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An *in vitro* comparison of cellular destruction and metabolic effects occurring in stored, leuco-reduced and irradiated red blood cells

By

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DECLARATION

I, Faieqa Adams, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

Biochemical and haematological changes occur in red blood cellular products during the recommended storage period of 35 to 42 days at 1°C to 6°C. The restriction of the sodium/potassium pump at specified temperatures result in low intracellular potassium ion levels while an increase in sodium ion levels are observed and acidosis occurs as a result of low pH concentrations due to glucose consumption. Structural and morphological changes occur such as the release of free haemoglobin, lactate dehydrogenase and potassium into the supernatant causing the formation of spheroechinocytes and osmotic fragility. All these factors negatively impact the rheological properties of blood. These changes that transpire in the red cells during the storage period are referred to as “storage lesions”.

Transfusion-associated graft versus host disease is an immunological and often fatal adverse transfusion reaction with gamma irradiation of cellular blood products used as a preventative measure. Gamma irradiation exacerbates storage lesions and of particular concern has been the increased potassium levels resulting in neonatal and infant hyperkalaemia. The storage lesions occurring in non-irradiated red blood cellular products are well documented although the literature regarding its irradiated counterparts has been less studied. A study of this nature has not previously been done in Cape Town, South Africa.

A total of 80 red blood cellular products were measured for biochemical and haematological indices to determine the levels of cellular destruction which occurred in non-irradiated and irradiated red blood cells. The red blood cellular products tested included whole blood, red blood cell concentrate (buffy-coat layer poor), leucocyte reduced red blood cell concentrate (prestorage) and paediatric red cell concentrate. Twenty units per product were randomly selected to form part of either the control group (non-irradiated products) or the test group (irradiated products).

Serial sample aliquots were taken of both the non-irradiated and irradiated products on Days 1, 7, 14, 21, 28, 35 and 42 respectively while storage temperature was maintained at 1° to 6°C**.** The evaluated haematological indices included haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentrate levels and a full blood count. The biochemical indices assessed included potassium, sodium, phosphate, glucose, lactate dehydrogenase, free haemoglobin (percentage plasma haemolysis) and pH levels.

The percentage plasma haemolysis results of the non-irradiated products up to Day 42 were all below 0.8%, as recommended by the European Council. However, the percentage plasma haemolysis results of the irradiated products indicated levels below 0.8% at Day 28 but above the recommended guidelines by Day 35. An increase in potassium and lactate dehydrogenase levels while a decrease in sodium and pH levels were observed during the storage period.

The outcome of this study confirms that gamma irradiation exacerbates the RBC storage lesion and that recipients should not be transfused with irradiated blood post Day 28. Although the South African blood transfusion establishment does not prescribe to the universal leucocyte-reduced policy, the products tested yielded similar results to other studies completed in developed countries and therefore, the South African transfusion medicine policy regarding irradiation should remain unchanged.

Keywords: Gamma irradiation, plasma haemolysis, leucocyte-reduced (prestorage), red blood cell concentrate

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DEDICATION

To my beloved parents
Gone but never forgotten.

My loving husband, Ismail
and precious children,
Faranaaz
Yusrah
Hifzul-Rahmaan

I am, because of you.

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LIST OF ABBREVIATIONS, SYMBOLS AND UNITS

2, 3 DPG	2, 3 Diphosphoglycerate
CPD	Citrate phosphate dextrose
FBC	Full blood count
g/dl	Grams per decilitre
Hb	Haemoglobin
IRR	Irradiated
LDH	Lactate dehydrogenase
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
mg/dl	Milligrams per decilitre
mmol/l	Millimoles per litre
NIRR	Non-irradiated
PRBCC	Paediatric red blood cells concentrate
RBC	Red blood cells
RBCC	Red blood cell concentrate
SCD	Sterile connecting device
Storage lesion	Changes occurring in red blood cells during storage for 35 to 42 days at 1°C to 6°C
U/L	Units per litre
WHO	World Health Organisation
WB	Whole blood
<	Less than
>	More than

CHAPTER ONE

INTRODUCTION

1.1 Research rationale

While storage lesions occurring in non-irradiated red blood cellular products (Zubair, 2010) are well documented, however literature regarding its irradiated counterparts has been less studied (Zimmermann *et al*., 2009). There are few systematic studies comparing the *in vitro* storage lesions of irradiated and non-irradiated red cell concentrates and it has been suggested that the impact of storage lesions on leucocyte-reduced red blood cell concentrate (RBCC) is incomplete (Zubair, 2010). Also, to date, a study of this nature had not previously been completed at a blood transfusion establishment in South Africa as the reference values used are usually those derived from studies completed in First World countries.

The purpose of this study was to investigate and compare the biochemical and haematological changes that occurred in gamma irradiated and non-irradiated red blood cellular products during the standard 35 to 42 day storage period at 1° to 6°C. The non-irradiated and irradiated blood components evaluated include whole blood, red blood cell concentrate with buffy coat removed, leucocyte-reduced red blood cell concentrate and paediatric red blood cell concentrate. The following hypothesis was addressed in this study:

1.2 Hypothesis

Biochemical and haematological indicators will establish that gamma irradiation exacerbates storage lesions when red blood cellular products are stored at 1°C to 6°C for up to 42 days.

1.3 Objectives

- To determine the cellular destruction which occur in non-irradiated and irradiated red blood cells by comparing the haematological changes. This included a full blood count composed of measuring the red blood cell (erythrocyte), white blood cell (leucocyte) and platelet (thrombocyte) counts. Other measured factors were haemoglobin (Hb), haematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular volume (MCV) and free haemoglobin (percentage plasma haemolysis) levels. In order to calculate the

percentage plasma haemolysis, a plasma haemoglobin test had to be completed to ensure valid calculations. The red blood cellular products tested were whole blood (WB), red blood cell concentrate (RBCC), prestorage leucocyte-reduced RBCC and paediatric RBCC. Ten units per product for the randomly selected Control Group (non-irradiated) and ten units per product for the Test Group (irradiated) were tested. Serial sample aliquots of red blood cell suspensions from the eighty units were taken on Days 1, 7, 14, 21, 28, 35 and 42 respectively while storage temperature was maintained at 1° to 6°C.

- To compare the biochemical changes which took place in non-irradiated and irradiated red blood cell concentrates during storage of up to 42 days. The analytes measured comprised of potassium, sodium, phosphate, glucose and lactate dehydrogenase (LDH). The pH concentration was another biochemical factor that was measured. Serial sample aliquots of RBC supernatant were taken on Days 1, 7, 14, 21, 28, 35 and 42 respectively while storage temperature was maintained at 1° to 6°C**.** The red blood cellular products tested were randomly selected WB, RBCC, prestorage leucocyte-reduced RBCC and paediatric RBCC of which ten units per product were placed in the Control Group (non-irradiated) while the remaining ten units per product became part of the Test Group irradiated).

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

In 1915, Roux and Turner discovered that refrigerated red blood cells (RBC) could be preserved for up to 4 weeks in a citrate-glucose solution with minimal haemolysis occurring. They discovered this while working on a storage solution to preserve rabbit cells for use in a syphilis heterophile agglutination test (Hess, 2006). Oswald Robertson, who worked with Roux and Peyton, used the citrate and glucose solution as an anticoagulant to store human blood for up to 26 days to revive soldiers during World War I. (Hess, 2006; Zimrin & Hess, 2009). Blood banking was introduced during World War II (1939-1945) when acid-citrate-dextrose (ACD) solution was used as an anticoagulant which allowed refrigerated blood to be stored in sterile vacuum glass bottles for 21 days. It also further allowed for the sterilisation of citrate and glucose. The rate of citrate toxicity observed in patients was significantly reduced as a lesser volume of ACD was needed and larger volumes of blood could be transfused (Hess, 2006; D'Amici *et al.*, 2012).

The addition of phosphates in the 1950s, replacement of plastic bags instead of glass bottles for whole blood collection in the 1960s, the addition of adenine to anticoagulant solutions during the 1970s and coupled with additive RBC nutritive solutions in the 1980s, all allowed for a longer than 21-day storage period of whole blood donations and subsequent component separation (Hess, 2006; Zimrin & Hess, 2009). Seepage of phosphates from stored RBC decreased once sodium phosphate was added to the ACD anticoagulant as this addition allowed the phosphate gradient to reach equilibrium and the citrate-phosphate-dextrose (CPD) solution is still used today (D'Amici *et al.*, 2012).

The transfusion of red blood cellular components is considered a vital treatment for patients suffering anaemia triggered by haemorrhage due to surgery or trauma injury, various haemoglobinopathies or malignancy (Sparrow, 2010). The administration of red blood cellular products is to enhance the intravascular oxygen-carrying capacity and subsequent improvement of tissue oxygenation. Approximately 108 million units of whole blood (WB) donations were collected globally in 2014 of which 76% of transfusions in high-income countries were patients over the age of 65 years whereas children under the age of 5 years accounted for 65% of blood transfusions in low-income countries. It is also reported that more than half of all the WB donations collected were from donors in high-income countries

and a growth of 8, 6 million WB donations from voluntary non-remunerated donors recorded from 2004 to 2012 (WHO, 2014).

Although transfusion of RBC products is a common procedure and the benefits are multiple, unfavourable biochemical and haematological changes occur during the storage of red blood cells and are referred to as “storage lesions”. Cytoplasmic RBC potassium levels increase with a reduction in sodium concentrations, adenotriphosphate (ATP) levels decrease, acidosis occurs due to a decrease in pH levels and 2, 3 diphosphoglycerate (2, 3 DPG) levels decline even though this is rapidly reversed *in vivo* post transfusion. This is in addition to the formation of spheroechinocytes caused by an increase in osmotic fragility leading to deformity of the cell membrane.

Haemoglobin, lactate dehydrogenase and potassium cations are released into the supernatant due to the oxidative damage caused by storage lesions (Chaudhary & Katharia, 2012). Lipid peroxidation and oxidative stress to band 3 structures (an integral glycoprotein and anion transport mediator across the RBC membrane) and partial membrane loss occur as a result of vesicle formation (D’Alessandro *et al*., 2010). Also, the consumption of glucose during storage causes the loss of structural integrity in the red cell membrane which furthermore allows microparticles to form containing cell-free haemoglobin.

The released free haemoglobin is a scavenger of intravascular nitric oxide (NO), an endogenous vasodilator and reduced NO levels may result in organ injury and decreased organ perfusion. The rheology is subsequently affected by the considerable changes that occur and may add to transfusion difficulties (Kim-Shapiro *et al*., 2011). While some storage lesions may occur within days or weeks, elevated potassium and LDH levels may be detected within hours of storage (D’Alessandro *et al*., and 2010).

2.2 Nutritive solutions

The development of saline-adenine-glucose preservative solution in the late 1970s with the later addition of mannitol in the 1980s allowed the red blood cell concentrate (RBCC) to be stored for 42 days at 1 ºC to 6 ºC and transfusion practices changed. The administration of this component allowed for increased volumes of blood to be transfused and clinicians observed a reduction in patients experiencing citrate toxicity observed (Zubair, 2010; Sparrow, 2012). The saline-adenine-glucose-mannitol (SAGM) solution is routinely used in Europe and in the South African western coastal region (e.g. Cape Town). In the South

African inland areas (e.g. Gauteng) however, a SAGM variant solution consisting of dextrose, adenine, mannitol and sodium chloride, also known as Adsol or AS-1, is used.

As the American Food and Drug Association (FDA) has yet to licence the use of SAGM, the blood transfusion centres use AS-1 and AS-5 (Optisol) while the third licensed additive solution is AS-3 (Nutricel). The latter solution is also used in certain areas of Canada. The AS-1 and AS-5 solutions are SAGM variants and contain the same constituents, albeit in differing quantities, whereas the AS-3 solution consists of dextrose, adenine, sodium phosphate, sodium citrate and sodium chloride (Sparrow, 2012). D'Amici and colleagues reported that the membrane protein profile of RBC stored in AS-3 appears more structured than those stored in SAGM even though fragmentation or vesiculation of the red cells occur in both solutions during storage (D'Amici *et* al., 2012).

When licensing additive solutions to be used in the production of RBC products, the European Council ruling is that the percentage haemolysis of stored RBC products should not be more than 0, 8% at the end of the storage period while the American FDA stipulates a less than 1% concentration with the addition of the "95/95" rule. This rule specifies that blood transfusion establishments must be able to demonstrate that 95% of their RBC products meet the recommended standards 95% of the time while both Councils insist on a 75% survival rate for transfused red blood cells 24 hours post-transfusion (D'Alessandro *et al*., 2010).

Once the plasma has been extracted and the buffy coat removed from the centrifuged whole blood (WB) unit, a nutritive solution is added to the RBC to maintain cellular functions during the storage period such as glucose and adenine while sodium chloride and mannitol lower haemolysis levels (D'Amici *et* al.,2012). The clinical consequences of additives such as glucose, sodium and citrate, however, exacerbate acidosis and the storage lesions contribute to hyperglycaemia, hypernatraemia and hypocalcaemia (Isbister, 2003).

2.3 Whole blood collection containers

For decades the scientific community has been embroiled in an on-going debate regarding lengthening the storage period of RBC products while ensuring the safety and efficacy of stored blood although clinicians agree that RBC deteriorates as soon as it leaves the donor's vein (Isbister, 2003).

The American FDA recommends that the blood bags used for whole blood (WB) donations be sterile, pyrogen-free and transparent. The material used to manufacture these containers should also not affect the efficacy and concentration of the contents (Kakaiya *et al.*, 2008). A normal WB donation volume is about 450-575ml and is collected into a bag containing the prescribed volume of anticoagulant (Bellairs and Ingram, 2013). In order to enhance oxygen permeability, increase bag suppleness and red cell survival, most storage bags used for WB collection consist of the colourless and odourless primary plasticiser 2-diethylhexylphthalate (DEHP). This plasticiser is added to polyvinyl chloride (PVC) but does not bind to PVC.

It has been reported that leaching occurs from these plastic containers into stored blood during prolonged storage (Bellairs and Ingram, 2014). After countless debates regarding the toxicity of DEHP, it has been reported that the plasticiser is an endocrine-disrupting compound capable of causing serious adverse health effects such as behavioural, reproductive and metabolic disorders (Shaz *et al*., 2011) but as exposure via blood transfusion is minimal, it is well below the toxicity range.

Despite the low concentrations of DEHP leaching into the plasma and RBC content, clinicians are concerned about the possible damaging effects on certain patient groups such as neonates, children, pregnant or nursing women (Shaz et al., 2011). At an international forum held in 2011, experts reported that while most countries did not have a programme to replace DEHP as tested alternatives revealed other problems, Sweden proved to be the most active in the anti-DEHP campaign. The American Red Cross uses the FDA-approved plasticiser *n*-butyryl-tri-n-hexyl citrate (BTHC)-PVC for 50% of WB collections (van der Meer *et al*., 2014) as BTHC-PVC has demonstrated low toxicity, a 24-hour post-transfusion survival rate and does not store in the body (Shaz *et al*., 2011). France reported that all medical devices containing DEHP are to be banned from maternity, paediatric and neonatal wards, effective 2015 although other countries were more reticent. The general global consensus is that DEHP should remain as a blood bag constituent until a suitable and similar alternative is found (van der Meer *et al*., 2014).

2.4 Whole blood (WB)

Whole blood is collected from the donor and refrigerated immediately with limited processing procedures performed and is also the primary material used for component processing. As the clotting factors and thrombocyte levels soon decline after donation, the expiry time of a WB unit is 35 days while that of other RBC products is 42 days. Other storage lesions

include the formation of micro-aggregates and the release of potassium from the RBC (Armstrong *et al.*, 2008).

This product is normally used for neonatal exchange transfusions or to rectify massive haemorrhage (Bellairs and Ingram, 2014). A neonatal exchange transfusion is administered to treat hyperbilirubinaemia as a result of haemolytic disease of the foetus and newborn (HDFN). This treatment decreases the neonatal bilirubin level by removing the Rh-D positive RBC and the circulating maternal allo-anti- D (Bellairs and Ingram, 2014).

A diagnosis of adult massive haemorrhage is made when more than 50% of total blood volume (TBV) is replaced to maintain haemostasis within 3 hours. This equates approximately to a transfusion of 10 RBCC within 24 hours and may be due to trauma, surgical or obstetrical cause. Patients suffering from massive haemorrhage may often be hospitalised with multifactorial early trauma–induced coagulopathy (ETIC) which occurs when the patient presents with a disseminated intravascular coagulation-like syndrome. This may be due to tissue injury as a result of trauma or surgery and indicated by hyperfibrinolysis and systemic anticoagulation (Pham & Shaz, 2013). Massive haemorrhage reduces haemostasis causing non-aggregation of thrombocytes which then results in anaemia (Adams *et al*., 2015). American military physicians working in a war zone prefer transfusing trauma patients suffering from hypothermia, acidosis and coagulation pathologies with fresh, warmed WB instead of using component therapy. This is due to logistical problems as the storage temperature for fresh frozen plasma and cryoprecipitate is -20°C but has to be thawed before use while a unit of platelets has to be kept constantly agitated until the 5-day expiry period (Repine *et al*., 2006). Transfusion practices changed with the advent of component therapy as WB was no longer the only available blood product. In an attempt to mimic WB *in vivo*, the transfusion ratio used to manage massive haemorrhage is equal parts red blood cell concentrate, plasma and platelets (viz.1:1:1 transfusion ratio) but this component combination contains approximately 180ml of added nutritive solutions compared to the lesser volume present in WB. The other advantages of using WB include reduced exposure to donor antigens, antibodies and infectious pathogens as well as optimum platelet function (Zielinski *et a*l., 2014).

2.5 Component therapy

The advent of component therapy altered blood transfusion guidelines and subsequently enhanced the practice of medicine. A WB donation is collected and using aseptic methods, may subsequently be processed into three major cellular components which include a red blood cellular concentrate, plasma and platelets (Kakaiya *et al.*, 2008). The administration of

component therapy is based on the transfusion of a specifically separated component. This allows more patients to benefit from a single WB donation while reducing the need for entire WB transfusions (Hardwick, 2008). For more than fifty years transfusion medicine has witnessed the technological development and use of automated equipment for component production from a whole blood donation. These products can be obtained by either processing a WB donation using centrifugation or by using the apheresis method (Rock *et al*., 2003; Devine & Howe, 2010).

Apheresis methodology is an automated procedure where an anticoagulant is added to donor WB prior to entering the machine chamber and the required component is then separated, leucocyte-reduced and removed. The remaining blood components are returned to the donor using either the same vein or via a vein in the other arm. While apheresis donors have to abide by the same guidelines as allogeneic blood donors, this technique is more costly as it requires expensive machinery and specialised trained staff (Smith & Burgstaler, 2008).

The standard procedure for blood product preparation is to use gravitational force. This is achieved by using centrifugation to separate the WB into the major components based on cellular size and density while maintaining a closed sterile system for maximum plasma removal.

The two most common methods used in component production are the platelet-rich plasma (PRP) and the buffy coat methods (Devine & Howe, 2010). The PRP technique is when a WB donation is centrifuged, RBC and leucocytes are separated and then extracted from the plasma and platelets. The buffy coat method is based on marked cellular separation via centrifugation where the buffy coat forms an enriched thrombocyte and leucocyte layer between the RBC and plasma. The three components are distributed between the sterile interconnected blood bags with the application of semi-automated pressure to the centrifuged bag containing the primary whole blood donation i.e. the extracted plasma and buffy coat are collected in separate satellite bags. A nutritive solution is then added to the remaining red blood cells in the primary collection bag and this allows the expiry to be increased top 42 days.

