatively higher than the findings of a study done in Mbarara Regional blood Bank, South Western Region Uganda that demonstrated a rate of 3.5% bacterial contamination (Matte Aloysius, Apecu, Richard, & Byarugaba, 2013), in Harare,

Zimbabwe;3.1% (Makuni et al., 2015), America; 0.2% (Roth, Kuehnert, Haley, Gregory, Schreiber, Arduino, Holt, Carson, Elder, 2001), United Kingdom; 0.15% (Williamson, Lowe, Love, Cohen, 1999), France; 0.1% (Wanger, Friedman, 1994). Our findings contradict the findings of a study done in Chandigarh India which demonstrated that none of the blood pints after three days storage interval at 2°C-6°C grew any organisms (Sharma, Subramanian, Kumar, Malkiat Singh, Meera Sharma, Agnihotri, 2004). The isolation of *Staphylococcus aureus* in this study might be attributable to asymptomatic donor bacteremia, blood donation collection technique, transportation of donated pints, and samples transfer technique. This study was not able to identify the source of bacterial contamination. Donor bacteremia, inexpedient disinfection of venipuncture site, contaminated blood bags, poor storage conditions, or unsuitable sample transfer technique could be the sources of bacterial contamination (Agzie, Niguse, 2019). Changes during inoculation of culture medium could the reason for not isolating any bacteria at day 7, day 14, and day 21 respectively. An increased blood pints surveillance during the 35 days reservation interval should be embraced to increase and ensure blood transfusion safety.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

The WBC count, platelets, RBC count, and indices are significantly altered in stored blood over the 35 days reservation interval. Biochemical parameters changes occur during the storage of blood despite the use of adequate storage conditions and may cause risks to patients during transfusion. Bacterial contamination with Staphylococcus aureus is possible in stored blood for transfusion which may be attributable to blood collection, transport, handling during storage, and sample transfer techniques.

6.2 Recommendations

The study recommends for fresh blood transfusion and the evaluation of cellular and biochemical changes that may occur in blood stored for blood transfusion especially for patients who suffer from renal failure, liver problems, and myocardial infarction. The study also recommends the putting of measures to protect the blood from being bacterially contaminated and increase bacterial surveillance of blood pints during the 35 days storage interval to increase blood safety. The study recommends further robust studies especially on bacterial blood contamination, level of unbound haemoglobin in stored blood supernatant, and various types of white blood cell changes.