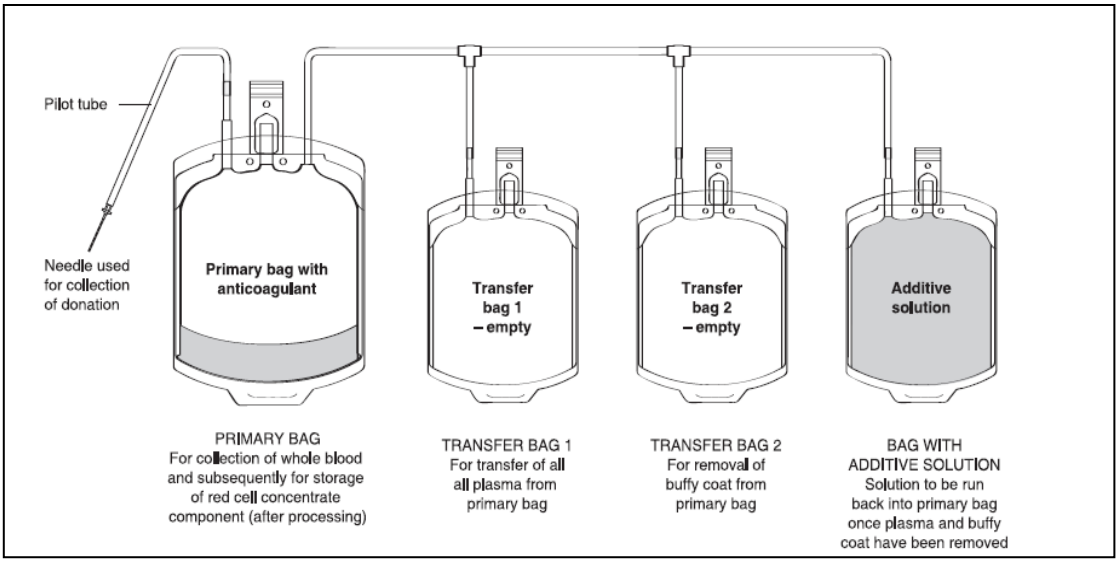

Figure 2.1: Diagram of quad bag used for whole blood collection
(Adapted from Hardwick, 2008)

A unit of pooled random donor platelets is produced when four to six blood group specific buffy coats are pooled after the red cells and leucocytes are extracted post-centrifugation. A unit of plasma belonging to one of the buffy coats is then added to the platelet pool.

Using the buffy coat method produces a better quality plasma yield due to the high centrifugal forces used and leucocyte levels in RBCC are reduced without filtration (Devine & Howe, 2010). The extracted plasma can be frozen to produce the fresh frozen plasma product or may be further processed into coagulation factor concentrates or plasma colloids such as albumin and immunoglobulins by using fractionation techniques.

2.6 Red blood cell concentrate (RBCC)

In order for a closed sterile system to be maintained during the RBCC processing procedure, a satellite bag containing the SAGM nutritive solution is added to the packed RBC after the removal of the plasma and buffy coat layer. This method decreases the number of viable leucocytes contained in the RBCC by approximately 70-80% with the product having a final volume of about 300ml. A distinct advantage of removing the buffy coat layer during the production of RBCC is the reduction of micro-aggregate formation during storage when compared to RBCC without the buffy coat removed (Armstrong *et al.,* 2008).

The RBCC component is indicated for patients suffering from anaemia, obstetric haemorrhage, surgery or patients with an acute blood loss of more than 30% TBV to improve oxygen delivery to body tissues. (Adams *et al*., 2015).
It has been reported that RBC transfusion should only be considered when patient haemoglobin levels fall below 7g/dl with a maintenance level of 7g/dl to 9g/dl although this does not apply to patients with cardiac pathologies (Sharma & Sharma, 2011).

The oldest indicator for transfusion is the "10/30" rule which refers to the haemoglobin level being maintained at 10g/dl or haematocrit levels more or equal to 30%. A recent American-Italian report provides a summary of current transfusion recommendations. This report indicates that instead of maintaining patient haemoglobin levels, clinicians should prevent ischaemia in patients while limiting donor exposure to improve patient outcome (Shander *et al*., 2013). Spahn & Vamvakas responded that best transfusion practice should also include best clinical practice as the suggested recommendations did not exclude the patient from incurring infectious or immunologic complications such as haemolytic and non-haemolytic adverse reactions associated with transfusion (Spahn & Vamvakas, 2013).
The buffy coat-poor RBCC is the standard product prepared for the majority of red blood cell transfusions in South Africa.

2.7 Paediatric red blood cell concentrate (PRBCC)

It is well known that blood stored for long periods contain storage lesions that are not well tolerated in neonates requiring multiple transfusions (Ratcliffe *et al*., 1986). Hyperkalaemia and arrhythmia have been associated with RBC transfusion of ill infants receiving large volumes of blood due to haemorrhage or those requiring surgery. These unfavourable outcomes may be due to metabolically active biochemical electrolytes such as high extracellular potassium levels present in stored RBC products, due to environmental heating or cellular degeneration caused by irradiation. It is therefore not uncommon to clinically supervise an infant with anaemia, hypotension and oligoanuria in a paediatric intensive care unit (Parshuram, 2003). The American Pediatric Perioperative Cardiac Arrest Registry reported that the most common cause of cardiac arrests in anaesthetised children from 1998 to 2004 was due to blood loss resulting in hypovolemia and hyperkalaemia caused by transfusion of stored blood (Bhananker *et al*., 2007).

However, it is reported that while symptomatic hyperkalaemia may be observed during rapid RBC transfusion or in children not able to withstand the potassium load, these adverse

effects seldom occur without any clinically significant changes in the patient's calcium, potassium, pH or lactate dehydrogenase levels observed (Parshuram, 2003).

Blood transfusion services produce the paediatric RBCC (PRBCC) and the infant RBCC (IRBCC) units in order to reduce cost and wastage due to small required volumes while delivering maximum benefit to infants and neonates requiring transfusion. In South Africa, an adult RBCC unit is leucocyte-reduced and then equally divided to produce 2 paediatric units of approximately 130ml each. The transfusion establishment found on the west coast also produces an IRBCC of approximately 55ml each when a leucocyte-reduced adult RBCC is divided between 4 transfer bags. The closed sterile system is maintained as the transfer bag and RBC filter is sterile-docked onto the adult RBCC pack. Several hospitals in London (United Kingdom), however, utilise multi-satellite bags to produce approximately seven small volume units for neonatal transfusion (Seghatchian & Krailadsiri, 2002). Clinicians who expect multiple transfusions to be administered to premature neonates with low birth weights of less than 1500g will place these patients on the neonatal limited donor exposure programme for IRBCC to be administered as patients will then be exposed to only I set of donor antigens (Josephson, 2008).

2.8 Pre-storage leucocyte-reduced red blood cell concentrate (LRBCPS)

Varying amounts of leucocytes are present in red blood cellular products and a normal RBCC unit may contain about 1 to 2 billion leucocytes which may be the cause of various transfusion-associated complications. This prompted the scientific community to develop leucocyte reduction methods such as removing the buffy coat from WB units post centrifugation, washing red blood cells, freezing RBC with glycerol and RBC filtration (Huh, 1993). Transfusion of non-leucocyte-reduced red blood cellular products may cause immunological complications or transmit leukotropic viruses from donor to patient which may cause immunosuppression and release toxic substances (Dzik, 1995).

Leucocyte-reduced and irradiated blood component products are prepared by blood transfusion establishments to reduce or prevent the adverse reactions caused by contaminating leucocytes or white blood cells. Filtration of blood should occur soon after collection or processing as granulocytes fragment during storage and the leucocytes present in RBC products rarely provide any therapeutic benefit to the patient. Instead, the white blood cells (WBC) are known to increase cellular damage and cause adverse transfusion reactions in recipients. These adverse transfusion reactions to allogeneic donor blood include allo-immunisation to human leucocyte antigens (HLA), non-haemolytic febrile

transfusion reactions (NHFTR), transfusion–associated Graft versus Host disease (TA-GvHD) and transfusion-associated lung injury (TRALI). Other adverse transfusion reactions associated with transfused leucocytes may be transfusion-related immunomodulatory (TRIM) effects which include post-operative infections leading to mortality due to multi-organ failure (van Hilten *et al.,* 2004). Blumberg (2005) concurred and reported that when only reporting on results from patients who have been transfused in clinical trials, then using leucocyte-reduced components decreased the chance of patients developing post-operative infections by approximately 50% compared to a 35% decrease when buffy coat-poor units were transfused (Blumberg, 2005). Vamvakas (2006) disagreed with Blumberg stating that meta-analyses should only include homogeneous study subsets and not across all clinical trial studies. He furthermore stated that the effects of transfusion immunomodulation cannot be proven "beyond a reasonable doubt." (Vamvakas, 2006).

Leucocytes may also be a pathogenic haven for certain transfusion-associated leukotropic viruses such as Human T-cell lymphotropic virus (HTLV types I and II), Epstein-Barr virus and cytomegalovirus (CMV). CMV is a monocyte-based DNA virus which may cause a transient illness in immunocompetent patients but may be lethal in CMV-seronegative patients with a compromised immune system. B-lymphocytes may also be regarded as vectors for the variant Creutzveldt-Jakob disease (vCJD) prions, alloimmunisation may be due to dendritic leucocytes acting as antigen-presenting cells that present major histocompatibility complexes classes I and II antigens (HLA) which the T lymphocytes recognise while granulocytes or granulocyte fragments may cause NHFTR (Pietersz *et al*., 1998).

RBC products may be leucocyte-reduced using different techniques and is dependent on the adopted policy regarding filtration, the requirements as well as the financial situation of the local government involved. There are a variety of methods to leucocyte-reduce red blood cellular methods such as inline filtration where the filter is built into the donation collection system to ensure that sterility is maintained. The inline filters may be used for WB filtration as this allows the RBCC and plasma products to be leucocyte-reduced but is not suitable for platelet production as the filter removes both leucocytes and platelets. Inline filtration may also be used to filter buffy-coat poor RBCC post WB component separation and this product is known as a pre-storage RBCC as the expiry time is unaffected.

Another filtration technique is to manually connect the filter to the buffy-coat poor RBCC although it requires the addition of a transfer bag attached to the filter. The spikes of the filter may either be physically inserted into the RBCC unit via an anterior-situated port on the bag

or the filter may be added using a sterile connecting device (SCD). Despite using a laminar flow cabinet, the former method is regarded as an “open” system and therefore, the expiry time of the product is reduced to 24 hours post filtration. When using an SCD, however, the expiry time of the filtered product is not affected as this method maintains sterility and is thus regarded as a “closed” system. These RBCC products are filtered within 48 hours post donation and is also known as a pre-storage RBCC. This component is usually provided when needed by transfusion establishments as only selected blood groups are processed instead of adopting the ULR policy due to the high cost involved (Armstrong *et a*l., 2008).

RBC products may also be completed at the patient’s bedside during transfusion where an inline leucocyte filter is a part of the RBC transfusion giving set. The bedside filtration process is the responsibility of the hospital staff and not the transfusion service. Adopting this procedure has a few disadvantages which may include lack of staff competency due to the occasional filtration request, leucocyte fragmentation and release of cytokines may occur as the storage period of the RBCC is not a considered factor and hospital staff may not be adept at RBC quality control methods used in a transfusion service. Not only is there a lack of good laboratory practice when undertaking bedside filtration but the transfused product becomes warm as it requires a slow blood flow rate. The warmer the blood product becomes, the fewer leucocytes are filtered which subsequently decreases filter performance and may have a negative impact on the recipient (Kumar *et al*., 2006). It is, therefore, more practical to complete this procedure in the processing laboratory as quality control measures for staff, equipment, procedures and completed products are maintained throughout component production. This also ensures product traceability with a standardised product upon completion.

A RBCC unit with the buffy coat removed contains approximately 8×10^8 leucocytes per unit (van Hilten *et al.*, 2004) whereas the American Association of Blood Banks (AABB) stipulates that a leucocyte-reduced RBCC product retains a minimum of 85% of the original RBC in 95% of the units tested and should contain less than 5×10^6 leucocytes. This is due to possible antibody stimulation by transfused histocompatibility antigens which may occur when the leucocyte level exceeds the specified parameters. The American FDA suggests that quality assurance testing should be completed on 1% of filtered units and that all tested units should not have more than 5×10^6 leucocytes (Kumar *et al*., 2006).

The European Council observe stricter guidelines and dictates that leucocyte content must be less than 1×10^6 post filtration (Sharma & Marwaha, 2010). The use of specific filters has reduced the leucocytes in red blood cellular products and platelets by 3 log ^{10} (99, 9%),

resulting in less than 3 x 10^6 in a 300ml RBCC component i.e. residual leucocytes is about 10 per microliter which is less than the recommended 5 x 10^6 leucocytes per unit (Bordin *et al*., 1994). Although many of the current filters used to reduce leucocytes remove 3 - 4 $\log^{10}$ of white blood cells, it does not eliminate all pathogens. A study using rodents indicated that filtering only removes 40-70% of infectivity and the remaining pathogens may therefore be transmitted to a recipient (Murphy and Pamphilon, 2009). It is reported that a leuco-reduced RBCC in SAGM yields lower glucose consumption, a reduced haemolysis rate and lower levels of LDH release with higher pH concentration when compared to a RBCC in SAGM with buffy coat removed (Pietersz *et al.,* 1998).

Although the universal leucocyte reduction (ULR) policy has been adopted by many First World countries as using filtered RBC products provide many advantages to the recipient, the major disadvantage of implementing a ULR policy in developing countries is the cost involved. However, a Dutch study reported that transfusing patients with leucocyte-reduced RBCC decreased hospital stay by 2, 4 days which would lead to a significant reduction in national hospital costs. They also observed a decline in multi-organ failure in gastrointestinal oncology and vascular surgery patients (van Hilten *et al*., 2004). Data from the French haemovigilance report shows a significant decline in NHFTRs and bacterial sepsis incidences following the initiation of the ULR program (Sharma & Marwaha, 2010). A Canadian study produced similar results to the French and also demonstrated a decrease in mortality with less febrile reactions which resulted in a decline in the antibiotic administration to high-risk recipients (Hébert *et al*., 2003). The Bowden study in 1995 determined no substantial difference regarding CMV transfusion transmission in bone marrow transplant patients when CMV-seronegative products were compared to leucocyte-reduced RBC products (Bowden *et al*., 1995). Although the data according to the British haemovigilance programme indicate that the transfusion of leucocyte-reduced RBCC has lowered the incidence of TA-GvHD, clinicians should be aware that the only recommended method to prevent this serious and often fatal disease is to filter and irradiate the RBCC used for transfusion (Zimmermann *et al*., 2009).

Canada implemented the ULR program in 1999 with Germany following suit in 2001 while leucocyte-reduced RBC products are transfused selectively in South Africa. The cost of adopting the ULR program in South Africa would create exorbitant costs and as the country is undergoing an ever escalating Human immunodeficiency virus (HIV) pandemic, each WB donation is tested using the expensive nucleic acid testing (NAT) for HIV, hepatitis B and hepatitis C. Therefore, although the authors acknowledge the benefits of implementing a ULR policy, there are other health concerns taking priority. There is also no evidence to

suggest that patients transfused with leucocyte-reduced RBCC will evade the transmission of vCJD and there has been no indication of recurrence of viral infections such as HIV or CMV in transfusion of non-leucocyte-reduced RBC (Bird and Crookes, 2006).
While using filtered RBC products has greatly reduced the occurrence of spongiform encephalopathy, it has not completely eradicated the transmission of this disease (Armstrong *et al.*, 2008).

In the Western Cape, a leucocyte-reduced RBCC component may be produced by using specific filters to reduce the number of leucocytes still present after the buffy coat extraction and can be accomplished via two methods. One such method is by sterile docking the filter onto a prepared RBCC using a sterile connection device within 48 hours of donation. This ensures an expiry of 42 days when stored at 1°C to 6°C and is known as a pre-storage leucocyte-reduced RBCC (LRBCPS). The second method is by using a laminar flow cabinet and physically inserting the filter into an opened port of a RBCC unit which is less than seven days old. Despite the use of the laminar flow cabinet, this method is regarded as an "open" system and therefore, this product has a 24-hour post filtration expiry in order to minimise bacterial contamination. The evaluated product used in the study is the pre-storage leucocyte-reduced RBCC with a 42-day expiry when stored at 1°C to 6°C.
Below is a brief summary of adverse transfusion reactions caused by leucocytes present in RBCC.

2.8.1 Non-haemolytic febrile transfusion reaction (NHFTR)

A non-haemolytic febrile transfusion reaction (NHFTR) occurs in nearly 1% of allogeneic RBC transfusions. It is defined as a rise in patient temperature by 1°C caused by HLA antibodies in the patient's plasma to antigens present on donor leucocytes. Most patients prone to NHFTR or multiply transfused patients have benefited from receiving leuco-reduced RBC products (Bordin *et al*., 1994).

The incidence of NHFTR may also be associated with high concentrations of tumour necrosis factor α (TNFα), interleukins 1β and 6 (IL-1β and IL-6), proving that the adverse reaction may not only be due to the formation of antigen-antibody immune complexes but may also be caused by cytokines (chemicals that affect the inflammatory response). These cytokines secreted by leucocytes accumulate during the storage period and therefore the depletion of leucocytes soon after WB collection may inhibit the release of cytokines (Bordin *et al*., 1994). The release of these cytokines is responsible for the symptoms associated with NHFTR which include fever, flushing and hypotension (Isbister, 2003).

2.8.2 Transfusion related acute lung injury (TRALI)

Transfusion related acute lung injury (TRALI) is the most common cause of adverse transfusion reactions associated with transfusion morbidity and mortality worldwide (Silliman et al., 2009). TRALI may be due to either antibody-mediated (immune TRALI) when passive transmission of donor anti-leucocyte antibodies react with patient leucocyte antigens and/or due to granulocyte-specific antibodies. Non-immune TRALI may be due to neutrophil-priming elements such as biologically active lipids (Bux, 2005). Donor leucocyte antibodies involved in immune TRALI reactions are thus either directed against human leucocyte antigens class I and II (HLA-A or HLA-B) or against human neutrophil antigens (HNA) present in the patient and may cause an adverse reaction even in immunocompetent individuals. However, the manifestation of non-immune TRALI may occur in a patient with a predisposing clinical pathology such as a haematological malignancy or cardiac disease after a RBC transfusion containing neutrophil-priming elements. The neutrophil is the effector cell in both antibody-mediated and non-immune TRALI which, when stimulated, damages the endothelial cells of the lung capillaries by releasing toxic enzymes and reactive oxygen radicals. The resultant pulmonary oedema is due to the fluid and protein exudation into the alveoli (Bux, 2005).
The symptoms include non-cardiogenic lung oedema, hypotension, cyanosis and hypoxemia which may occur within 2 to 6 hours of transfusion as well as dyspnoea. Although the symptoms demonstrated are very similar to adult respiratory distress syndrome (ARDS), clinicians should be aware that the pulmonary infiltrates associated with TRALI are transient and in 81% of affected patients, are resolved within 48 to 96 hours (Bordin *et al*., 1994). The formation of microaggregates may be implicated in TRALI but may also be a risk factor for the development of ARDS (Isbister, 2003).

Blood components implicated in TRALI reactions include red blood cellular products, platelets, fresh frozen plasma and cryoprecipitate**.** While washing RBC products to remove TRALI mediators present in the plasma is an expensive and time-consuming procedure especially when critically ill patients need to be considered, pre-storage leucocyte reduction of RBC products may decrease the risk of immune TRALI but does not inhibit non-immune TRALI (Silliman *et al*., 2009). The antibody responsible for causing antibody-mediated TRALI may be present in male donors but are more common in multiparous compared to nulliparous female donors as this may be due to previous exposure to paternal leuco-agglutinins present on foetal cells during pregnancy. The multiparous donor cannot be excluded from donating as the donor pool would decrease considerably (Bux, 2005)**.** While screening donor cells for HLA and HNA antibodies appear to be a prudent measure, the

readily available assays are more sensitive to organ transplantation than transfusion medicine while the assays are also laborious and expensive.

2.8.3 Transfusion-associated Graft versus Host Disease (TA-GvHD)

This immunological and often fatal adverse transfusion reaction is caused when viable immunocompetent allogeneic donor T lymphocytes are not cleared by the patient post transfusion. These T lymphocytes subsequently become engrafted into patient tissue resulting in proliferation in the recipient's bone marrow. The survival of the transfused lymphocytes may be due to recipients with either acquired or congenital immunodeficiencies allowing the proliferation of the T cell lineages as the donor lymphocytes are not recognised as foreign and therefore, are not destroyed. Donor lymphocytes may also not be rejected due to HLA similarities when the donor is homozygous for an HLA haplotype while the patient is heterozygous and may occur in immunocompetent transfusion patients. Another factor may be the actual number of viable lymphocytes in the blood component and although lymphocyte viability is not constant during the storage period, the highest risk to the recipient is 3 days post transfusion (Australian and New Zealand Society of Blood Transfusion (ANZSBT), 2011). Transfusion associated Graft versus Host disease (TA-GvHD) occurs when the cytotoxic T lymphocytes and natural killer (NK) cells of the allogeneic donor become the primary effector cells and the haematopoietic stem cells and epithelial cells signify the host target cells.

The tissue damage occurring in the host may be due to TNF-α, TNF-β and IL-1 released by NK cells and donor cytotoxic T lymphocytes although cytolysis is caused by direct cell contact as a result of activated NK cells (Bordin *et al*., 1994). The most common products associated with TA-GvHD are WB and RBCC but may also include platelets or fresh frozen plasma and thus the clinical indications for blood component transfusion should be according to relevant transfusion medicine guidelines. Gamma irradiation of cellular components is the primary preventative measure to avoid TA-GvHD as it inhibits lymphocyte proliferation while maintaining component integrity. Although pathogen reduction may be considered as an alternative for plasma and platelets, it is currently not being used to inhibit lymphocyte proliferation in RBC products (Hauck *et al*., 2015).

2.9 Irradiation

The available literature concerning leucocyte-reduced RBCC is extensive although data regarding the effects of gamma irradiation on pre-storage leucocyte-reduced RBCC is considerably less since the trend of First World countries adopting the ULR policy. The aim of irradiating blood prior to transfusion is to prevent TA-GvHD by eliminating the proliferation of the lymphocytes present in cellular products while preserving the quality of the other cells.

A study in 2009 reported significant differences in the potassium, plasma haemolysis, ATP and 2, 3 DPG concentrations when irradiated and non-irradiated leucocyte-reduced RBCC were compared. These variances were observed whether the leucocyte-reduced RBCC were prepared after component separation or prepared from filtered WB. Differences were also observed in the mean cell volume results although the percentage plasma haemolysis rate for both groups did not exceed 0, 8 percent (Zimmermann *et al.,* 2009). However, while a recent study indicated similar biochemistry results, the concentrations for percentage plasma haemolysis were higher than the recommended 0, 8% (Winter *et al*., 2015).

Although filtration of RBC products considerably reduces the number of viable lymphocytes, a few leucocytes still remain in the pack. While these remaining leucocytes would normally not be problematic for immunocompetent recipients unless they are receiving family directed/designated donations, the outcome of transfusing the viable leucocytes to immunocompromised recipients may cause TA-GvHD as the eligible patients in this category are increasing (Mintz & Anderson, 1993; Agarwal *et al.,* 2005).

It is also imperative that recipients receiving bone marrow transplants, undergoing chemotherapy or in uterine transfusions receive irradiated blood (Leitner *et al.,* 2001). Irradiation causes the inactivation of T lymphocytes as proliferation is inhibited once the nucleated cells are penetrated by ionising gamma rays, causing damage to the lymphocyte deoxyribonucleic acid (DNA) and therefore, leucocyte reduction via filtration is not the same as irradiation. Granulocyte and platelet function are not impaired as a result of gamma irradiation as this damage mainly affects RBC components.

Red blood cells require an efficient metabolism in order to maintain the biconcave shape and membrane permeability of the RBC during storage. It has been reported that gamma irradiation impacts negatively on the potassium/sodium-ion gradient as it changes the intracellular nucleotide levels which may be due to degeneration of the RBC lipid bilayer caused by irradiation instead of the inactive potassium/sodium pump (Brugnara & Churchill, 1992; Leitner *et al*., 2001).

RBC membrane impairment due to irradiation causes an increase in supernatant potassium ions, plasma haemoglobin and lactate dehydrogenase levels and a decrease in pH concentration. The elevated potassium concentration post irradiation is inversely proportional to sodium ion levels and this ion exchange causes the ATP levels to decrease.
These factors indicate that gamma irradiation exacerbates storage lesions (Mintz & Anderson, 1993; Agarwal *et al*., 2005). When storage lesions occur as a result of irradiation, it causes the levels of free haemoglobin to increase but is disregarded as being clinically significant unless patients present with renal failure or hyperkalaemia.

There is a general consensus within the transfusion medicine community regarding the types of recipients who have to receive irradiated blood and these include cellular donations from blood relatives for transfusion, donors who are homozygous for shared HLA haplotypes, neonatal intrauterine or exchange transfusions, patients with congenital or acquired immunodeficiency syndromes such as Di George Syndrome or Wiscott Aldrich Syndrome, premature neonates weighing less than 1200g, allogeneic bone marrow transplant recipients or those harvesting their stem cells for possible later re-infusion, patients having aggressive chemotherapy due to haematological malignancies or being treated with purine analogue drugs as well as patients with Hodgkin lymphoma (Shaz & Hillyar, 2009).

There are a few variations in current international clinical practice guidelines regarding the transfusion of irradiated RBC components. These clinical practices arise from statistics based on non-filtered irradiated red blood cellular products despite the adoption of ULR policy in most countries (Zimmermann *et al*., 2011).

The American FDA recommends a maximum storage period of 28 days or the primary expiry date for irradiated components regardless of product storage date for the irradiation process whereas the Chinese State Food and Drug Administration follows a 35-day storage time (Ran *et al*., 2011). The British Committee for Standards in Haematology Blood Transfusion Task Force, the ANZSBT as well as the Clinical Guidelines for the use of blood products in South Africa recommend that RBC components may be irradiated up to 14 days post WB collection with an additional 14 days storage after irradiation date and that irradiation dosage is between 25 grays (Gy) to 50Gy. These guidelines also caution that irradiated RBC products be infused within 24 hours post irradiation should a patient present with a risk of hyperkalaemia. The Council of Europe's guidelines stipulates that RBC components may be irradiated up to 28 days post collection but may not be transfused after the 14-day post irradiation process. A recent study has indicated similar cellular damage was observed

whether RBCC was irradiated on Day +14 or Day +3. It was also recommended that the transfusion of irradiated blood should be completed at the earliest opportunity to patients with increased potassium sensitivity such as premature infants or renal failure patients while those less sensitive may be transfused with stored blood for up to 28 days post irradiation date (Hauck *et al*., 2015).

While clinicians are aware that irradiation and storage cause cellular degeneration and a subsequent increase in percentage haemolysis, they should also be aware that even "fresh" blood consists of an assortment of RBC at different stages of maturity. The oldest RBC will haemolyse due to shear stress while the more flexible younger RBC may remain undamaged and thus, the storing of irradiated blood for 28 days post irradiation may not be best clinical practice for the recipient (Harm *et al*., 2012).

It has been established that as a result of metabolic pathologies resulting in hyperkalaemia, children are more prone to cardiovascular diseases (Bhananker *et al*., 2007). Hyperkalaemia may be reduced by washing the irradiated RBC cellular product by using a cell saver for neonates undergoing cardiopulmonary bypass surgery although it is ineffective in reducing hyperlactaemia associated with postoperative morbidity and mortality (Swindell *et al*., 2007). However, if the neonate is hyperkalaemic then the guidelines also stipulate that blood used for either an exchange or intrauterine transfusion be less than 5 days old and infused within 24 hours after irradiation (Treleaven *et al*., 2010, ANZSBT, 2011; Ingram & Bellairs, 2014). According to the Japanese national guidelines, blood for transfusion may optimally be irradiated up to 14 days post donation with irradiated dosage prescribed between 15Gy to 50Gy and as their usual expiry period post WB collection is 21 days, the irradiated blood may be used for other patients provided the elevated potassium level will not harm the recipient. Caution is advised against irradiating blood fresher than 3 days old because the risk of TA-GvHD is higher. Due to the limited haplotypes present, the Japanese also irradiate a wider range of blood components compared to other national guidelines (Asai *et al*., 2000).

According to Pelszynski and colleagues (1994) who used the limiting dilution analysis (LDA) to measure viable T lymphocytes, TA-GvHD may be prevented when there is a reduction of more than 2 $\log_{10}$ viable cells post gamma irradiation. Despite storage time reducing the number of proliferative T cells, they reported that even though the application of 15Gy resulted in inactivation of more than 4 $\log_{10}$, viable cells remained while irradiation at 25Gy and 30Gy indicated no growth (Pelszynski *et al*., 1994). Using flow cytometry and LDA methodology 12 years later, Góes and colleagues (2006) also demonstrated T lymphocyte growth when blood was irradiated at 15Gy but that proliferation was reduced by 5 $\log_{10}$ when

blood was exposed to 25Gy. This study demonstrated that to prevent TA-GvHD, a dose of at least 25Gy using sources such as ^{60}Cobalt, ^{137}C or X-rays produced by a linear accelerator should be applied (Góes *et al*., 2006). As irradiation is the preferred method to prevent TA-GvHD, most blood transfusion establishments use the free-standing ^{137}C irradiator where the cellular components are placed within a metal container situated on a rotating turntable and it is recommended that routine dose mapping is completed on an annual basis or post major repair (Moroff & Luban, 1997). It has been reported that even though irradiation is currently used to prevent TA-GvHD, the use of ultraviolet (UV) irradiation is being considered as the potassium concentrations were similar but no significant differences demonstrated in haemoglobin, haematocrit, mean cell haemoglobin concentration, mean cell volume, the mean cell haemoglobin, RBC and platelet levels of pre- and post-irradiation samples. There was, however, a significant difference in leucocyte levels as a decrease in the UV post-irradiated sample was observed (Golsa & Suhaimi, 2012).

2.10 Red cell haemolysis

The red cell undergoes a series of biochemical and morphological changes during the storage period of up to 42 days and the application of irradiation aggravates these storage lesions. The presence of haemolysis is observed when the plasma or supernatant of a blood sample shows pink to red discolouration as a result of the RBC membrane integrity disruption and subsequent haemoglobin release. Percentage haemolysis (also known as plasma free haemoglobin) is a biochemical storage lesion indicator and is used to measure various factors such as extracellular haemolysis which may occur as a result of storage for up to 42 days at 1°C to 6°C or the presence of leucocytes in unfiltered blood. It may also be used to evaluate mechanical injury during filtration, bacterial contamination occurring during donation, component processing practices as well as using incorrect transportation procedures (Han *et al*., 2009). Other factors influencing the presence of haemolysis include temperature deviation during collection and processing procedures, using non-DEHP blood bags or haemoglobinopathies negatively affecting RBC deformability such as sickle cell anaemia, congenital spherocytosis and glucose-6-phosphate dehydrogenase deficiency. The evidence of plasma free haemoglobin may also be due to RBC exposure to hypertonic, hypotonic and pH fluctuations, donors on high concentrations of drugs such as penicillin, alpha-methyl-dopa and vitamin C as well as using gamma irradiation on RBC products to prevent TA-GvHD (Sowemimo-Coker, 2002). It has been reported that while the rate of haemolysis is higher in room temperature-held blood compared to the rapidly cooled blood prior to component production, it is even higher when RBCC are produced from room temperature-held blood stored in SAGM (Eckstein *et al*., 2015).

The presence of haemolysis increases the oxygen affinity of haemoglobin which negatively affects the levels of 2, 3 DPG and subsequently, not only reduces oxygen transfer to the tissues but also causes a lower pH concentration. The biochemical reaction of free haemoglobin and nitric oxide may cause endothelial dysfunction leading to intravascular thrombosis, leucocyte adhesion and possible vasoconstriction (Aubron *et al*., 2013). Observation of haemolysis in the supernatant or plasma may indicate that the RBC have either been ruptured or is due to loss of membrane-bound haemoglobin micro-vesicles. The transfusion of red blood cellular components containing free haemoglobin may cause oxidation-reduction (redox) damage to bodily tissue, to the endothelium or to the renal proximal tubule. Procoagulant and proinflammatory surfaces may appear due to the infusion of microvesicles affecting the microcirculation and consequently, impact on systemic haemodynamics (Hess *et al*., 2009). Leucocyte reduction of RBC and the addition of membrane stabilisers such as citrate or mannitol may decrease the level of plasma haemolysis.

Red cell haemolysis is determined by measuring the free haemoglobin released into the supernatant in relation to the total haemoglobin concentration within the product. There are various methods used to establish quantitative percentage haemolysis results such as photometric, spectrophotometric and microplate techniques. However, no standardised method exist as each criterion may be measured in various ways as was reported in a study that proved a coefficient of variation of approximately 55% between 14 laboratories measuring percentage haemolysis (Han *et al*., 2009).

A common practice is to visually check red blood cellular products before being issued from the blood bank. This visual method of evaluating levels of plasma haemolysis in a RBC product is often incorrect, subjective and may result in gross overestimation.
This may subsequently lead to these units being discarded as dark pink supernatant discoloration may demonstrate plasma haemoglobin levels as low as 0, 09% haemolysis (Sawant *et al*., 2007).

The American FDA specifies that percentage haemolysis in a RBC product should not more than 1% at the end of the storage period with a 75% survival rate for transfused red blood cells 24 hours post transfusion. They later added that blood transfusion establishments must be able to demonstrate that 95% of their RBC products meet the recommended standards and that it is statistically achieved 95% of the time (Hess *et al*., 2009, D'Alessandro *et al*., 2010). This latter addition became known as the "95/95 rule" and was implemented due to

the difference in free haemoglobin occurring between donors in addition to safeguarding transfusion recipients such as sickle cell anaemic and pulmonary hypertensive patients from free haemoglobin overload (Sparrow *et al*., 2009).

The recommendation by the Council of Europe however, stipulate that the level of free haemoglobin (percentage plasma haemolysis) in a RBC product should not go beyond the 0, 8% level. A recent study revealed a difference in percentage haemolysis between RBCC components stored in Adsol (AS-1) and SAGM. The lower mean haemolysis level demonstrated in the Adsol units may be attributed to Adsol containing 150% more glucose and 750mg/dl mannitol in addition to 50% more adenine than SAGM or that the PRP method was used in the SAGM-containing bags (Makroo *et al*., 2011).

Measuring haemolysis became one of the first indicators used to assess the quality of RBC when Roux and Turner initially used it in 1915 and it is still currently being used (Hess *et al*., 2009).

2.11 Fresh blood versus older blood

Storage lesions are associated with RBC alterations as well as the accumulation and leakage of harmful substances into the plasma causing a reduction in oxygen transportation to tissues and therefore, transfusing old blood versus fresh blood in critically ill patients is a constant topic of debate.

Aubron and colleagues (2013) reported on various studies related to this discussion but whether the study has been observational, prospective or retrospective, the outcomes have been shrouded in controversy due to contradictory conclusions. After examining 32 studies regarding critically ill recipients receiving fresh versus old blood, they concluded that after receiving older blood, patients in the intensive care unit (ICU) experienced a longer length of stay in the hospital and cardiac patients suffered higher post-operative infections after receiving older blood. Some studies also indicated that trauma recipients developed an increased risk of developing multi-organ failure and renal dysfunction while the occurrence of deep vein thrombosis and mortality were observed in others. They also reported that 44% of the studies did not demonstrate any causal relationship between storage time of blood and nosocomial infections, mortality, period of mechanical ventilation, renal dysfunction and length of ICU stay as the transfused fresh blood were all less than 8 days old (Aubron *et al*., 2013). A few prospective trials have been completed to investigate possible adverse effects of stored blood (Zimring, 2013).

The Red Cell Storage Duration Study (RECESS) is a clinical trial supervised from 2010 to 2014 where cardiac surgical patients received blood either stored for up to 10 days versus blood stored for 21 days or more to demonstrate a possible difference in patient clinical outcomes. Steiner and colleagues (2014) reported no differences when transfusing fresh filtered RBCC (stored for 10 days or less) or transfusing older filtered RBCC (stored at 21 days or more) to cardiac patients requiring surgery as no transfusion reactions, changes in multiple-organ dysfunction scores nor mortality at day 28 when the clinical trial ended, were observed (Steiner *et al*, 2014).

The Age of Blood Evaluation (ABLE) study was started in 2011 to determine whether the transfusion of fresh (up to 8 days old) leucocyte-reduced RBCC will improve the 90-day mortality rate and reduce morbidity in critically ill recipients when compared to using filtered blood stored for up to 35 days (mean for transfusion is 18-21 days). Other aspects of this study will consider including a 30-day and a 6-month month mortality rate, a reduction in nosocomial infections, a decrease in organ dysfunction and adverse transfusion reactions. Pending results due in 2015, a positive study result will validate the requirement for blood banks to manage the available red blood cellular stock products more effectively and improve storage techniques while a negative outcome will confirm and reassure clinicians regarding the safety and effectiveness of transfusing critically ill patients with standard- issue blood (Lacroix *et al*., 2011).

The Age of Red Cells in Premature Infants (ARIPI) was a comparative study using fresh blood versus older blood in premature neonates with birth weights less than 1250g to determine the possible decrease of neonatal pathologies related to organ dysfunction, organ failure or nosocomial infections. The transfused fresh blood was stored for less than 7 days (mean storage of 5.1 days) while the older blood had a mean storage time of 14.6 days. None of the candidates of the ARIPI study had blood older than 15 days transfused even though the results of the study indicated no difference in clinical outcomes between the transfusions of fresh or older blood (Fergusson *et al*., 2012).

Clinicians have raised a few concerns at using older blood in neonatal transfusions and the accompanying implication that older blood does not affect necrotising enterocolitis, a common morbidity in premature infants. Also, a liberal transfusion practice was followed as haemoglobin levels were not stated prior to transfusion although each infant received approximately 5 RBCC aliquots of about 14ml per aliquot, the results of the trial may be challenging to establishments using a more conservative transfusion practice. Furthermore,

the older blood used for neonatal transfusion had a mean storage time of 14.6 days whereas the average storage period of a RBCC in American centres is about 18 days. The authors recommended caution to blood transfusion establishments outside Canada before implementing the outcome of this study (Patel, 2013).

Clinicians administer RBC transfusion for various clinical indications and the exposure to the biochemical changes occurring in stored blood and will have a different effect depending on the pathophysiology of the patient. Current literature has determined that there is a difference between fresh and old blood as the storage lesions occurring in stored blood up to 42 days clearly indicate RBC degeneration and deformity (Zimring, 2013). The question begs to ask: "is it relevant?" and the response is vague. This is as a result of variability in retrospective study reports and therefore, no consensus regarding the storage period of RBC components, morbidity and mortality have been reached. This may be attributed to most patients who participated in these retrospective studies received both fresh and old blood (Vandromme *et a*l., 2009).

Most of the associations reported in clinical trials are from studies completed in North America and none from Europe or developing countries. The variances indicated in these studies may also be due to differences in component production methods, nutritive solutions or cellular composition of blood products used. The results from ongoing prospective trials and additional research into RBC storage techniques may provide the required answers regarding transfusion practices although global research is needed should these results become applicable to blood transfusion services worldwide.

CHAPTER THREE

RESEARCH DESIGN AND METHODOLOGY

3.1 Ethical Considerations

The study proceeded according to the World Medical Association Declaration of Helsinki (1964) and good clinical practice. All ethical and scientific standards were maintained regarding the collection of whole blood units donated by voluntary, non-remunerated donors as well as the testing and the correlation of test results. Dr. G.R.M. Bellairs (CEO/ Medical Director) at Western Province Blood Transfusion Service (WPBTS) provided a letter of consent for the research study to take place at WPBTS Headquarters in Ndabeni, Cape Town (see Appendix A).