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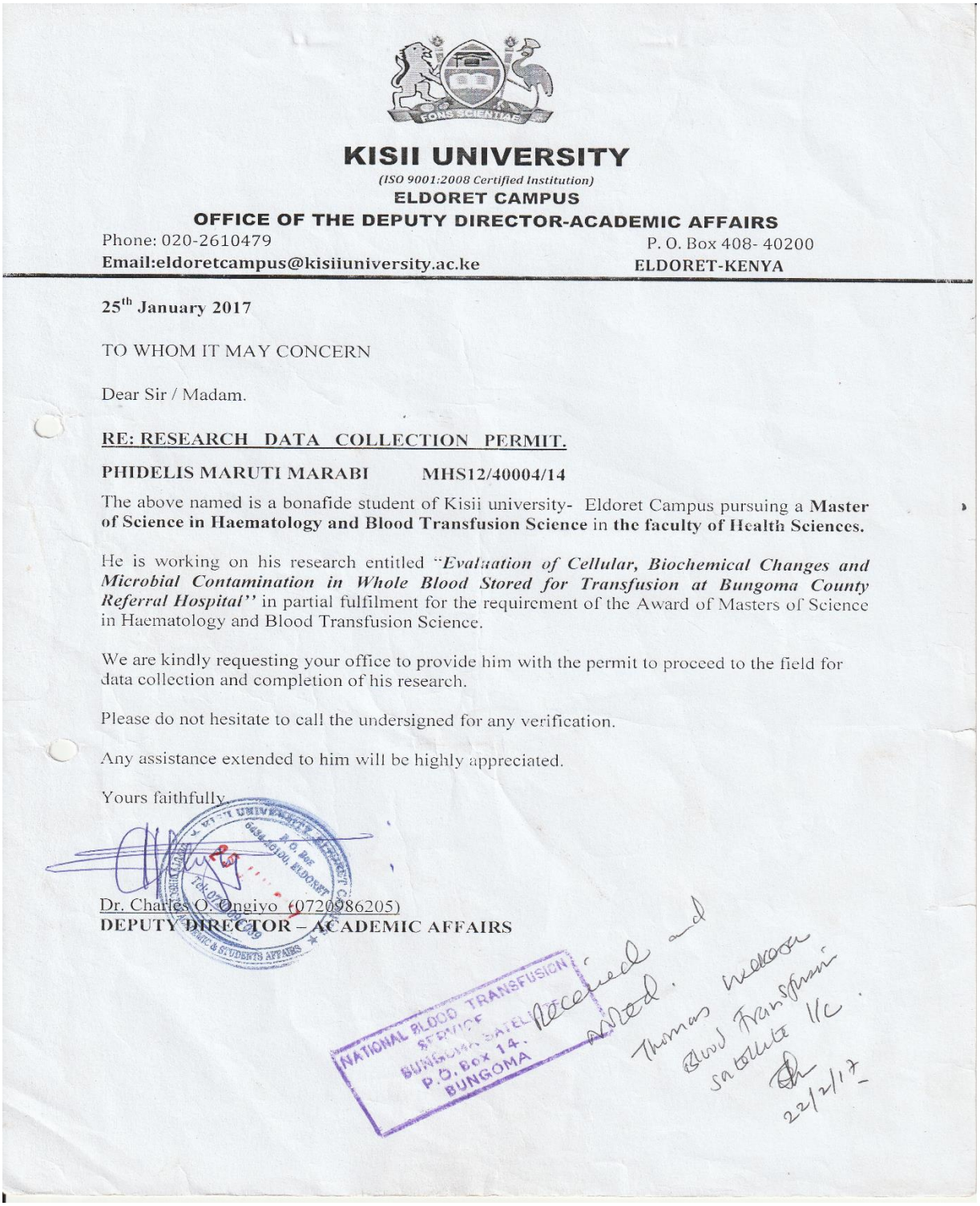
APPENDICES

APPENDIX XI: PERMISSION LETTER FROM KISII UNIVERSITY FOR DATA COLLECTION.



KISII UNIVERSITY

(ISO 9001:2008 Certified Institution)

ELDORET CAMPUS

OFFICE OF THE DEPUTY DIRECTOR-ACADEMIC AFFAIRS

Phone: 020-2610479 P. O. Box 408- 40200

Email:eldoretcampus@kisiiuniversity.ac.ke **ELDORET-KENYA**

25th January 2017

TO WHOM IT MAY CONCERN

Dear Sir / Madam.

RE: RESEARCH DATA COLLECTION PERMIT.

PHIDELIS MARUTI MARABI MHS12/40004/14

The above named is a bonafide student of Kisii university- Eldoret Campus pursuing a **Master of Science in Haematology and Blood Transfusion Science** in **the faculty of Health Sciences.**

He is working on his research entitled "***Evaluation of Cellular, Biochemical Changes and Microbial Contamination in Whole Blood Stored for Transfusion at Bungoma County Referral Hospital***" in partial fulfilment for the requirement of the Award of Masters of Science in Haematology and Blood Transfusion Science.

We are kindly requesting your office to provide him with the permit to proceed to the field for data collection and completion of his research.

Please do not hesitate to call the undersigned for any verification.

Any assistance extended to him will be highly appreciated.

Yours faithfully,

Dr. Charles O. Ongiyo (0720986205)

DEPUTY DIRECTOR – ACADEMIC AFFAIRS

NATIONAL BLOOD TRANSFUSION SERVICE BUNGOMA SATELLITE P.O. Box 14. BUNGOMA

Received and filed. Thomas Wekesa Blood Transfusion Satellite I/C 22/2/17

APPENDIX XII: PERMISSION LETTER FROM KENYA NATIONAL BLOOD TRANSFUSION SERVICES (KNBTS) FOR DATA COLLECTION.