Donors completed a donor questionnaire per standard procedure prior to routine whole blood donation (see Appendix B). The purpose of the proposed research study was to improve the safety and efficacy of transfusion practices and therefore individual informed consent from voluntary, non-remunerated donors is not applicable for this study as they completed a donor questionnaire prior to routine donation (see point 6 in the Declaration section of the donor questionnaire). The investigator also ensured that available levels of blood stock were not compromised in any way. To ensure and maintain confidentiality, barcoded serial numbers were used instead of personal information as all donor information is regarded as confidential.

Ethical approval from the Health and Wellness Sciences Research Ethics Committee (HWS-REC) of Cape Peninsula University of Technology was obtained in 2013 (CPUT/HW-REC 2013/H30) with the approval of an extension application in 2014 (CPUT/HW-REC 2014/H10). The study was a double-blind, quantitative design.

3.2 Research Setting

WPBTS was initially known as the Cape Peninsula Blood Transfusion Services with a donor enrolment of approximately 200 donors. During the1930's, the practice of blood transfusion was very basic. Donors were contacted if a patient needed blood and with only a screen separating them, donor blood was directly infused into the patient. The organisation opened its first blood bank during 1939 and this allowed the military hospitals to be supplied with whole blood during the Second World War.

The organisation changed its name during 1949 when they started to recruit donors from the outskirts of Cape Town and by October 1943, the donor base consisted of 1394 active donors. By 1959, the organisation expanded its premises to include the technical facets of transfusion medicine. WPBTS was actively involved with the first heart transplant that took place at Groote Schuur Hospital in 1966 and by 1969, approximately 7000 pints of whole blood were collected monthly.

Nowadays, more than 130 000 units of safe blood are collected annually with approximately 500-550 red blood cellular products issued daily to treat patients while the donor database indicates about 69 000 active donors. It is a community-based health establishment and provides the safest blood products by using the latest technology (nucleic acid testing) and research to safeguard both donors and patients. All of this is accomplished while complying with blood transfusion standards per the World Health Organisation, the South African Human Tissue Act and the Occupational Health and Safety Act. The blood transfusion establishment is also accredited by the South African National Accreditation System (SANAS) and all laboratory staff are members of the Health Professional Council of South Africa. Globally, WPBTS is well-respected within the transfusion medicine community and is also regarded as one of the most technologically innovative blood transfusion institutions in Africa.

3.3. Study design: From pilot study to completion of study

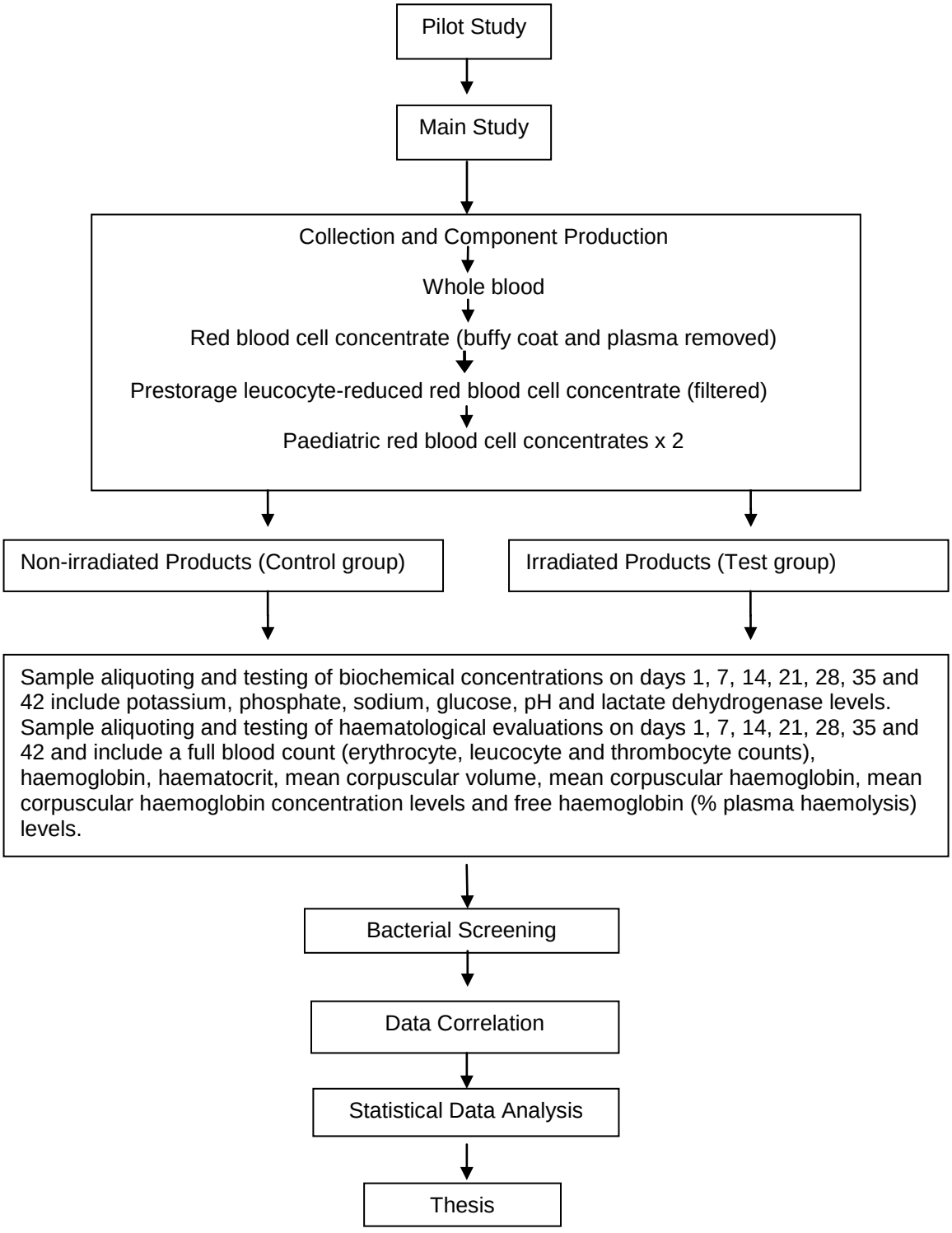

Figure 3.1: Study design: From pilot study to completion of study

3.4 Pilot study

Prior to starting the research study, a pilot study was initiated to include the serial aliquoting of two units of adult red blood cell concentrates (RBCC) on days 1, 7, 14, 21 28, 35 and 42 by using an aseptic technique.

3.4.1 Aim of the pilot study

The aim of the pilot study was to identify the time it would take to complete the sample aliquoting of each unit and the subsequent separation of aliquoted samples on the proposed days. A broad spectrum bacterial screening was completed on the used bags after the final aliquot. This was completed to assess the possibility of bacterial contamination during the 42-day storage period. The ABX Pentra 400 biochemistry analyser had to be validated prior to starting the study as part of good clinical practice and according to the Quality Management System used at WPBTS.

3.4.2 Methodology

3.4.2.1 Sample aliquoting

Two adult red blood cell concentrates were collected from the Components Laboratory at WPBTS on the 7th November 2013. Additional serial number labels for each unit were printed and were affixed on 6 x 4ml red top tubes, without added anticoagulant. This process was repeated on Days 1, 7, 14, 21 28, 35 and 42 respectively. The 6 labelled tubes required was to test for the following:

- 1 aliquot for testing the haematological indices and pH levels.
- 1 aliquot for testing the plasma haemoglobin plus 1 x 4ml tube for the separated plasma.
- 1 aliquot for the metabolic analytes plus 1 x 4ml tube for separated plasma
- 1 aliquot for testing the glucose levels.

A duplicate serial number label was then placed on the diversion pouch i.e. the tubes and diversion pouch had corresponding labels attached. The diversion pouch used in the aliquoting procedure is an unused by-product of the random donor platelet pooling kit. It was removed from the kit using a Tube Sealer AC-155 (Terumo, Colorado, U.S.A.) which hermetically seals the tubing and thus ensuring sterility of the diversion pouch.

The unit was gently but thoroughly agitated to ensure a homogenous mixture of the unit contents. The pouch was aseptically attached to the RBC unit by using a TSCD® II Sterile Tubing Welder (Terumo, Colorado, U.S.A.). The TSCD® II wafer uses heat up to 300 ˚C and therefore sterility is maintained during the cutting and welding procedure without allowing particulates or chemical residue to form in the tubing. The wafers are automatically discarded after a single use into a built-in disposal box as this avoids possible cross contamination between units. An alarm sounded to indicate that the container for the discarded wafers was full and had to be discarded according to WPBTS Health and Safety guidelines.

Prior to breaking the seal between the diversion pouch and the tubing of the unit, the unit was gently agitated again. The seal was broken, allowing the contents of the unit to flow into the sterile diversion pouch. The diversion pouch is able to hold ±75ml of blood and it was ascertained that filling the diversion pouch to approximately a third full of aliquoted blood (± 25ml) was sufficient volume for the blood samples needed for the required testing.

After the pouch was filled with the required amount of blood, the tubing of both the unit and the diversion pouch was sealed using the tube sealer. The seal between the unit and the diversion pouch was then cut to separate the two bags. The unit was returned to the fridge, thus maintaining storage temperatures of between 1° to 6°C. The seal of the diversion pouch was cut and the labelled tubes were filled. The tubes were centrifuged for 5 minutes at 3000rpm, the plasma separated and placed in the labelled tubes. A new diversion pouch was sterile docked to the product whenever sample aliquoting was required.

After the final aliquot on Day 42, the used RBCC bags were sent to the WPBTS Microbiology laboratory for aerobic and anaerobic bacterial screening to determine the possible occurrence of bacterial contamination during the storage period. The sample aliquots, as well as the products used in the pilot study, were stored at 1° to 6°C for a period up to 42 days.

3.4.2.2 The ABX Pentra 400 biochemistry analyser

This analyser was validated for sensitivity, specificity and reproducibility. Ten random donors were asked to supply 2 whole blood (WB) samples each to complete the validation. The 10 WB samples were collected in SST tubes and were centrifuged for 5 minutes at 3000rpm. The separated plasma was used to test potassium, phosphate, lactate dehydrogenase (LDH) and sodium levels.

The other 10 WB samples were collected in glucose tubes with added anti-glycolytic sodium fluoride from the 10 donors were centrifuged for 5 minutes at 3000rpm. The separated plasma was used to test glucose levels.

Random samples were used to test the ABX Pentra 400 analyser for reproducibility. Data was correlated and a report submitted to the research study supervisors. The investigator ensured that all equipment used in the pilot study was calibrated and serviced prior to use. All used materials and disposable equipment were discarded in labelled biohazardous containers according to the Occupational Health and Safety Act and per WPBTS Work Instructions.

3.5 Study Population

The donor division at WPBTS collects whole blood units from various donation clinics daily. This is to maintain a healthy supply of blood and plasma products needed by patients. Both the control and test groups of forty units each were randomly selected from a pool of healthy, voluntary and non-remunerated blood donors residing in the Western Cape. All donors fulfilled the criteria as listed in the donor study profile.

3.5.1 Donor Study Profile

There are certain criteria used to evaluate the possibility of a donor donating and these are documented in the Standards of Practice for Blood Transfusion in South Africa. This document, as well as the Clinical Guidelines for the use of Blood Products in South Africa, is due to a combined effort by the Chief Executive Officer (CEO) of WPBTS as well as the CEO of the South African National Blood Transfusion Service. Listed below are the donor inclusion and exclusion criteria used for the study.

3.5.1.1 Inclusions

It is imperative that a blood transfusion establishment protect their donors from being compromised and certain criteria were followed to protect the donor.

- All donors were voluntary and non-remunerated.
- Informed consent: First time/new donors are given verbal or written information about the whole blood donation procedure. A donor questionnaire was completed and signed by all prospective donors.
- Gender: Either male or female.

- Age: Blood donors were older than 16 years of age but younger than 65 years.
- Donation interval: The interval between consecutive blood donations was not less than 56 days.
- General health: Prior to donation, each donor was verbally questioned to confirm that he/she enjoyed normal health and has not suffered, nor is suffering from any serious illness. All information was suitably documented.
- Drug Therapy: A registered nurse or medical practitioner evaluated prospective donors who were taking medications to determine their suitability to donate blood. A list of drugs requiring deferral from donation was available.
- Haemoglobin and haematocrit concentration: The haemoglobin level of each suitable donor was not less than 12, 5 g/dl (125 g/L) and the haematocrit value was not less than 0,38L/L.
- Pulse: The pulse rate of each donor did not indicate any irregularity and was between 50 to 100 beats per minute, except in highly-trained athletes where a lower pulse rate was acceptable.
- Blood Pressure: The systolic blood pressure of a donor was between 90mm Hg and 180 mm Hg while the diastolic pressure was between 50mm Hg and 100mm Hg.
- Pregnancy: Known existing pregnant female donors were precluded from routine donation until 3 months post birth of the baby.
- Donor Weight: Donors did not weigh less than 50 kg.

(Bellairs & Mpuntsha, 2013: 14-16)

3.5.1.2 Exclusions

- All those donors who were unable to fulfil the blood donor criteria as listed in the “inclusions”. A document detailing circumstances for donor deferral was available.
- No incarcerated or mentally-challenged individuals.
- No individuals younger than 16 years or older than 65 years were allowed to donate.
- Any person who appeared to be under the influence of alcohol or any drug having a narcotic or any other adverse effect was not accepted as a blood donor.
- Unexplained weight loss of a significant degree e.g. more than 10% of body weight, was a reason for exclusion.
- First-time donors were excluded.
- Donors were deferred due to certain medical conditions, medication, immunisations, vaccinations and donor exposure to possible transfusion transmissible diseases. The

same applied to prospective donors who were unable to provide reliable answers to the medical history or other questions.

3.6 Main Study

This was a prospective, double-blind, quantitative research design and consisted of 4 types of the red blood cellular products with 20 units per product. These included whole blood (WB), buffy coat poor red blood cell concentrate (RBCC), prestorage leucocyte reduced RBCC (LRBCPS) and the paediatric RBCC. Eighty WB units were collected on 11.08.2014. All units were randomly selected for subsequent processing into the different products and were part of either the Control Group or Test Group.

Twenty units of WB units were left unprocessed i.e. to be used as WB. Ten WB units were randomly used as the Control Group while the remaining 10 x WB units were irradiated and used as the Test Group.
The remaining 60 units were processed into buffy coat poor RBCC on 12.08.2014. Twenty RBCC were kept aside to be used as RBCC units. Ten RBCC units were randomly placed in the Control Group while the remaining 10 x RBCC units were irradiated and used as the Test Group.
The residual 40 x RBCC units were leucocyte-reduced using filtration methodology and this modified product is known as prestorage leucocyte-reduced RBCC. Twenty filtered units were retained as prestorage leucocyte-reduced RBCC. Ten prestorage leucocyte-reduced RBCC (LRBCPS) units were used as the Control Group and the remaining 10 x LRBCPS units were irradiated and placed in the Test Group.
The remaining 20 filtered units were further modified and processed into paediatric RBCC. An adult filtered unit is divided into 2 and known as a paediatric RBCC. Ten x 2 paediatric RBCC units were randomly placed in the Control Group and the last 10 x 2 paediatric RBCC units were irradiated and used as the Test Group (Each adult LRBCPS was divided into 2 bags viz. PRBC 1 and PRBC 2).

Irradiation of units placed in the test group took place on 12.08.2014. Testing for the haematological and biochemical factors commenced 24 hours after the irradiation procedure i.e. 13.08.2014, also known as Day 1. Each group was tested for haematological and biochemical changes on Days 1, 7, 14, 21, 28, 35 and 42 while maintaining the storage temperature of 1° to 6°C.

3.6.1 Donation procedure

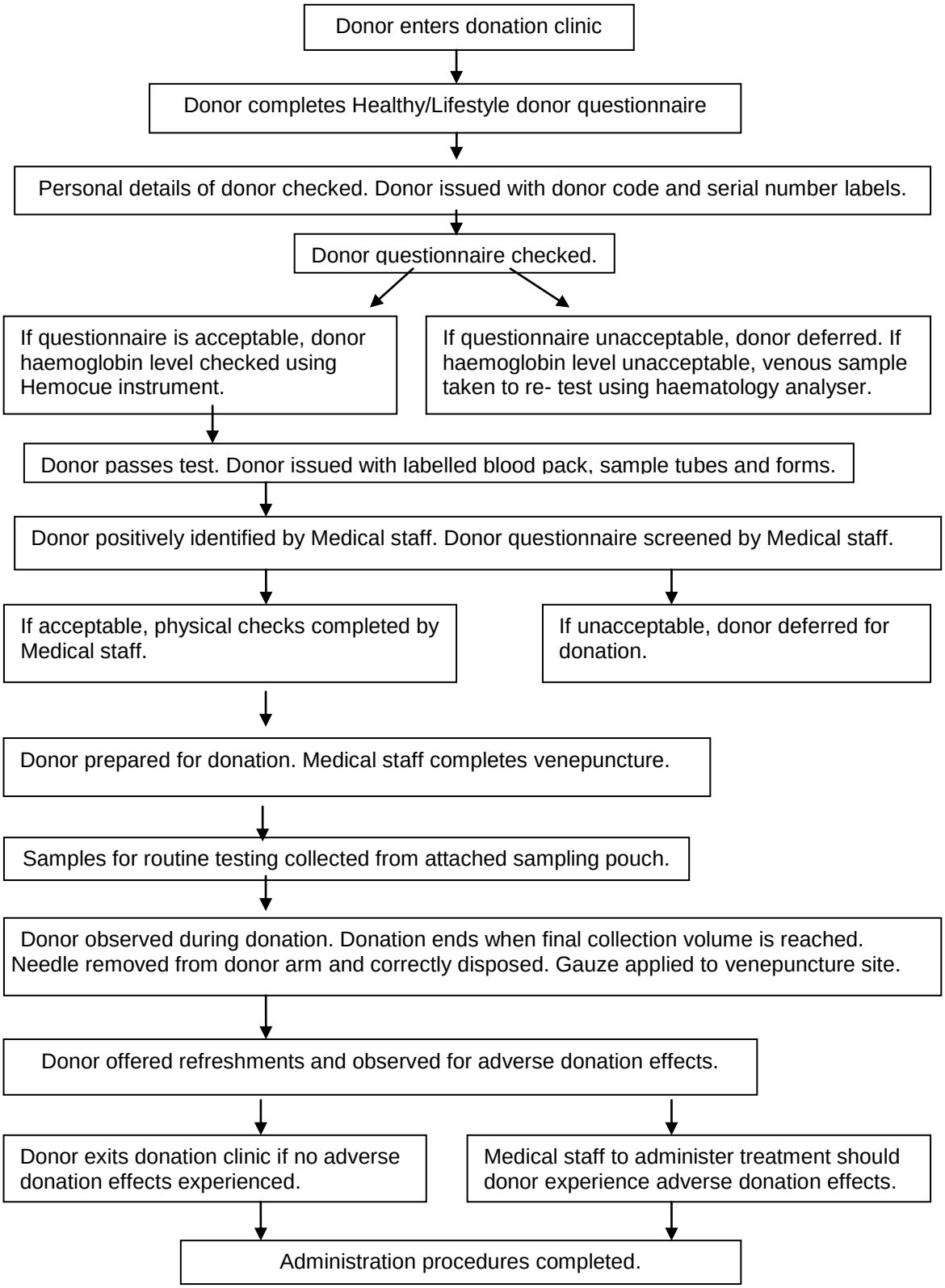

Figure 3.2: Donation procedure

3.6.2 Whole blood collection procedure

Qualified staff of donor division are adept at WB collection procedures and are trained to reduce the incidence of phlebotomy adverse reactions such as haematomas, from occurring. The whole blood volume collected from the accepted donor was between 475ml and 575ml. It was collected in a sterile, pyrogen-free collection bag with a unique batch (lot) number containing citrate phosphate dextrose (CPD) with 3 satellite bags attached viz. the primary collection bag, 1 satellite bag containing the nutritive solution and 2 empty satellite bags. Each donor was given a sticker card with serial numbers attached that was only used for that particular donor for that specific donation. Staff attached a serial number from the sticker card to the form containing the matching donor code. This was to link donor code to the serial number in order to maintain donor confidentiality. A serial number from the same sticker card was also placed on the primary collection bag as well as on each of the sample tubes collected for routine laboratory testing. Blood and test samples were collected from all donors who succeeded to donate. All WB units, together with test samples from each donor and relevant documentation pertaining to the collection were packed appropriately in blood crates. This was done to prevent accidents from happening during transportation from donor collection clinics to the central collection laboratory. Each blood crate was labelled with a tag indicating the collection venue and area relating to the collection.