MINISTRY OF HEALTH

Telephone: 020-2012867
Hotline: +254 716775245
Email: info@nbtskenya.or.ke
Website: www.nbtskenya.or.ke
When replying please quote:

NATIONAL BLOOD TRANSFUSION SERVICE - HQS
LOCATION: KENYATTA NATIONAL HOSPITAL, NPHLS GROUNDS
P.O.BOX 29804-00202
NAIROBI

To the Chairman

23/11/2016

Ethical Review Committee

Kisii University

RE; AUTHORIZATION LETTER FOR USE OF BLOOD SAMPLES FROM KNBTS FACILITY

This is to confirm that authority has been given to Mr Phidelis Maruti Marabi of Kisii University admission number MHS12/40004/14 to use blood samples from Bungoma Satellite Blood Transfusion Center for study titled "EVALUATION OF CELLULAR, BIOCHEMICAL CHANGES AND MICROBIAL CONTAMINATION IN WHOLE BLOOD STORED FOR TRANSFUSION AT BUNGOMA COUNTY REFERRAL HOSPITAL

This office wishes him all the best and requests that his study findings will be shared with KNBTS before any publications

Thank you

Dr Margaret Oduor

Director KNBTS

APPENDIX XIII: PERMISSION LETTER FROM MINISTRY OF EDUCATION, BUNGOMA COUNTY FOR DATA COLLECTION.

REPUBLIC OF KENYA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY
State Department of Education – Bungoma County

When Replying please quote
e-mail: bungomacde@gmail.com

Ref No: BCE/DE/19/VOL.1/159

County Director of Education
P.O. Box 1620-50200
BUNGOMA

Date: 20th January, 2019

TO WHOM IT MAY CONCERN

RE: AUTHORITY TO CARRY OUT RESEARCH – PHIDELIS MARUTI MARABI - REF: NACOSTI/P/18/14947/26117

The bearer of this letter Phidelis Maruti Marabi of Kisii University has been authorized to carry out research on ***"Evaluation of cellular, biochemical changes and microbial contamination in whole blood stored for transfusion at Bungoma county Referral Hospital",*** for a period ending ***17th January, 2020.***

Kindly accord him necessary assistance.

For: COUNTY DIRECTOR OF EDUCATION
BUNGOMA
P.O. Box 1620,
BUNGOMA - 50200

CALLEB OMONDI
For: COUNTY DIRECTOR OF EDUCATION
BUNGOMA COUNTY

APPENDIX XIV: PERMISSION LETTER FROM MINISTRY OF HEALTH, BUNGOMA COUNTY FOR DATA COLLECTION.

COUNTY GOVERNMENT OF BUNGOMA

MINISTRY OF HEALTH

Telegrams: "MEDICAL", BUNGOMA
Telephone: (055) 30230 Fax: (055) 30650
E-mail: sylvestermutoro@gmail.com
When replying please quote

COUNTY DIRECTOR OF HEALTH,
BUNGOMA COUNTY
P O BOX 18-50200
BUNGOMA

Our ref no. CG/BGM/CDH/RES/VOL.1/(12)

DATE: 06th April, 2017

PHIDELIS MARUTI MARAMBI MHS12/40004/14
KISII UNIVERSITY- ELDORET CAMPUS
P.O BOX 408-40200
ELDORET- KENYA

RE: RESEARCH AUTHORISATION

The Health Research Commitee is happy to inform you that your research In Bungoma County entitled **'Evaluation Of Cellular, Biochemical Changes And Microbial Contamination In The Whole Blood Stored For Transfusion At Bungoma County Referral Hospital''** has been approved.

The Commitee recommends you submit two(2) copies of your findings for feedback and retention purposes.

We wish you all the best.

Beverlyne Shitakule
For: COUNTY DIRECTOR HEALTH
BUNGOMA COUNTY.

CC
MedSupt - BCRH

APPENDIX XV: APPROVAL LETTER FROM JOOTRH ETHICAL REVIEW COMMITTEE.