3.6.3 Procedure at central collection laboratory

Crates containing blood received from the collection clinics were checked to ensure that the blood was transported correctly and staff established that arrival temperatures were between 2 – 37 °C. Blood crates were placed in a refrigerator at 1°C – 6 °C within 8 hours of bleeding while those WB units used to produce random donor platelets, in addition to the RBCC and plasma product, were placed in a 20–24 °C controlled environment within 6 hours of bleeding for a maximum of 24 hours. Each blood collection was checked to ensure that the number of WB blood collections documented on the corresponding form also correlated with the number of test samples received. Staff also checked that the serial numbers attached to the WB unit matched the serial numbers on the corresponding sample tubes.

The refrigerated blood was not kept for longer than an hour at room temperature when staff had to segment the tubing of the WB unit. Prior to segmentation, a light pressure was applied to the outside of the tubing which ensured that the contents of the tubing were moved into the primary WB bag. The WB unit was then gently agitated to ensure a homogenous mixture and the pressure on the tubing was subsequently released. This allowed the contents of the

tubing and the primary WB unit to be the same. The tubing attached to the WB unit was segmented using a T-Seal II sealer (TerumoBCT, Colorado, U.S.A.) with a corresponding serial number attached to each of the 2 segments. One WB segment was used for routine testing while the other segment was packaged according to collection venue and retained for 42 days at 1 – 6 °C from the date bled. The latter WB segments are retained as a sample of the primary collection bag and may be used later for testing, for example, when a patient suffers an adverse transfusion reaction and the product being investigated does not have segments attached for testing. All segments were discarded in a labelled biohazardous waste container according to Health and Safety regulations after the retention period.

Labels were printed indicating date bled and expiry dates and placed on each primary collection bag. The satellite bags had specific product labels with the corresponding serial numbers, bleeding dates and expiry dates specific to the product, attached. All collection data were captured onto the computer system. The WB units were returned to the refrigerator to await further processing. The samples collected from donors were centrifuged using the Heraeus Megafuge 40 centrifuge (Thermo Fisher Scientific, Lagenselbold, Germany) and delivered to the respective laboratories for testing.

3.6.4 Routine testing of donor samples

The responsibility of a transfusion institution is not only about processing blood components according to global quality standards but to also include the accurate testing of blood donation units in the quest to provide safe, sufficient, quality blood products to recipients.

All testing was successfully accomplished by qualified, trained and competent staff, ensuring that all equipment were serviced and calibrated prior to use. Various methods for automated and manual testing were used. Methodology, together with the corresponding reagents and all equipment, were used following the manufacturers' instructions and WPBTS Work Instructions. All samples used were labelled with barcoded serial numbers to maintain donor confidentiality and none of the samples tested required any confirmatory testing to be done. Results were sent to the host computer where serial numbers and corresponding results were automatically uploaded.

The Olympus PK7300 analyser was used to test all donor samples to determine the ABO blood group and Rh status. This analyser was also used to phenotype the donor samples for red cell antigens such as C, c, E, e, Kell, Jk^a, Jk^b, Fy^a, Fy^b, S and s by using the

corresponding antisera.. Testing also included determining the anti-A, B titre and samples were screened for the possible presence of irregular antibodies in the plasma.

Syphilis is a transfusion-transmitted infection and was tested using the indirect *Treponema Pallidum* haemagglutination (TPHA) method by the Olympus PK7300 analyser as well as the Venerable Disease Research Laboratory/ Rapid Plasma Reagin (VDRL/RPR) method. A system for syphilis confirmatory testing is in place using the VDRL/RPR test method and the Axis Shield kit. Anticoagulated WB samples were automatically de-capped using an Olympus AutoMate before placed in the Olympus PK7300 analyser. Samples were stored for 7 days at 1°C – 6 °C after testing. The ABO and Rh blood groups as well as anti-A, B titres were confirmed with no irregular antibodies detected in all samples tested. All donor samples tested negative for syphilis.

Other transfusion transmitted infections routinely tested included the Human immunodeficiency virus (HIV), hepatitis B and hepatitis C. The antigen or antibody testing of these viral markers were completed using a chemiluminescent immunoassay (Abbott Prism System) and nucleic acid testing by means of genomic (transcription-mediated) amplification technology (Procleix Panther System). A system for confirmatory testing is in place using a chemiluminescent microparticle immunoassay (Architect i2000RS analyser). The Safe Sampling System-3S is a bio-sampler (Ilex, Saint Ouen l'Aumône, France) and was used to aliquot 1000µl of plasma from routine negative donor samples and subsequently stored for 2 years at -80°C. Plasma aliquots from confirmed positive reactive donors are also stored at -80°C, albeit in separate containers with an indefinite storage period for look-back or research purposes.

Table 3.1: Routine testing methods used

TEST	ANALYSER	MANUFACTURER
ABO and Rh blood groups Irregular antibodies Syphilis –TPHA test	Olympus PK7300 analyser	Beckman Coulter, California, U.S.A.
Syphilis – VDRL/RPR Syphilis – Axis-Shield RPR	Kit provided Kit provided for confirmatory tests	Davies Diagnostics, Scotland, U.K
HIV 1 and HIV 2 antibodies Hepatitis B surface antigen Hepatitis C antibody	Prism System using chemiluminescence immunoassay technology	Abbott Diagnostics, Illinois, U.S.A.
HIV 1 and HIV 2 (RNA) Hepatitis B (DNA) Hepatitis C (RNA)	Procleix Panther System - NAT via TMA technology	Gen-Probe, California, U.S.A.
HIV Combo (antigen & antibody) Anti-HCV HBsAg Anti-HBs, Anti-HBc & Anti-HBc (IgM)	Architect i2000RS analyser - chemiluminescence microparticle immunoassay technology for confirmatory testing	Abbott Diagnostics Illinois, U.S.A.

3.6.5 Preparation procedures of red blood cellular products

Production of products was successfully accomplished by qualified, trained and competent staff who ensured that all equipment were serviced and calibrated prior to use. Various methods for automated and manual testing, as well as equipment, were used following the manufacturers' instructions and WPBTS Work Instructions. All equipment used were serviced and calibrated per the service and maintenance schedules prior to procedures. The samples used for testing were labelled with barcoded serial numbers to maintain donor confidentiality. Materials and disposable equipment used during testing were discarded in labelled biohazardous containers according to the Occupational Health and Safety Act and per WPBTS Work Instructions.

3.6.5.1 Whole blood

Twenty whole blood (WB) units were randomly selected to form part of either the control group (non-irradiated products) or the test group (irradiated products). The units were placed in a blood crate as no further processing needed to be done. After the routine testing was complete, the WB units were labelled indicating the name of the product, the donor's ABO blood group and Rh status, as well as the date bled, expiry date and Anti-A, B titre. The shelf life of WB is 35 days when stored at 1°C to 6°C.

3.6.5.2 Red blood cell concentrate

The remaining sixty WB units were moved to the processing laboratory where all processing procedures are standardised according to Good Manufacturing Practices and WPBTS Work Instructions detailing said procedures.

WB consists of a variety of cellular elements all having different densities. When centrifuged, the red blood cells (RBC) settle to the bottom of the bag due to the applied centrifugal force as they have a higher cellular density component. This is closely followed by the platelets, granulocytes and leucocytes due to a lower cellular density and these components form the buffy coat layer which settles above the red blood cells. The final layer is the plasma mixed with the anticoagulant which settles above the buffy coat layer as this component has the lowest cellular density.

It is important to choose the correct centrifugation speed and time period when processing a specific component. Should the required product be platelet rich plasma, then centrifugation speed is lower for a longer period (moderate centrifugation) compared to when processing cell-free plasma. The latter method would yield a densely packed RBC and clear plasma and therefore, the centrifugal force applied is higher for a shorter time. Both methods were used to process the RBCC used in the study. The cellular components of a WB unit were separated according to cellular density using a Sorvall RC 12 BP Plus refrigerated centrifuge (Thermo Fisher Scientific, Massachusetts, U.S.A). These refrigerated centrifuges are large floor-standing machines, able to centrifuge up to twelve WB units at a time and assist in maintaining the cold chain of the red blood cellular products.

Cellular components of a WB unit was separated via centrifugation at 3140rpm (3273rcf) for 12 minutes at 4°C for the production of a RBCC, fresh frozen plasma and cryoprecipitate but was centrifuged at 3140rpm (3273rcf) for 10 minutes at 22°C for the production of RBCC, fresh frozen plasma (FFP) and platelets. In order to avoid the plasma being contaminated by red blood cells, the centrifuged WB units were carefully moved to the T-ACE II extractor (TerumoBCT, Colorado, U.S.A.) for automated component separation. The appropriate programme was selected to control the blood flow between the primary and satellite bags. The multiple satellite bag system ensured that sterility was maintained throughout the processing procedure as the cellular components were easily transferred from the primary collection bag to the attached labelled satellite bags. This was achieved via the access ports between the bags. The nutritive, SAGM (composed of saline, adenine, glucose and mannitol), was added to the packed RBC after the plasma and buffy coat was removed to

complete the processing procedure. Compared to manual plasma extraction, the T-ACE II extractor is subjected to minimal human error as the operator only had to load and unload the device.

Each unit was gently agitated to ensure a homogenous mixture and the processing of the RBCC units were complete. The empty SAGM bag was hermetically sealed and left attached for the production of paediatric RBCC units. The twenty RBCC units that were randomly selected to form part of either the control group (non-irradiated products) or the test group (irradiated products) had the empty SAGM bags removed. The tubing of the RBCC unit was segmented using a using a T-Seal II sealer (TerumoBCT, Colorado, U.S.A.). Two barcoded serial number stickers were printed via the computer programme with 1 serial number attached to the first segment of the product. This segment was cut and placed in a test tube with a duplicate serial number attached and sent to the Blood Grouping laboratory for product batch grouping testing to be done. Batch group testing is done to confirm the ABO blood group and Rh status of the modified product. The completed unit was placed in a labelled blood crate and stored at 1°C to 6°C with a shelf-life of 42 days. The remaining forty RBCC units were placed in a separately labelled crate and also refrigerated at 1°C to 6°C. The RBCC processing procedure was completed within the recommended 72 hours post donation period.

The remaining plasma product was packaged, packed in a crate and placed at minus 40°C. Where applicable, the removed buffy coats were sorted according to blood group, Rh status, date and time bled, as only WB units bled for up to 12 minutes are suitable for the production of buffy coats during component processing. The appropriate buffy coats were pooled to produce donor platelet products (according to WPBTS Work Instructions) and were placed at 20°C to 24°C with constant gentle agitation. All the completed products were labelled with a sticker indicating the name of the product, the donor's ABO blood group and Rh status. The label also indicated the bleeding date, the expiry date and where applicable, the Anti-A, B titre status prior to use.

3.6.5.3. Pre-storage leucocyte-reduced red blood cell concentrate

Twenty processed RBCC were randomly selected to be leucocyte-reduced. Prior to the units being filtered, the empty SAGM bag was labelled with a sticker indicating the date bled, the expiry date and that the contents of the bag were filtered.

An Immugard leucocyte removal filter (Terumo, Colorado, U.S.A) with an attached transfer bag was aseptically attached to the RBCC unit using a TSCD® II Sterile Tubing Welder (Terumo, Colorado, U.S.A.) to maintain sterility. The tubing of the RBCC unit was placed in a

slot on the sterile connecting device (SCD) while the tubing of the filter was placed in another parallel-placed slot. The machine superheated the disposable copper wafer and as the two ends of tubing became heated, the slots moved to align the two ends of the tubing. The tubing of the RBCC connected to the filter tubing via the SCD and the withdrawn wafer was collected in a built-in plastic container.

The RBCC unit was gently agitated to ensure a homogenous mixture, the sterile tubing welds opened and the blood in the RBCC unit passed through the filter into the transfer bag. After the air in the transfer bag was removed and the tubing hermetically sealed, the filter with the attached empty RBCC bag was removed and discarded in a labelled biohazardous container. The tubing of the final product was segmented using a T-Seal II sealer. Two barcoded serial number stickers were printed via the computer programme with 1 serial number attached the first segment of the product. This segment was cut and placed in a test tube with a duplicate serial number attached and sent to the Blood Grouping laboratory for product batch grouping testing to be done. Batch group testing is done to confirm the ABO blood group and Rh status of the modified product. The completed unit was placed in a labelled blood crate and stored at 1°C to 6°C. Maintaining sterility during the processing procedure allows the product expiry to be 42 days when stored at 1°C to 6°C. The filtered units were not kept for longer than 30 minutes at room temperature and all units were labelled with the donors' ABO blood group and Rh status after the routine testing was completed. The processing procedure was completed within the recommended 72 hours post donation period.

3.6.5.4 Paediatric red blood cell concentrate

The final 20 units of processed RBCC were used to produce 20 x 2 paediatric RBCC units. An empty transfer bag and an Immugard filter were sterile-docked onto the tubing of the RBCC using a SCD device. The empty SAGM bag was labelled as paediatric RBCC 1 (green label) and the transfer bag was labelled as paediatric RBCC 2 (blue label) with the duplicate serial number, date bled and expiry date attached. The RBCC unit was gently agitated, the sterile tubing welds opened and the blood in the RBCC unit passed through the filter into the transfer bag. After the air in the transfer bag was removed, the blood volume was equally divided between the transfer bag and the empty SAGM bag and all tubing hermetically sealed. The filter with the attached empty RBCC bag was removed and discarded in a labelled biohazardous container. Two barcoded serial stickers were printed via the computer programme with 1 serial number attached the first segment of the product. This segment was

cut and placed in a test tube with a duplicate serial number attached and sent to the Blood Grouping laboratory for product batch grouping testing to be done.

This preparation method allows the completed paediatric units to be stored for 42 days at 1°C to 6°C. The completed units were placed in a labelled blood crate and stored at 1°C to 6°C. The filtered paediatric units were not kept for longer than 30 minutes at room temperature and all units were labelled with the donors' ABO blood group and Rh status after the routine testing was completed and results uploaded to the mainframe computer. The RBCC processing procedure was completed within the recommended 72 hours post donation period.

3.6.6 Irradiation

All red blood cellular products which were randomly selected for the test group were irradiated using a Gammacell 3000 irradiator (Elan, Ottawa, Canada). Irradiation was done to eradicate the mitotic potential of circulating lymphocytes as it inactivates the immuno-competent T-lymphocyte cells. This prevents the cells from engrafting and initiating an immunological response against the host. The indicated dose of gamma irradiation should be between 2500cGy – 5000cGy (25-50 Gy) anywhere on the irradiated product. The irradiator machine-cycle printout was attached to each irradiated product and it was determined that the central dose was 2932cGy with a minimum dose of 2574cGy. The irradiator is routinely used in the blood bank and therefore it was not necessary to complete a validation.

3.6.7: Procedure from donor collection to the irradiator

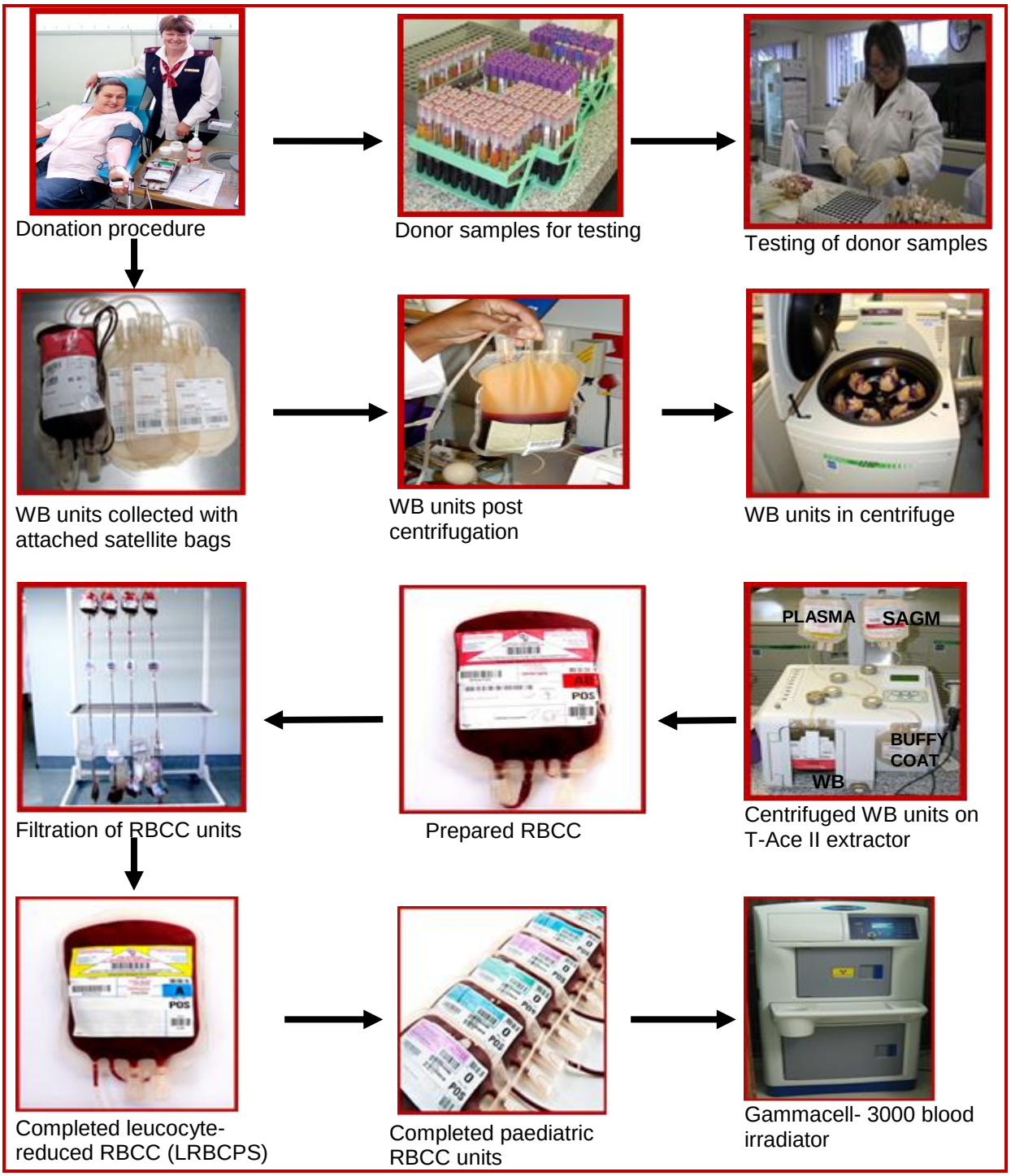

Figure 3.3: Procedure from collection to irradiaton

Table 3.2: Specifications for component pro=duction

	GENERAL DESCRIPTION	SOLUTIONS	SPECIFICATION	CONTAINER
WHOLE BLOOD	• Blood collected from healthy, non-remunerated donors. • No processing of unit done.	Anticoagulant: CPD	Quadruple pack weighing 733g-839g (525 ± 50ml including anticoagulant)	• Terumo quadruple pack with diversion pouch Code: BB*AGQ456ES2
RED BLOOD CELL CONCENTRATE **(Buffy coat removed)**	• RBCC produced from WB donation. • Plasma and buffy coat removed post centrifugation. • Additive solution (SAGM) is added.	Anticoagulant: CPD Additive solution: SAGM	• Volume: 300 ± 50ml • Haematocrit:0,6 ± 0,1 L/L	• Terumo quadruple pack with diversion pouch Code: BB*AGQ456ES2
PRESTORAGE LEUCOCYTE-REDUCED RED BLOOD CELL CONCENTRATE	• A unit of RBCC (less than 48 hours). • Leucocyte removal via filtration • Filter with attached transfer bag sterile docked onto pack	Anticoagulant: CPD Additive solution: SAGM	• Volume: 260 ± 50ml • Haematocrit:0,6 ± 0,1 L/L • Pre filtration: weight/volume, WBC and total WBC count per unit • Post filtration: weight/volume, HCT, Flow cytometer WBC count, unit total leucocyte count	• Terumo quadruple pack with diversion pouch Code: BB*AGQ456ES2 • Terumo leucocyte filter Code: F*IP1602ES (with transfer bag)
PAEDIATRIC RED BLOOD CELL CONCENTRATE x 2	• Unit of RBCC in SAGM. • Leucocyte removal via filtration. • Blood volume equally divided between SAGM & transfer pack. • Filter & separate transfer bag sterile docked onto filtered RBCC unit	Anticoagulant: CPD Additive solution: SAGM	• Volume: ±130ml per unit	• Terumo quadruple bag with diversion pouch Code: BB*AGQ456ES2 • Terumo 600ml transfer pack Code: BB*T060CB • Terumo leucocyte filter Code: F*IR1002ND

3.6.8 Testing of samples for study

The 80 WB units were bled on the 11/08/2014 and processed on 11/08/2014 to 12/08/2014. The RBC units from the Test Group were irradiated from late afternoon until evening on the 12/08/13. Both the Control and Test units were aliquoted and tested on the morning of the 13/08/2014. Therefore, the documented Day 1 of testing is, in fact, Day 3.