MINISTRY OF HEALTLH

Telegrams: "MEDICAL", Kisumu
Telephone: 057-2020801/2020803/2020321
Fax: 057-2024337
E-mail: ercjootrh@gmail.com
When replying please quote

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Ref: ERC.IB/VOL.1/454

Date: 27th July, 2018

KISII UNIVERSITY,

Dear Phidelis Maruti Marabi,

RE: FORMAL APPROVAL OF THE PROTOCOL STUDY ENTITILED:-
"EVALUATION OF CELLULAR, BIOCHEMICAL CHANGES AND MICROBIAL CONTAMINATION IN WHOLE BLOOD STORED FOR TRANSFUSION AT BUNGOMA COUNTY REFERRAL HOSPITAL." FOR MASTERS DEGREE.

The JOOTRH ERC reviewed your protocol and found it ethically satisfactory. You are therefore permitted to commence your study immediately. Note that this approval is granted for a period of one year (w.e.f. 27th July, 2018 to 27th July, 2019). If it is necessary to proceed with this research beyond approved period, you will be required to apply for further extension to the committee.

Also note that you will be required to notify the committee of any protocol amendment(s), serious or unexpected outcomes related to the conduct of the study or termination for any reason.

In case the study site is JOOTRH, kindly report to the Chief Executive Officer before commencement of data collection.

Finally, note that you will also be required to share the findings of the study in both hard and soft copies upon completion.

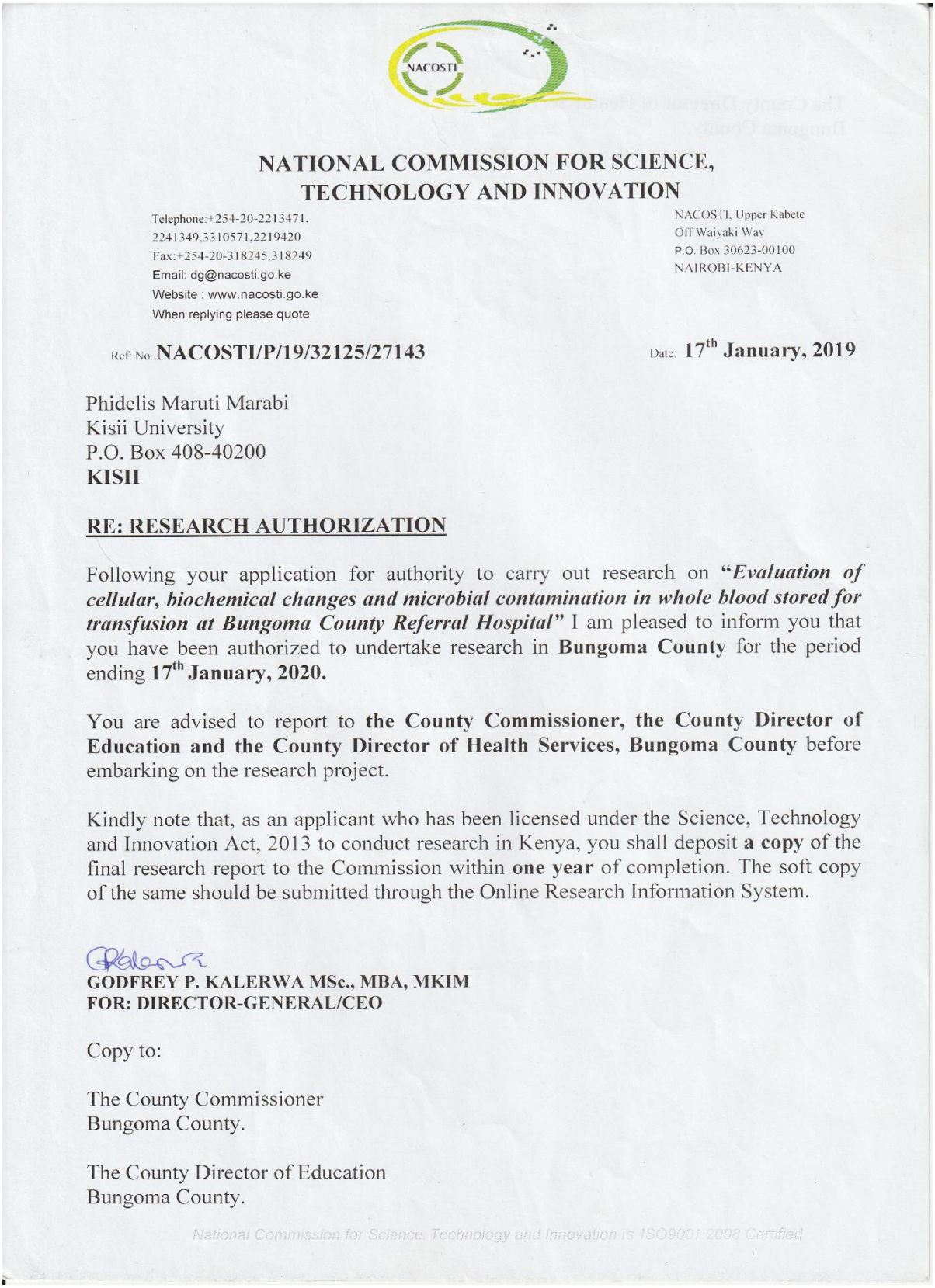
The JOOTRH – IERC takes this opportunity to thank you for choosing the Institution and wishes you the best in your future endeavours.

Yours sincerely,

WILBRODA N. MAKUNDA
SECRETARY- IERC
JOOTRH - KISUMU

JOOTRH ETHICS & REVIEW COMMITTEE
P. O. Box 849 - 40100
KISUMU

APPENDIX XVI: NACOSTI RESEARCH AUTHORIZATION LETTER.



NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email: dg@nacosti.go.ke
Website : www.nacosti.go.ke
When replying please quote

NACOSTI, Upper Kabete
Off Waiyaki Way
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No. **NACOSTI/P/19/32125/27143**

Date: **17th January, 2019**

Phidelis Maruti Marabi
Kisii University
P.O. Box 408-40200
KISII

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Evaluation of cellular, biochemical changes and microbial contamination in whole blood stored for transfusion at Bungoma County Referral Hospital"*** I am pleased to inform you that you have been authorized to undertake research in **Bungoma County** for the period ending **17th January, 2020.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Director of Health Services, Bungoma County** before embarking on the research project.

Kindly note that, as an applicant who has been licensed under the Science, Technology and Innovation Act, 2013 to conduct research in Kenya, you shall deposit **a copy** of the final research report to the Commission within **one year** of completion. The soft copy of the same should be submitted through the Online Research Information System.

GODFREY P. KALERWA MSc., MBA, MKIM
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Bungoma County.

The County Director of Education
Bungoma County.

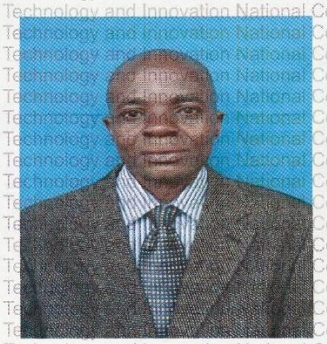
APPENDIX XVII: NACOSTI RESEARCH PERMIT.

THIS IS TO CERTIFY THAT:
MR. PHIDELIS MARUTI MARABI
of KISII UNIVERSITY, 0-50200
BUNGOMA,has been permitted to
conduct research in *Bungoma County*

on the topic: *EVALUATION OF CELLULAR, BIOCHEMICAL CHANGES AND MICROBIAL CONTAMINATION IN WHOLE BLOOD STORED FOR TRANSFUSION AT BUNGOMA COUNTY REFERRAL HOSPITAL*

for the period ending:
17th January,2020

Permit No : NACOSTI/P/19/32125/27143
Date Of Issue : 17th January,2019
Fee Recieved :Ksh 1000



.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation

APPENDIX XVIII: DONOR INFORMATION AND CONSENT FORM.

This informed consent form has two parts:

1. Facts sheet (to share details about the study)
2. Certification of consent

PART 1: INFORMATION SHEET

Introduction

My name is Mr. Phidelis Maruti Marabi, a postgraduate student in the Department of Health Sciences at Kisii University. I am conducting this study to evaluate cellular and biochemical changes in whole blood stored for transfusion.