The products of both the control and test groups were collected, routinely tested and then processed within the prescribed 72 hours post donation period. Routine donor testing included determining ABO blood group and Rh status, anti-A,B titre, detection of possible irregular antibodies, antibodies to Human Immunodeficiency Virus I and II, testing for Hepatitis B, Hepatitis C as well as syphilis Once the components were processed, the designated products in the Test Group were irradiated.

After gentle mixing via multiple inversions of the blood bag and using an aseptic technique, sample aliquots were taken of all products in both the control and test groups on Days 1, 7, 14, 21, 28, 35 and 42 respectively. The principle investigator followed the same method for aliquoting the weekly samples per the pilot study i.e. the sample aliquots were obtained by using diversion pouches which were aseptically docked to the tubing of the red blood cellular unit using a sterile connecting device. The tubing of the pouch was cut and once the RBC unit and diversion pouch were separated, the unit was returned to the fridge. A weekly sample aliquot consisting of ±30ml was taken from each red blood cellular product and it was divided as follows:

- 1 aliquot for testing the haematological indices (1 x EDTA tube)
- 1 aliquot for testing the pH levels (1 x EDTA tube)
- 1 aliquot for testing the plasma haemoglobin plasma (1x EDTA tube).
- 1 aliquot for the metabolic analytes (1x gel SST tube)
- 1 aliquot for testing the glucose levels (1 x glucose tube with added anti-glycolytic sodium fluoride)

All tubes were labelled with a barcoded serial number (same as on the original blood bag), the product, whether Test or Control Group, the date of aliquoting and testing. Samples were centrifuged for 5 minutes at 3000rpm and where indicated, the plasma was removed and transferred to another labelled tube.

Testing of the various red blood cell markers occurred on the same day as the serial sample aliquoting. Both the aliquots and the products used in the study were stored at 1°C to 6°C up to 42 days of storage. After the storage period was completed, all the blood bags

used in both the control and test groups were sent to the WPBTS Microbiology laboratory for bacterial screening.

3.6.8.1 Evaluation of biochemical and red blood cell indices

The biochemical and haematological levels were performed to determine the metabolic effects and cellular destruction that red blood cellular products undergo during the 42-day storage period when stored at 1° to 6°C. The various markers were tested on Days 1, 7, 14, 21, 28, 35 and 42. The screening included the following:

a) Biochemical evaluation
- Potassium
- Phosphate
- Sodium
- Glucose
- Lactate dehydrogenase
- pH

b) Red blood cell (haematological) indices
- Full Blood Count (included erythrocyte, leucocyte and thrombocyte counts)
- Haemoglobin (Hb)
- Haematocrit (HCT)
- Mean corpuscular volume (MCV)
- Mean corpuscular haemoglobin (MCH)
- Mean corpuscular haemoglobin concentration (MCHC)
- Percentage plasma haemolysis (free haemoglobin)

3.6.8.2 Methods Used

a) Biochemical testing of electrolytes

The biochemical changes that occurred during the storage period of up to 42 days in non-irradiated and irradiated red blood cell concentrates were evaluated. These tests were performed on the serial aliquots processed weekly from Day 1 to Day 42.

Sodium, potassium, phosphate, glucose and lactate dehydrogenase

The sodium, potassium, phosphate, glucose and lactate dehydrogenase levels were quantitatively determined using an ABX Pentra 400 chemistry analyser (ABX Horiba, France). The analyser was used in conjunction with corresponding reagents from the same company. The reagents were all prepared according to the International Federation of Clinical Chemistry guidelines and ready for use on the analyser. However, the positive and

negative controls, including the calibrator reagent, were lyophilised and had to be reconstituted using distilled water. The analyser could not be used for testing samples if the on-board controls and calibrator reagent were not validated while the ISE module automatically completed 1-point and 2-point calibrations. These quality control processes were completed whenever samples had to be tested. A plasma sample was used for measuring the analyte levels.

This benchtop analyser uses potentiometry, colorimetric and turbidimetry technologies together with a serum/plasma sample to determine the analysis of the different electrolytes tested. Potentiometry is the determination of a solute concentration by using two electrodes with different potentials and a high impedance voltmeter to measure the potential difference between the two. One of the two electrodes is a reference electrode with a known potential while the other is a test electrode. Turbidimetry is the process of measuring the amount of light transmitted and subsequently absorbed by suspended particles in a solution. This absorbed light is collected by a photoelectric cell and measured using a spectrophotometer. The spectrophotometer measured the enzyme reactions and substrate concentrations after the colour-reaction occurred.

The sodium and potassium concentrations were measured using the ion-selective electrode (ISE) technique which includes a reference electrode. The measurement of the electric potential between the specific ISE flowed by the plasma sample whereas the reference ISE flowed by a particular reference solution. The additional two known standard solutions are stored by the analyser and used to measure the electrode slopes. The sodium ISE consists of a glass membrane selective to sodium ions and the potassium ISE contains a plastic membrane selective to potassium ions. The undiluted plasma reaches the ISE block via an air sensor which has the ability for air detection and was therefore able to demonstrate the end of the sampling process. The electric potential is thus the measurement between the ISE and reference electrodes and made possible via the ion concentration.

Glucose was measured via an enzymatic technique using glucose oxidase together with peroxidase (Trinder method) by colorimetry. The enzyme, lactate dehydrogenase (LDH), consists of five iso-enzymes which catalysed the interconversion of lactate and pyruvate via a kinetic technique according to the recommendations of the German Society of Clinical Chemistry. Phosphate was tested based on a UV methodology using phosphomolybdate.

The pH concentration of each product aliquot was used to measure the alkalinity or acidity of the aliquoted anticoagulated blood samples. The constant glycolysis that occurs during the storage period leads to the decrease in the pH of blood and subsequently causes the 2,

3 diphosphoglycerate and adenotriphosphate to decrease. The desktop pH meter (Amtech Laboratory Services, Cape Town, South Africa) was used.

A pH meter measures the concentration of hydrogen ion (H^+) in a substance as both hydroxide ($OH^{-)}$ and H^+ ions are always present in any solution. If there is an excess of OH^- ions in the solution, the substance is regarded as being alkaline as the pH is more than 7. However, if the H^+ ions are in excess, the solution is viewed as being acidic as the pH will be less than 7. Only pure water is neither acidic nor basic and is regarded as neutral because the pH will be 7. A visual check of the pH meter was completed prior to testing to ensure the instrument was undamaged. In addition to the visual observation, a quality control was done using 2 buffer solutions (pH 4 and pH 7) while the probe was rinsed with distilled water after it was removed from its potassium chloride storage solution. Testing only commenced once calibration was completed and the results are digitally presented without further calculations. All testing was done in accordance with the manufacturer's instructions and WPBTS Work Instructions.

b) Haematological indicators

The haematological factors were evaluated weekly to determine the degree of cellular destruction in irradiated and non-irradiated red blood cellular products during the storage period of up to 42 days.

The ABX Pentra XL 80 (Horiba, France) is a haematology analyser which operates on the multi-distribution and double hydrodynamic sampling systems. The multi-distribution technology allowed for minimum sample volume to be split into separate aliquots and diverted to specific chambers within the analyser. The double hydrodynamic sampling system measures the cellular content and cell volume by using cytochemistry, polychromatic light absorbance measurement and cytometry. The analyser measurements of RBC, leucocytes and platelets is based on the impedance principle. Electrical impedance is when cells pass through a micro-aperture that is placed between two electrodes which only allows one cell to pass through at a time. As the cell passes through, the impedance changes which is proportional to the cell volume. This results in the volume being measured and a cell count is done. Cells that passed through the micro-aperture were classified according to volume and light absorbance while the haemoglobin was measured by a spectrophotometer at 540nm wavelengths after the RBC had haemolysed.

Full blood count, haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentrate. The RBC haematological indices included the measurement of a full blood count (FBC) such as the erythrocyte,

leucocyte and thrombocyte counts. This analyser was also used to determine the total haemoglobin (Hb), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) levels.

Plasma haemoglobin

The plasma haemoglobin level was quantitatively tested using the Hemocue® Plasma/Low Hb System (Ängelholm, Sweden) together with an undiluted plasma sample. This system is calibrated using the International Council for Standardisation in Haematology (ICSH) reference method. It is comprised of an analyser with a built-in photometer which measures wavelengths at 570nm and 880nm to compensate for any possible turbidity. Microcuvettes are used as both a measuring cuvette and a pipette. A modified azide-methaemoglobin reaction occurs in the cuvettes when haemoglobin is released due to haemolysis. The haemoglobin binds to sodium nitrate and converts to methaemoglobin. The methaemoglobin then combines with the azide which results in azide-methaemoglobin. The haemoglobin concentration and absorbance levels are calculated when the absorbance is directly proportional to the haemoglobin levels. The results are digitally presented without further calculations.

Percentage plasma haemolysis

This RBC cell marker was calculated by using the levels of plasma haemoglobin, haematocrit (HCT) and the total haemoglobin (Hb) of each aliquoted sample. The following formula was applied to calculate the level of percentage plasma haemolysis per sample:

$$\text{Percentage Haemolysis} = \frac{(100 - \text{Haematocrit}) \times \text{Plasma Haemoglobin}}{\text{Total Haemoglobin}}$$

Percentage plasma haemolysis calculation

3.7 Statistical analysis

Data was documented using an Excel spreadsheet (Excel 2013, Microsoft Corporation, Redmond, Washington, U.S.A). Statistical analyses of the test results were performed using Excel 2013 as well as Prism 5, 2007 (GraphPad Software, Inc. San Diego, California, U.S.A). Central tendencies of the validation results of ABX Pentra 400 chemistry analyser used in the pilot study were ascertained per descriptive data. Data expressed as mean ± standard deviation (SD), 75% quartile range, 95% confidence interval and p-value of less than 0.05 (< 0.05) was regarded as significant. Statistical comparison of the non-irradiated (control group) and irradiated (test group) was completed using paired t-test for single time-

points and analysis of variance (ANOVA) for multiple time-points during the storage period. Multi-comparisons to define specific differences between the control and test groups at specific time-points were performed using the *post hoc* Bonferroni statistical calculations. Results are expressed as mean ± standard deviation (SD), 95% confidence interval and a p-value of < 0.05 was considered significant.

CHAPTER FOUR

RESULTS

4.1 Pilot study

The pilot study indicated that the aliquoting procedure and attainment of the supernatant required for testing was completed in approximately 10 minutes per unit. After the weekly serial aliquoting was complete, the used bags were sent to the WPBTS Microbiology laboratory to test for possible bacterial contamination. A broad spectrum culture medium was used to do the bacterial screening and the results from the WPBTS Microbiology laboratory were negative. This indicated an absence of bacterial contamination following the serial aliquoting and weekly storage at 1 °C to 6°C i.e. sterility was maintained.

Whole blood samples were collected from random donors to validate the reproducibility and specificity of test results using the analyser. The validation of the ABX Pentra 400 biochemistry analyser using corresponding reagents viz. potassium, sodium, glucose, phosphate and lactate dehydrogenase (LDH), was successful and deemed suitable for use. The results are presented below in tabular representation.

Table 4.1: ABX Pentra 400 analyser validation for specificity

Analyte	Date	Mean ± SD	75% Percentile	Confidence Interval (95%)	p-Value
Potassium (mmol/L)	14.04.14	4.3 ± 0.2	4.5	4.2 – 4.4	0.6
	16.04.14	4.3 ± 0.2	4.5	4.2 – 4.4	0.6
	29.04.14	4.3 ± 0.3	4.5	4.1- 4.6	0.4
Sodium (mmol/L)	14.04.14	143 ± 2.0	4.5	141 – 144	0.4
	16.04.14	142 ± 1.0	4.5	141 – 143	0.7
	29.04.14	143 ± 1.0	4.5	142 – 144	0.1
Glucose (mmol/L)	14.04.14	6.2 ± 1.7	7.4	5.0 – 7.4	0.4
	16.04.14	5.3 ± 1.1	6.0	4.5 – 6.1	0.9
	29.04.14	5.1 ± 1.0	5.7	4.4 - 5.8	0.4
Phosphate (mmol/L)	14.04.14	1.2 ± 0.2	1.4	4.2 – 4.4	0.7
	16.04.14	1.1 ± 0.2	1.3	4.2 – 4.4	0.6
	29.04.14	1.2 ± 0.2	1.3	4.1 - 4.6	0.5
LDH (U/L)	14.04.14	301 ± 41	340	271 – 330	0.9
	16.04.14	308 ± 43	335	278 – 339	0.8
	29.04.14	280 ± 40	311	251 - 309	0.3

Key: Quartile - 75% percentile
SD - Standard deviation
p-Value - Significant if < 0.05
Confidence Interval - 95% of the mean
N = 10

Table 4.2: ABX Pentra 400 validation – Reproducibility results

Analyte	Sample Number	75% Percentile	Mean ± SD	Confidence Interval (95%)	p-Value
Potassium (mmol/L)	2	4.1	4.1 ± 0.01	4.08 – 4.10	0.5
Phosphate (mmol/L	2	1.2	1.9 ± 0.01	1.17 – 1.20	0.5
Sodium (mmol/L)	2	143	143 ± 0.2	142.8 – 143.0	0.8
Glucose (mmol/L)	15	5.5	5.5 ± 0.06	5.4 – 5.5	0.5
LDH (U/L)	10	279	277± 2.0	276 - 279	1.0

Key: Quartile - 75% percentile
SD - Standard deviation
p-Value - Significant if < 0.05
Confidence Interval - 95% of the mean
N = 10

4.2 Main study

The main study consisted of testing 80 units of red blood cellular (RBC) products collected from random donors. Ten units per group were randomly selected for irradiation and these units became the Test Group whereas the non-irradiated RBC products became the Control Group. The irradiated and non-irradiated blood components evaluated include whole blood, red blood cell concentrate with buffy-coat layer removed, leucocyte-reduced red blood cell concentrate and paediatric red blood cell concentrate. The processing of the relevant products, routine testing and subsequent irradiation all occurred within 72 hours post collection date. WB units were collected and relevant units processed on 11.08.2014. Irradiation occurred the following day and testing started on 13.08.2014. All products, as well as weekly aliquots, were stored at 1° to 6°C during the 42-day storage period.

4.2.1 Whole blood (WB): Biochemistry and haematology results

The potassium (mmol/L) ion results for the non-irradiated (NIRR) WB and irradiated (IRR) WB units presents with a significant increase during the 35-day storage period at 1 °C to 6 °C (p values < 0.05). The potassium 95% Confidence Interval (95% CI) for IRR WB ranges from 17.5 – 21.3 on Day 1 to 37.9 – 43.1 on Day 35 while the range of NIRR WB is 8.4 – 9.6 on Day 1 to 25.9 – 29.8 on Day 35. The comparison of the extracellular sodium (mmol/L) ion levels between NIRR WB and IRR WB from Day 1 to Day 35 is considered significant as the p-value is less than 0.05 ($P<0.05$) throughout the storage period. The phosphate and LDH levels show an increase while that of the glucose and pH concentrations indicate a decrease although no statistically significant differences were detected during the 35-day storage period.

Day 1, 14 and 21 for erythrocytes, haemoglobin and HCT, display statistically significant differences (p-values < 0.5). The percentage plasma haemolysis results exhibit significant differences on Day 28 (p-value =0.02) and Day 35 (p-value = 0.05).

The Day 35 result for IRR WB percentage plasma haemolysis result is higher than the 0.8% per recommended guidelines allow (1.06% ± 0.47%). However, it should be noted that according to the Clinical Guidelines for the use of blood products in South Africa, blood may be irradiated up to 14 days post collection date and then stored for an additional 14 days i.e. blood only kept until Day 28.