You are requested to take part in this research. In case you do not understand any words used in this facts sheet and have any inquiries, please inquire from me to stop and explain.

Type of Research Intervention:

This research involves the analysis of blood reserved for transfusion to determine cellular and biochemical changes. We will collect blood pints following a normal blood donation procedure. The blood will then be sent to the laboratory to measure the cellular and biochemical changes up to 42 days of storage. The unit will then be discarded after the 42 days monitoring.

Participant Selection:

We aim to recruit participants from the volunteer donors donating blood meant for transfusion.

Voluntary Engagement:

Your taking part in this experimentation is optional and it is your option whether to take part or not. Whether you pick to take part or not, all the intention of donating to save a life will continue with no change whatsoever.

Series of steps and Protocol:

Eligible participants will be requested to join the study. All will then be requested to sign a consent form. Thereafter 450 mLs will be collected following a normal donation procedure. You will feel a little pain. Your blood unit will then be taken to the laboratory to measure for cellular and biochemical changes up to 42 days. If selected into the study, the unit will not be transfused to a patient as it will have expired after 42 days.

Side Effects:

There are no side effects in this experiment.

Risks:

There will be no threats expected with this experiment. Sometimes a hematoma may form at the site of the needle prick but this should subside within a few days.

Benefits:

You will benefit from the normal donor benefits including' knowing your blood group, weight, and Haemoglobin level. If critical results are noted at baseline, the donor will be notified for further investigation.

Reimbursements:

You will not be given any money or handouts to get involved in this experiment.

Confidentiality:

All participants will be identified using a number (names will not be used). All information shared by you during this study will be viewed by the researchers only.

Sharing the Results:

We will publish the outcomes so that other interested parties may acquire knowledge from it. However, your identity will never be revealed.

Request to participate in the study

Kindly indicate whether you are interested in joining this study. If you are willing to join the study I kindly request you to fill the consent certificate provided.

Right to Refuse:

Should you decline to participate in this study, this will not exert influence on your donation intention in any way. You will still have all the satisfactions that you would have or else.

Who to get in touch with:

If you have any inquiries regarding this experiment at any time you may correspond with the investigator below:

PHIDELIS MARUTI MARABI

MOB NUMBER: 0729614980

PART II: CERTIFICATE OF CONSENT

I have read the preceding facts, or it has been interpreted to me. I have the chance to raise any inquiries about my participation in the experiment, and any inquiries that I have inquired have been responded to my gratification. My rights have been explained to me and I consent my own unbound will to participate in this study experiment.

Participant’s name:

Participant’s signature:

Date:

If unable to read or write:

A witness who can read and write must put a signature (preferably, this witness should be selected by the person taking part in the stud and should have no relationship to the research team). Participants who are not able to read or write should include their thumbprint as well.

I have witnessed the precise interpretation of the consent form to the probable participator, and the individual has had the chance to make inquiries. I authenticate that the individual has given consent unboundly.

Witness name:

Witness signature:

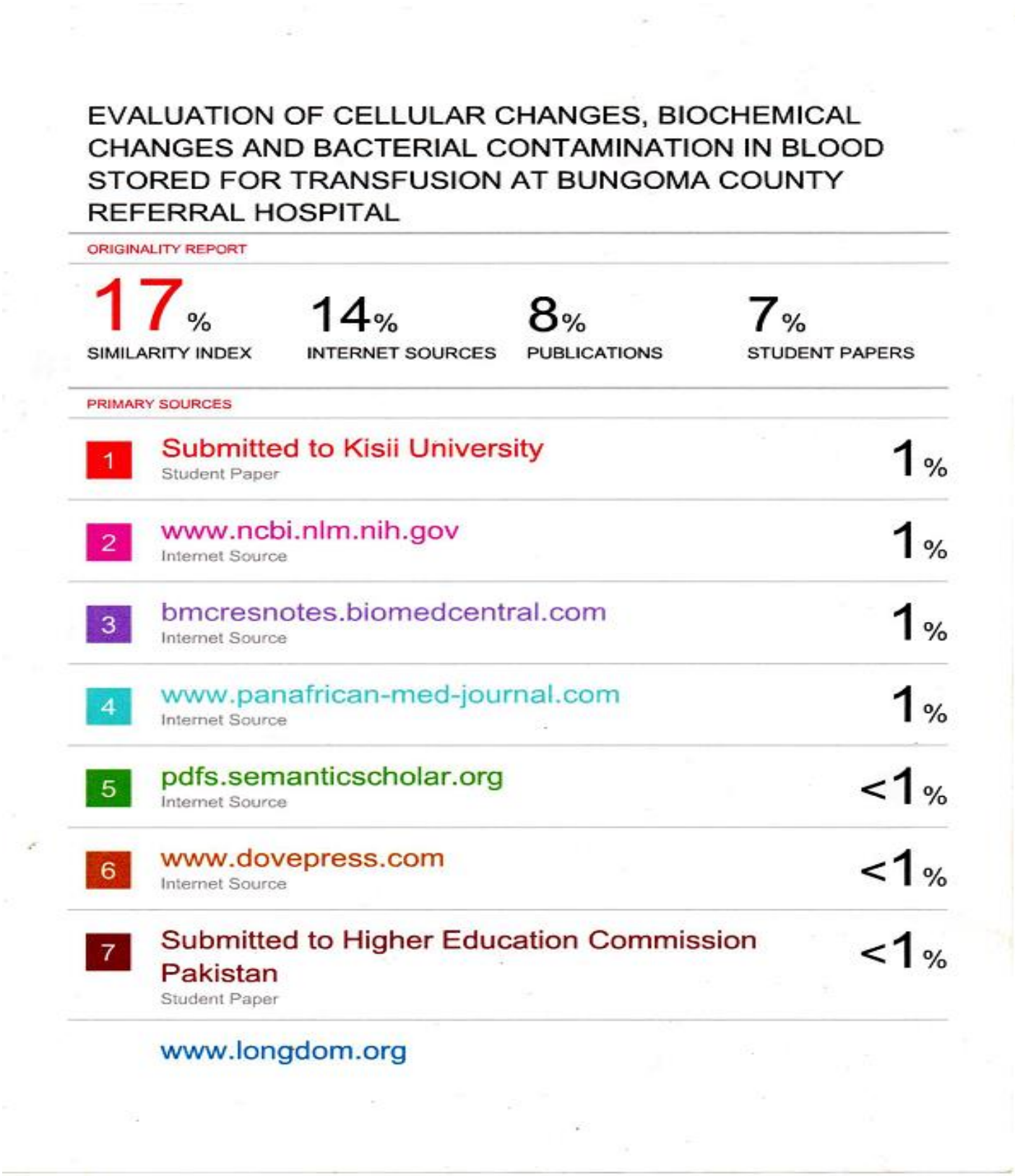
Date:

Participant thumb Print:

APPENDIX XIX: STUDY DATA COLLECTION TOOL/WORKSHEET.

BLOOD UNIT NUMBER……							
CELLULAR CHANGES							
	WEEK1 (Baseline)	WEEK 2	WEEK3	WEEK 4	WEEK 5	WEEK6	
Red cell count							
White cell count							
Platelet count							
Haematocrit							
MCH							
MCHC							
MCV							
BIOCHEMICAL CHANGES							
Potassium level							
Sodium level							
pH							
BACTERIAL CONTAMINATION (Specify the micro-organism if isolated)							

APPENDIX XX: PLAGIARISM REPORT



EVALUATION OF CELLULAR CHANGES, BIOCHEMICAL CHANGES AND BACTERIAL CONTAMINATION IN BLOOD STORED FOR TRANSFUSION AT BUNGOMA COUNTY REFERRAL HOSPITAL

ORIGINALITY REPORT

17%	14%	8%	7%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Submitted to Kisii University Student Paper	1%
2	www.ncbi.nlm.nih.gov Internet Source	1%
3	bmcresnotes.biomedcentral.com Internet Source	1%
4	www.panafrican-med-journal.com Internet Source	1%
5	pdfs.semanticscholar.org Internet Source	<1%
6	www.dovepress.com Internet Source	<1%
7	Submitted to Higher Education Commission Pakistan Student Paper	<1%
	www.longdom.org	