Table 4.3: Irradiated vs Non-irradiated Whole Blood: Biochemistry results

Biochemistry Indicators	Storage Period	Irradiated (IRR)		Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p – Value
Potassium Levels (mmol/L)	Day 1	19.4 ± 2.7	17.5 – 21.3	9.1 ± 0.8	8.4 – 9.6	0.002*
	Day 7	30.1 ± 3.4	27.7 – 32.5	14.9 ± 1.6	13.7 – 16.1	0.002*
	Day 14	29.5 ± 2.9	27.4 – 31.6	16.7 ± 1.5	15.7 – 17.8	0.002*
	Day 21	38.1 ± 3.8	35.3 – 40.8	24.6 ± 4.7	21.2 – 27.9	0.01*
	Day 28	41.3 ± 4.6	38.1 – 44.6	34.6 ± 4.5	31.3 – 37.8	0.01*
	Day 35	40.5 ± 3.6	37.9 – 43.1	27.8 ± 2.7	25.9 – 29.8	0.002*
Sodium Levels (mmol/L)	Day 1	155 ± 2.0	154 - 156	157 ± 2.0	156 – 159	0.02*
	Day 7	159 ± 3.0	157 – 161	166 ± 3.0	164 – 169	0.01*
	Day 14	147 ± 2.0	146 - 148	155 ± 2.0	154 – 157	0.01*
	Day 21	145 ± 2.0	144 - 146	153 ± 2.0	152 - 155	0.01*
	Day 28	147 ± 1.0	146 - 148	160 ± 2.0	158 - 162	0.01*
	Day 35	140 ± 2.0	139 - 141	148 ± 2.0	146 - 149	0.01*
Phosphate Levels (mmol/L)	Day 1	3.8 ± 0.1	3.7 – 3.9	3.7 ± 0.2	3.5 – 3.8	0.34
	Day 7	4.1 ± 0.2	3.9 – 4.3	4.1 ± 0.4	3.8 – 4.4	0.84
	Day 14	4.6 ± 0.2	4.5 – 4.8	4.8 ± 0.5	4.5 – 5.2	0.33
	Day 21	5.9 ± 0.3	5.6 – 6.1	5.6 ± 0.6	5.4 – 6.1	0.68
	Day 28	5.3 ± 0.4	5.1 – 5 6	5.3 ± 0.5	5.0 – 5.6	0.77
	Day 35	6.1 ± 0.3	5.9 – 6.4	5.8 ± 0.5	5.4 – 6.2	0.26
Glucose Levels (mmol/L)	Day 1	19.8 ± 1.1	19.1 – 20.6	20.8 ± 1.4	19.8 – 21.9	0.13
	Day 7	18.5 ± 1.1	17.7 – 19.3	19.3 ± 1.6	18.1 – 20.4	0.42
	Day 14	17.0 ± 1.1	16.2 – 17.8	17.5 ± 1.6	16.3 – 18.7	0.72
	Day 21	14.8 ± 1.0	14.1 – 15.5	14.9 ± 1.8	13.6 – 16.2	0.96
	Day 28	14.8 ± 1.0	13.1 – 14.6	13.7 ± 2.0	12.3 – 15.1	0.72
	Day 35	12.6 ± 1.1	11.8 – 13.3	12.1 ± 1.9	10.8 – 13.4	0.50
Lactate Dehydrogenase Levels (U/L)	Day 1	397 ± 178	267- 524	429 ± 146	324 - 533	0.85
	Day 7	786 ± 156	675 - 898	731 ± 184	599 - 863	0.31
	Day 14	899 ± 140	799 - 1000	906 ± 176	780 - 1032	1.00
	Day 21	1132 ± 194	993 - 1271	1059 ± 166	940 - 1177	0.70
	Day 28	1270 ± 222	1111 - 1429	1179 ± 175	1054 - 1304	0.23
	Day 35	1251 ± 238	1081 - 1421	1134 ± 163	1017- 1251	0.43

Table 4.3.1: Irradiated vs Non-irradiated Whole Blood: Biochemistry results

Biochemistry Indicators	Storage Period	Irradiated (IRR)		Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p – Value
pH Levels	Day 1	7.26± 0.08	7.20 - 7.32	7.28 ± 0.08	7.23 - 7.34	0.02*
	Day 7	6.91 ± 0.07	6.86 - 6.96	6.90 ± 0.08	6.84 - 6.96	0.48
	Day 14	6.80 ± 0.05	6.77 - 6.83	6.82 ± 0.04	6.79 - 6.85	0.12
	Day 21	6.75 ± 0.05	6.71 - 6.79	6.75 ± 0.05	6.71 - 6.78	1.00
	Day 28	6.76 ± 0.05	6.72 - 6.80	6.75 ± 0.03	6.73 - 6.77	0.68
	Day 35	6.77 ± 0.05	6.74 - 6.81	6.75 ± 0.04	6.75 - 6.78	0.13

Key: CI - Confidence interval
SD - Standard deviation
p-Value - Significant if < 0.05
N = 10

Table 4.4: Irradiated vs Non-irradiated Whole Blood: Haematology results

Haematology Indicators	Storage Period	Whole Blood Irradiated (IRR)		Whole Blood Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p - Value
Erythrocyte Count	Day 1	3.8 ± 0.4	3.6 - 4.1	4.4 ± 0.4	4.1 – 4.6	0.03*
	Day 7	4.3 ± 0.7	3.8 – 4.9	4.5 ± 1.8	3.2 – 5.8	0.61
	Day 14	3.6 ± 0.5	3.2 – 4.0	4.8 ± 0.9	4.2 – 5.5	0.01*
	Day 21	3.7 ± 0.7	3.2 – 4.2	4.6 ± 0.4	4.4 – 4.9	0.01*
	Day 28	3.5 ± 0.7	3.0 – 3.9	4.1 ± 0.5	3.8 – 4.4	0.05
	Day 35	3.9 ± 0.9	3.3 – 4.6	4.0 ± 0.4	3.7 – 4.3	0.08
Leucocyte Count	Day 1	7.4 ± 1.8	6.1 – 8.6	6.0 ± 1.1	5.2 – 6.8	0.10
	Day 7	5.5 ± 2.8	3.5 – 7.5	4.5 ± 1.4	3.5 – 5.5	0.33
	Day 14	4.6 ± 1.8	3.4 – 5.9	3.7 ± 1.3	2.8 – 4.7	0.19
	Day 21	3.9 ± 1.1	3.0 – 4.7	3.1 ± 1.2	2.3 – 4.0	0.10
	Day 28	3.5 ± 1.2	2.7 – 4.4	2.8 ± 1.2	2.1 – 3.5	0.19
	Day 35	3.6 ± 1.3	2.7 – 4.6	2.7 ± 0.8	2.1 – 3.2	0.08
Thrombocyte Count	Day 1	215 ± 33	191 – 239	211 ± 36	186 – 237	0.84
	Day 7	147 ± 37	121 – 174	136 ± 29	115 – 17	0.49
	Day 14	180 ± 28	160 – 200	149 ± 32	126 – 171	0.06
	Day 21	175 ± 26	156 – 193	148 ± 29	127 – 169	0.11
	Day 28	167 ± 26	149 – 186	141 ± 23	124 – 158	0.06
	Day 35	110 ± 46	77 – 143	140 ± 28	120 – 161	0.12
Haemoglobin (Hb) Levels	Day 1	11.4 ± 1.0	10.6 – 12.1	12.7 ± 0.8	12.1 – 13.3	0.02*
	Day 7	13.0 ± 2.4	11.2 – 14.7	13.4 ± 0.7	13.0 – 13.9	0.59
	Day 14	10.7 ± 1.5	9.7 – 11.8	13.2 ± 1.0	12.4 – 13.9	0.02*
	Day 21	11.0 ± 1.9	9.6 – 12.3	13.2 ± 1.1	12.4 – 14.0	0.02*
	Day 28	10.2 ± 2.0	8.7- 11.6	11.8 ± 1.3	10.8 – 12.8	0.07
	Day 35	11.7 ± 2.2	10.1 – 13.3	11.7 ± 0.6	11.2 – 12.1	0.96

Table 4.4.1: Irradiated vs Non-irradiated Whole Blood: Haematology results

Haematology Indicators	Storage Period	Whole Blood Irradiated (IRR)		Whole Blood Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p - Value
Haematocrit (HCT) Levels	Day 1	33.8 ± 2.7	31.9 – 35.8	37.7 ± 2.3	36.1 – 39.3	0.01*
	Day 7	38.2 ± 6.5	33.6 – 42.9	40.8 ± 3.8	38.1 – 43.5	0.35
	Day 14	32.0 ± 4.2	29.0 – 35.0	41.8 ± 7.6	36.4 – 47.3	0.01*
	Day 21	32.7 ± 5.2	29.0 – 36.4	39.7 ± 2.9	37.6 – 41.7	0.01*
	Day 28	30.2 ± 5.3	26.5 – 34.0	35.2 ± 3.6	32.7 – 37.8	0.24
	Day 35	34.3 ± 6.4	29.7 – 38.9	34.3 ± 1.7	33.0 – 35.5	0.99
Mean Corpuscular Volume (MCV) Levels	Day 1	88.5 ± 3.3	86.1 – 90.9	86.9 ± 6.7	82.1 – 91.7	0.68
	Day 7	88.2 ± 3.5	85.7 – 90.7	90.2 ± 4.8	86.8 – 93.7	0.31
	Day 14	89.0 ± 3.7	86.4 – 91.6	87.3 ± 6.7	82.5 – 92.1	0.72
	Day 21	87.6 ± 3.3	85.2 – 90.0	85.0 ± 6.6	80.3 – 89.7	0.54
	Day 28	88.4 ± 3.6	85.8 – 91.0	86.8 ± 6.7	82.0– 91.6	0.72
	Day 35	87.6 ± 3.6	85.0 – 90.2	86.6 ± 7.0	81.6 – 91.6	0.96
Mean Corpuscular Haemoglobin (MCH) Levels	Day 1	29.7 ± 1.2	28.8 – 30.6	29.3 ± 2.7	27.3 – 31.3	1.00
	Day 7	29.8 ± 1.5	28.7 – 30.9	30.2 ± 1.5	29.2 – 31.3	0.41
	Day 14	29.9 ± 1.5	28.8 – 30.9	29.4 ± 2.7	27.5 – 31.3	0.84
	Day 21	29.4 ± 1.4	28.4 – 30.4	28.3 ± 2.6	26.5 – 30.2	0.51
	Day 28	29.5 ± 1.6	28.4 – 30.6	29.0 ± 2.7	27.1 – 31.0	1.00
	Day 35	29.8 ± 1.4	28.8 – 30.8	29.5 ± 2.7	27.5 – 31.4	0.88
Mean Corpuscular Haemoglobin Concentrate (MCHC) Levels	Day 1	33.6 ± 0.5	33.2 – 34.0	33.7 ± 0.7	33.2 – 34.1	0.59
	Day 7	33.8 ± 0.7	33.3 – 34.3	33.4 ± 0.5	33.0 – 33.8	0.39
	Day 14	33.5 ± 0.5	33.2 – 33.9	33.6 ± 0.8	33.0 – 34.2	0.48
	Day 21	33.6 ± 0.6	33.1 – 34.0	33.3 ± 0.7	32.8 – 33.8	0.38
	Day 28	33.5 ± 0.9	32.9 – 34.2	33.5 ± 0.8	32.9 – 34.0	1.00
	Day 35	34.1 ± 0.5	33.7 – 34.4	34.0 ± 0.7	33.5 – 34.5	0.77
Plasma Haemoglobin Levels	Day 1	0.03 ± 0.01	0.02 – 0.03	0.03 ± 0.01	0.02 – 0.03	1.00
	Day 7	0.07 ± 0.05	0.04 – 011	0.05 ± 0.03	0.03 – 0.07	0.33
	Day 14	0.08 ± 0.03	0.05 – 0.10	0.07 ± 0.03	0.05 – 0.09	0.51
	Day 21	0.09 ± 0.04	0.06 – 0.12	0.08 ± 0.06	0.03 – 0.12	0.70
	Day 28	0.13 ± 0.06	0.0 – 0.18	0.10 ± 0.03	0.08 – 0.12	0.24
	Day 35	0.18 ± 0.07	0.13 – 0.22	0.12 ± 0.03	0.10 – 0.13	0.05
Percentage Plasma Haemolysis Levels	Day 1	0.16 ± 0.04	0.13 – 0.18	0.13 ± 0.05	0.10 – 0.17	0.34
	Day 7	0.33 ± 0.22	0.17 – 0.49	0.23 ± 0.12	0.14 – 0.31	0.26
	Day 14	0.48 ± 0.14	0.38 – 0.58	0.30 ± 0.13	0.21 – 0.39	0.22
	Day 21	0.53 ± 0.26	0.35 – 0.72	0.34 ± 0.27	0.14 – 0.53	0.14
	Day 28	0.76 ± 0.31	0.54 – 0.99	0.55 ± 0.15	0.44 – 0.65	0.02*
	Day 35	1.06 ± 0.47	0.73 – 1.40	0.65 ± 0.13	0.56 – 0.75	0.05*

Key: CI - Confidence interval
SD - Standard deviation
p-Value - Significant if < 0.05
N = 10

4.2.2 Red Blood Cell Concentrate (RBCC): Biochemistry and Haematology Results

The comparison of potassium (mmol/L) ion levels of irradiated - and non-irradiated RBCC show an increasing significance with $p<0.05$ during the 42-day storage period. The potassium result for irradiated RBCC for Day 1 is 48.2 ± 7.7mmol/L (mean ± SD) and Day 35 is 182.7± 50.4 while the non-irradiated RBCC Day 1 result is 19.0 ± 2.9mmol/L and Day 35 is 65.9 ± 5.7mmol/L. The sodium (mmol/L) ion levels indicate a significant reduction (p value < 0.05). Phosphate, glucose, LDH and pH results of the irradiated and non-irradiated RBCC were not significantly different (p-value > 0.05).

The evaluated haematological factors such as erythrocytes, thrombocytes, leucocytes, haemoglobin, MCV, HCT, MCH and MCHC indices which were evaluated during the storage period, show no significant differences. However, significant differences (p-value <0.05) were observed from Day 7 to Day 42 in the evaluation of plasma haemoglobin and percentage plasma haemolysis indicators. The outcomes of the percentage plasma haemolysis evaluated on Day 35 (0.97% ± 0.16%) and Day 42 (1.14% ± 0.23%) are considerably higher that the recommended 0.8% but it should be noted that irradiated blood is only stored until Day 28 or according to the original expiry date if it is sooner.

Table 4.5: Irradiated vs Non-irradiated Red Blood Cell Concentrate: Biochemistry Results

Biochemistry Indicators	Storage Period	RBCC Irradiated (IRR)		RBCC Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p- Value
Potassium Levels (mmol/L)	Day 1	48.2 ± 7.7	42.7 - 53.7	19.0 ± 2.9	42.7 – 53.7	0.002*
	Day 7	66.5 ± 6.0	62.2 – 70.8	26.9 ± 4.0	62.2 – 70.8	0.002*
	Day 14	65.7 ± 3.1	63.4 – 67.9	33.6 ± 3.8	63.4 – 67.9	0.002*
	Day 21	75.3 ± 3.3	72.9 – 77.7	44.2 ± 4.3	72.9 – 77.7	0.002*
	Day 28	85.9 ± 3.4	83.5 – 88.3	56.7 ± 3.7	83.5 – 88.3	0.002*
	Day 35	70.6 ± 2.5	68.9 – 72.4	50.4 ± 4.2	68.9 – 72.4	0.002*
	Day 42	182.7± 50.4	146.7 – 218.7	65.9 ± 5.7	146.7 – 218.7	0.002*
Sodium Levels (mmol/L)	Day 1	128 ± 3	125 – 130	147 ± 2	146 – 148	0.002*
	Day 7	123 ± 3	121 – 126	148 ± 2	146 -149	0.006*
	Day 14	110 ± 3	108 – 112	134 ± 3	132 – 136	0.002*
	Day 21	112 ± 2	110 – 113	134 ± 2	133 – 136	0.002*
	Day 28	119 ± 2	117 – 120	137 ± 4	135 – 140	0.002*
	Day 35	105 ± 2	104 – 106	121 ± 3	119 – 123	0.006*
	Day 42	108 ± 2	107 - 109	122 ± 3	120 - 124	0.004*
Phosphate Levels (mmol/L)	Day 1	2.1 ± 0.3	1.8 – 2.3	1.9 ± 0.5	1.6 – 2.2	0.43
	Day 7	3.1 ± 0.3	2.8 – 3.3	2.7 ± 0.5	2.4 – 3.1	0.20
	Day 14	4.1 ± 0.5	3.8 – 4.5	4.0 ± 0.6	3.5 – 4.4	0.92
	Day 21	5.5 ± 0.4	5.2 – 5.8	5.5 ± 0.8	5.0 – 6.0	0.85
	Day 28	6.4 ± 0.7	5.9 – 6.9	6.1 ± 1.3	5.2 – 7.0	0.56
	Day 35	6.8 ± 0.6	6.4 – 7.3	6.4 ± 0.8	5.8 - 6.9	0.28
	Day 42	7.2 ± 1.1	6.5 – 8.0	6.5 ± 1.1	5.7 – 7.2	0.22

Table 4.5.1: Irradiated vs Non-irradiated Red Blood Cell Concentrate: Biochemistry Results

Biochemistry Indicators	Storage Period	RBCC Irradiated (IRR)		RBCC Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p- Value
Glucose Levels (mmol/L)	Day 1	28.7 ± 2.7	26.7 – 30.6	29.3 ± 1.8	28.1 – 30.6	0.77
	Day 7	27.2 ± 1.4	26.2 – 28.2	27.6 ± 1.9	26.3 – 29.0	0.70
	Day 14	24.1 ± 2.9	22.0 – 26.1	25.3 ± 2.5	23.6 – 27.1	0.38
	Day 21	22.2 ±2.5	20.4 - 24.0	23.3 ± 2.7	21.4 – 25.2	0.32
	Day 28	19.2 ± 2.6	17.4 – 21.1	20.3 ± 1.4	19.3 – 21.3	0.26
	Day 35	17.0 ± 2.9	15.0 – 19.1	18.3 ± 3.3	16.0 – 20.7	0.32
	Day 42	16.5 ± 3.2	14.2 – 19.8	18.1 ±3.8	15.4 – 20.7	0.23
Lactate Dehydrogenase Levels (U/L)	Day 1	503 ± 222	332 – 673	607 ± 321	377 – 836	0.38
	Day 7	745 ± 254	564 – 927	716 ± 269	523 – 909	1.00
	Day 14	1222 ± 243	1049 – 1396	1174 ± 301	959 – 1390	0.56
	Day 21	1614 ± 464	1282 – 1946	1482 ± 510	1118 – 1847	0.49
	Day 28	2458 ± 708	1951 – 2965	2028 ± 603	1597 – 2459	0.32
	Day 35	2378 ± 517	2009 – 2748	2081 ± 560	1681 – 2482	0.19
	Day 42	3082 ± 281	2881 - 3283	2770 ± 438	2457 – 3083	0.11
pH Levels	Day 1	7.17 ± 0.05	7.13 – 7.21	7.14 ± 0.09	7.07 – 7.20	0.31
	Day 7	6.99 ± 0.11	6.91 – 7.07	7.06 ± 0.09	6.99 – 7.13	0.26
	Day 14	6.81 ± 0.04	6.78 – 6.84	6.86 ± 0.06	6.82 – 6.90	0.07
	Day 21	6.70 ± 0.10	6.63 – 6.77	6.71 ± 0.04	6.68 – 6.74	0.73
	Day 28	6.62 ± 0.05	6.59 – 6.66	6.65 ± 0.05	6.61 -6.68	0.24
	Day 35	6.62 ± 0.5	6.58 – 6.66	6.65 ± 0.05	6.61 – 6.68	0.44
	Day 42	6.54 ± 0.06	6.50 – 6.58	6.56 ± 0.03	6.54 – 6.59	0.23

Key: CI - Confidence interval
SD - Standard deviation
p-Value - Significant if < 0.05
N = 10

Table 4.6: Irradiated vs Non-irradiated Red Blood Cell Concentrate (RBCC): Haematology Results

Haematology Indicators	Storage Period	RBCC Irradiated (IRR)		RBCC Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p - Value
Erythrocyte Count	Day 1	6.7 ± 0.2	6.6 – 6.9	6.8 ± 0.4	6.5 – 7.1	0.51
	Day 7	6.6 ± 0.4	6.4 – 6 9	6.9 ± 0.2	6.7 – 7.1	0.18
	Day 14	6.9 ± 0.3	6.7 – 7.1	6.6 ± 0.4	6.4 – 6.9	0.92
	Day 21	6.6 ± 0.6	6.1 – 7.0	6.7 ± 0.2	6.6 – 6.9	0.19
	Day 28	7.1 ± 0.3	6.8 – 7.3	6.6 ± 0.3	6.3 – 6.8	0.12
	Day 35	7.1 ± 0.5	6.7 – 7.4	6.7 ± 0.3	6.5 – 6.9	0.23
	Day 42	7.0 ± 0.2	6.8 – 7.2	6.5 ± 0.5	6.1 – 6.8	0.24
Leucocyte Count	Day 1	4.8 ± 1.4	3.7 – 5.8	1.9 ± 1.1	1.1 – 2.6	0.16
	Day 7	5.8 ± 1.6	4.6 – 6.9	1.3 ± 0.6	0.8 – 1.7	0.71
	Day 14	2.5 ± 2.5	0.7- 4.2	1.3 ± 1.6	0.9 – 1.7	0.24
	Day 21	2.8 ± 1.6	1.7 – 4.0	1.1 ± 0.5	0.7 – 1.4	0.33
	Day 28	1.7 ± 1.2	0.8 – 2.6	1.1 ± 0.5	0.8 – 1.5	0.82
	Day 35	2.4 ± 1.2	1.5 – 3.3	0.9 ± 0.5	0.6 – 1.3	0.62
	Day 42	1.4 ±1.2	0.5 – 2.3	0.9 ± 05±	0.5 – 1.3	0.88
Thrombocyte Count	Day 1	97 ± 42	67 – 126	58 ± 29	38 – 79	0.44
	Day 7	114 ± 48	80 – 146	40 ± 19	26 – 54	0.60
	Day 14	61 ± 44	30 – 92	42 ± 20	28 – 56	0.37
	Day 21	70 ± 46	37 – 103	41 ± 17	29 – 53	0.16
	Day 28	61 ± 33	38 – 84	29 ± 11	21 – 37	0.78
	Day 35	72 ± 30	50 – 93	37 ± 17	25 – 49	0.08
	Day 42	49 ± 21	34 - 64	35 ± 21	20 - 50	0.78
Haemoglobin (Hb) Levels	Day 1	20.2 ± 0.8	19 7 – 20.8	19.6 ± 1.5	18.5 – 20.6	0.25
	Day 7	19.8 ± 1.7	18.6 – 21.1	20.9 ± 1.0	20.2 – 21.6	0.11
	Day 14	20.7 ± 1.1	19.9 – 21.5	19.6 ± 1.6	18.4 – 20.7	0.44
	Day 21	19.4 ± 2.0	18.0 – 20.8	20.3 ± 0.8	19.7 – 20.9	0.10
	Day 28	21.5 ± 1.3	20.6 – 22.4	19.9 ± 1.1	19.1 – 20.7	0.14
	Day 35	21.0 ± 2.0	19.6 – 22.4	20.0 ± 0.8	19.4 – 20.6	0.35
	Day 42	20.5 ± 0.9	19.8 – 21.1	19.1 ± 1.5	18.1 – 20.2	0.07
Haematocrit (HCT) Levels	Day 1	59.0 ± 1.6	57.4 – 59.8	51.5 ± 16.2	39.9 – 63.1	0.69
	Day 7	58.1 ± 3.5	55.6 – 60.7	60.7 ± 2.6	59.7 – 62.8	0.18
	Day 14	60.7 ± 2.6	58.8 – 62.5	58.0 ± 4.8	55.2 – 60.7	0.40
	Day 21	58.0 ± 4.8	54.5 – 61.4	60.5 ± 2.0	59.1 – 62.0	0.14
	Day 28	63.5 ± 3.2	61.2 – 65.8	58.0 ± 2.1	56.6 – 59.5	0.03*
	Day 35	62.3 ± 4.9	58.7 – 65.8	61.2 ± 2.2	59.6 – 62.8	0.03*
	Day 42	60.3 ± 2.0	58.9 – 61.7	57.5 ± 4.1	54.6 – 60.5	0.01*
Mean Corpuscular Volume (MCV) Levels	Day 1	87.3 ± 4.3	84.8 – 89.8	84.2 ± 2.7	82.3 – 86.1	0.46
	Day 7	88.3 ± 2.5	86.6 – 90.1	89.5 ± 4.2	86.5 – 92.5	0.57
	Day 14	88.0 ± 3.9	85.2 – 90.8	87.3 ± 2.8	85.3 – 89.3	0.89
	Day 21	88.8 ± 2.3	87.1 – 90.5	90 .0 ± 3.9	87.2 – 92.8	0.11
	Day 28	88.9 ± 3.8	86.2 – 91.7	87.6 ± 2.6	85.8 – 89.5	0.12
	Day 35	88.7 ± 2.7	86.8 – 90.6	90.9 ± 4.4	87.8 – 94.0	0.09
	Day 42	86 3 ± 3.7	83.7 – 88.9	89.2 ± 2.6	87.3 – 91.1	0.33
Mean Corpuscular Haemoglobin (MCH) Levels	Day 1	30.2 ± 1.6	29.1 – 31.3	28.9 ± 1.5	27.8 – 30.0	0.76
	Day 7	30.0 ± 1.7	28.8 – 31.2	29.9 ± 1.4	28.8 – 30.9	0.45
	Day 14	30.1 ± 1.6	28.9 - 31.2	29.5 ± 1.5	28.4 – 30.6	0.57
	Day 21	29.6 ± 1.5	28.5 – 30.7	30.2 ± 1.6	29.0 – 31.4	0.50
	Day 28	30.3 ± 1.5	29.2 – 31.3	30.0 ± 1.6	28.9 – 31.1	0.56
	Day 35	29.9 ± 1.6	28.7 – 31.1	29.8 ± 1.4	28.8 – 30.8	0.81
	Day 42	29.3 ± 1.5	28.2 – 30.3	29.7 ± 1.3	28.7 – 30.6	0.81

Table 4.6.1: Irradiated vs Non-irradiated Red Blood Cell Concentrate (RBCC): Haematology Results

Haematology Indicators	Storage Period	RBCC Irradiated (IRR)		RBCC Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p - Value
Mean Corpuscular Haemoglobin Concentrate (MCHC) Levels	Day 1	34.5 ± 0.6	34.1 – 34.9	34.3 ± 0.9	33.7 – 34.9	0.29
	Day 7	34.1 ± 1.2	33.2 – 34.9	33.3 ± 0.9	32.7 – 34.0	0.30
	Day 14	34.1 ± 0.6	33.7 – 34.5	33.8 ± 0.7	33.2 – 34.3	0.31
	Day 21	33.4 ± 0.9	32.7 – 34.1	33.5 ± 0.6	33.1 – 33.9	0.36
	Day 28	33.9 ± 0.7	33.4 – 34.4	34.2 ± 0.9	33.5 – 34.9	0.37
	Day 35	34.3 ± 0.9	33.7 – 34.9	32.7 ± 0.4	32.4 – 33.1	0.09
	Day 42	33.9 ± 0.5	33.6 – 34.3	33.3 ± 0.6	32.8 – 33.7	0.13
Plasma Haemoglobin Levels	Day 1	0.05 ± 0.02	0.04 – 0.07	0.04 ± 0.01	0.03 – 0.05	0.21
	Day 7	0.08 ± 0.01	0.07 – 0.09	0.05 ± 0.03	0.03 – 0.07	0.02*
	Day 14	0.18 ± 0.04	0.15 – 0.21	0.12 ± 0.07	0.07 – 0.17	0.04*
	Day 21	0.30 ± 0.06	0.25 – 0.34	0.16 ± 0.08	0.10 – 0.21	0.001*
	Day 28	0.40 ± 0.07	0.35 – 0.45	0.21 ± 0.08	0.15 – 0.27	<0.001*
	Day 35	0.50 ± 0.09	0.44 – 0.56	0.23 ± 0.13	0.14 – 0.33	<0.001*
	Day 42	0.59 ± 0.15	0.49 – 0.70	0.25 ± 0.12	0.17 – 0.33	<0.001*
Percentage Plasma Haemolysis Levels	Day 1	0.10 ± 0.04	0.07 – 0.13	0.09 ± 0.03	0.07 – 0.11	0.36
	Day 7	0.15 ± 0.03	0.13 – 0.17	0.10 ± 0.05	0.07 – 0.13	0.04*
	Day 14	0.31 ± 0.09	0.24 – 0.37	0.21 ± 0.13	0.12 – 0.31	0.10*
	Day 21	0.57 ± 0.10	0.50 – 0.65	0.37 ± 0.17	0.25 – 0.49	0.01*
	Day 28	0.74 ± 0.10	0.67 – 0.81	0.46 ± 0.20	0.32 – 0.60	0.002*
	Day 35	0.97 ± 0.16	0.86 – 1.08	0.48 ±0.27	0.29 – 0.68	<0.001*
	Day 42	1.14 ± 0.23	0.97 – 1.31	0.54 ± 0.21	0.39 – 0.70	<0.001*

Key: CI - Confidence interval p Value - Significant if < 0.05
SD - Standard deviation n = 10

4.2.3 Leucocyte-reduced Red Blood Cell Concentrate pre-storage (LRBCPS): Biochemistry and haematology results

The potassium ion results for the non-irradiated (NIRR) LRBCPS and irradiated (IRR) LRBCPS units show a significant increase during the 35-day storage period as most of the p values are less than 0.05 except for Day 21 due to an included outlier. The potassium 95% Confidence Interval for IRR LRBCPS ranges from 41.3 – 47.4 mmol/L on Day 1 to 84.3 – 91.2mmol/L on Day 35 while the range of NIRR LRBCPS is 16.2 – 20.3 mmol/L on Day 1 to 67.2 – 76.0 mmol/L on Day 35. The comparison of the extracellular sodium (mmol/L) ion levels in the control and test groups from Day 1 to Day 35 is considered significant ($p<0.05$) throughout the storage period. The phosphate levels show an increase while that of the glucose and pH concentrations indicate a decrease although no statistically significant differences are detected during the 35-day storage period.

The results for thrombocyte count, plasma haemoglobin and percentage plasma haemolysis indicate p-values less than 0.05 throughout the 42-day storage period and thus considered significant. The percentage plasma haemolysis results on Day 35 (0.83% ±

0.19%) and Day 42 (1.12% ± 0.32%) for IRR LRBCPS are higher than the 0.8% per recommendation by the Clinical Guidelines for use of blood products in South Africa but it should be noted that blood may be irradiated up to 14 days post collection date and then stored for an additional 14 days i.e. blood only kept until Day 28.

Table 4.7: Irradiated vs Non-irradiated leucocyte–reduced RBCC pre-storage (LRBCPS): Biochemistry Results

Biochemistry Indicators	Storage Period	LRBCPS Irradiated (IRR)		LRBCPS Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p - Value
Potassium Levels (mmol/L)	Day 1	44.4 ± 4.3	41.3 – 47.4	18.3 ± 2.9	16.2 – 20.3	0.002*.
	Day 7	68.2 ± 5.7	64.1 – 72.3	32.2 ± 4.8	28.8 – 35.7	0.002*
	Day 14	61.3 ± 3.4	58.8 – 63.8	35.3 ± 3.8	32.6 – 38.1	0.002*
	Day 21	178.0 ± 14.3	167.8 – 188.3	97.7 ± 22.2	81.8 – 113.5	0.432
	Day 28	115.1 ± 54.7	75.9 – 154.2	83.5 ± 30.0	62.0 – 104.9	0.002*
	Day 35	72.8 ± 4.0	69.9 – 75.6	56.6 ± 5.4	52.7 – 60.5	0.002*
	Day 42	87.71 ± 4.8	84.3 – 91.2	71.6 ± 6.2	67.2 – 76.0	0.002*
Sodium Levels (mmol/L)	Day 1	126 ± 2	124 – 128	142 ± 2	141 – 143	0.002*
	Day 7	126 ± 2	127 – 127	155 ± 4	152 – 158	0.002*
	Day 14	109 ± 2	108 – 110	133 ± 2	131 – 134	0.002*
	Day 21	111 ± 2	109 – 112	132 ± 3	130 – 134	0.002*
	Day 28	122 ± 4	119 – 125	139 ± 5	135 – 142	0.002*
	Day 35	106 ± 2	104 – 107	119 ± 3	117 – 121	0.002*
	Day 42	110 ± 2	108 - 111	121 ± 3	119 - 123	0.006*
Phosphate Levels (mmol/L)	Day 1	2.0 ± 0.03	1.8 – 2.2	1.6 ± 0.4	1.4 – 1.9	0.059
	Day 7	3.0 ± 0.4	2.8 – 3.3	2.7 ± 0.6	22 – 3.1	0.129
	Day 14	4.8 ± 0.5	4.5 – 5.2	4.1 ± 0.6	3.7 – 4.5	0.020
	Day 21	5.8 ± 0.6	5.3 – 6.2	5.5 ± 0.7	5.0 – 6.0	0.375
	Day 28	6.2 ± 0.5	5.8 – 6.6	8.3 ± 2.7	6.3 – 10.2	0.049
	Day 35	6.7 ± 0.6	6.3 – 7.1	6.7 ± 0.8	6.1 – 7.3	1.000
	Day 42	6.7 ± 0.5	6.4 – 7.0	6.9 ± 1.0	6.2 – 7.6	0.057
Glucose Levels (mmol/L)	Day 1	29.4 ± 1.0	28.6 – 30.1	29.4 ± 1.2	28.6 – 30.3	0.921
	Day 7	27.6 ± 1.4	26.5 – 28.6	26.9 ± 1.5	25.8 – 28.0	0.492
	Day 14	24.5 ± 2.6	22.6 – 26.4	24.9 ± 2.3	23.2 – 26.6	0.769
	Day 21	22.8 ± 2.2	21.2 – 24.4	21.9 ± 2.3	20.2 – 23.5	0.432
	Day 28	20.0 ± 2.6	18.1 – 21.9	19.0 ± 2.5	17.2 – 20.8	0.375
	Day 35	17.3 ± 2.7	15.4 – 19.2	16.0 ± 2.6	14.1 – 17.8	0.322
	Day 42	16.9 ± 3.0	14.8 – 19.0	15.4 ± 3.0	13.2 – 17.6	0.432
Lactate Dehydrogenase Levels (U/L)	Day 1	151 ± 26	133 – 170	118 ± 36	92 – 143	0.052
	Day 7	255 ± 64	209 – 301	209 ± 71	158 – 259	0.123
	Day 14	299 ± 88	236 – 362	200 ± 45	168 – 233	0.002*
	Day 21	427 ± 153	318 – 536	243 ± 60	201 – 286	0.004*
	Day 28	554 ± 199	412 – 697	212 ± 53	174 – 250	0.002*
	Day 35	545 ± 228	282 – 708	207 ± 62	163 – 251	0.002*
	Day 42	804 ± 336	564 - 1045	304 ± 94	237 - 372	0.002*
pH Levels	Day 1	7.15 ± 0.05	7.11 – 7.19	7.18 ± 0.05	7.14 – 7.21	0.343
	Day 7	6.94 ± 0.07	6.88 – 6.99	6.92 ± 0.05	6.89 – 6.96	0.742
	Day 14	6.85 ± 0 08	6.79 – 6.91	6.86 ± 0.06	6.82 – 6.90	0.838
	Day 21	6.71 ± 0.06	6.67 – 6.75	6.72 ± 0.04	6.69 – 6.75	0.799
	Day 28	6.65 ± 0.07	6.60 – 6.70	6.63 ± 0.04	6.61 – 6.66	0.695
	Day 35	6.59 ± 0. 06	6.55 – 6.64	6.62 ± 0.04	6.59 – 6.64	0.283
	Day 42	6.50 ± 0.07	6.45 – 6.55	6.51 ± 0 05	6.48 – 6.54	0.858

Key: CI - Confidence interval p Value - Significant if < 0.05
SD - Standard deviation n = 10

Table 4.8: Irradiated vs Non-irradiated leucocyte–reduced RBCC Pre-storage (LRBCPS): Haematology Results

Haematology Indicators	Storage Period	LRBCPS Irradiated (IRR)		LRBCPS Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p - Value
Erythrocyte Count	Day 1	6.6 ± 0.4	6.3 – 6.9	6.6 ± 0.3	6.4 – 6.8	0.908
	Day 7	6.6 ± 0.4	6.3 – 6.9	6.9 ± 0.5	6.6 – 7.2	0.162
	Day 14	6.7 ± 0.5	6.3 – 7.1	6.6 ± 0.3	6.3 – 6.8	0.650
	Day 21	6.9 ± 0.5	6.5 – 7.2	6.9 ± 0.4	6.6 – 7.1	0.854
	Day 28	6.7 ± 0.3	6.5 – 6.9	6.8 ± 0.5	6.4 – 7.1	0.667
	Day 35	6.4 ± 0.4	6.1 – 6.7	6.4 ± 0.4	6.2 – 6.7	0.949
	Day 42	6.5 ± 0.4	6.3 – 6.8	6.6 ± 0.3	6.3 – 6.8	0.880
Leucocyte Count	Day 1	0.7 ± 0.2	0.55 – 0.87	1.0 ± 0.27	0.85 – 1.23	0.015*
	Day 7	0.3 ± 0.2	0.19 – 0.45	0.7 ± 0.9	0.07 – 1.35	0.229
	Day 14	0.12 ± 0.04	0.09 – 0.15	0.3 ± 0.6	0.12 – 0.74	0.346
	Day 21	0.02 ± 0.04	0.01 – 0.05	0.2 ± 0.3	0.07 – 0.37	0.226
	Day 28	0.04 ± 0.05	0.00 – 0.08	0.07 ± 0.05	0.04 – 0.10	0.193
	Day 35	0.07 ± 0.05	0.04 – 0.10	0.07 ± 0.05	0.04 – 0.10	1.000
	Day 42	0.04 ± 0.05	0.00 – 0.08	0.08 ± 0.04	0.05 – 0.11	0.104
Thrombocyte Count	Day 1	10.7 ± 4.8	7.3 – 14.1	20.1 ± 7.1	15.1 – 25.2	0.006*
	Day 7	1.0 ± 0.9	0.3 – 1.7	16.3 ± 7.0	11.3 – 21.3	<0.001*
	Day 14	0.6 ± 0.7	0.1 – 1.1	13.3 ± 6.7	8.5 – 18.1	0.002*
	Day 21	0.7 ± 0.5	0.4 – 1.0	6.7 ± 8.2	0.8 – 12.6	0.004*
	Day 28	0.2 ± 0.4	0.1 – 0.5	7.6 ± 4.6	4.3 – 10.9	0.001*
	Day 35	0.3 ± 0.5	0.05 – 0.7	2.6 ± 1.8	1.3 – 3 9	0.002*
	Day 42	0.1 ± 0.3	0.13 - 03	0.7 ± 0.7	0.2 – 1.2	0.024*
Haemoglobin (Hb) Levels	Day 1	19.9 ± 1.6	18.7 – 21.0	20.3 ± 1.3	19.3 – 21.2	0.549
	Day 7	20.0 ± 1.5	19.0 – 21.1	21.2 ± 2.0	19.8 – 22.7	0.170
	Day 14	20.3 ± 1.9	19.0 – 21.7	20.2 ± 1.5	19.1 – 21.3	0.880
	Day 21	20.2 ± 1.8	18.9 – 21.5	20.6 ± 1.5	19.5 – 21.7	0.603
	Day 28	20.2 ± 1.6	19.0 – 21.4	20.5 ± 1.5	19.4 – 21.5	0.686
	Day 35	19.5 ± 1.6	18.3 – 20.7	20.0 ± 1.5	19.0 – 21.1	0.430
	Day 42	19.6 ± 1.6	18.4 – 20.7	19.9 ± 1.4	18.9 – 20.9	0.618
Haematocrit (HCT) Levels	Day 1	57.9 ± 3.6	55.4 – 60.4	59.3 ± 2.5	57.5 – 61.1	0.300
	Day 7	58.8 ± 3.1	56.6 – 61.0	62.3 ± 4.3	59.3 – 65.4	0.060
	Day 14	60.1 ± 4.3	57.0 – 63.1	60.0 ± 3.3	57.6 – 62.4	0.963
	Day 21	57.5 ± 3.7	54.9 – 60.1	57.9 ± 3.5	55.4 – 60.4	0.806
	Day 28	60.3 ± 3.3	57.6 – 62.3	60.4 ± 3.2	58.1 – 62.6	0.761
	Day 35	58.1 ± 3.6	55.5 – 60.7	58.6 ± 3.3	56.2 – 61.0	0.713
	Day 42	59.9 ± 3.7	57.3 – 62.6	59.9 ± 3.1	57.7 – 62.0	0.952
Mean Corpuscular Volume (MCV) Levels	Day 1	87.9 ± 4.8	84.5 – 91.4	89.7 ± 5.1	86.0 – 93.4	0.444
	Day 7	88.8 ± 5.0	85.3 – 92.4	90.7 ± 5.2	87.0 – 94.4	0.444
	Day 14	89.6 ± 4.8	86.1 – 93.1	91.0 ± 5.4	87.2 – 94.8	0.564
	Day 21	84.1 ± 4.6	80.8 – 87.4	84.1 ±5.7	80.0 – 88.2	1.000
	Day 28	89.6 ± 4.6	86.3 – 92.9	8.9 ± 5.5	86.0 – 93.8	0.895
	Day 35	90.5 ± 4.7	87.1 – 93.9	90.2 ± 5.4	86.3 – 94.1	0.899
	Day 42	91.7 ± 4.9	88.2 – 95.2	91.3 ± 5.4	87.5 – 95.1	0.866

Table 4.8.1: Irradiated vs Non-irradiated leucocyte–reduced RBCC pre-storage (LRBCPS): Haematology Results

Haematology Indicators	Storage Period	LRBCPS Irradiated (IRR)		LRBCPS Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p - Value
Mean Corpuscular Haemoglobin (MCH) Levels	Day 1	30.1 ± 2.1	28.6 – 31.7	30.7 ± 2.2	29.1 – 32.3	0.605
	Day 7	30.3 ± 2.1	28.8 – 31.8	30.8 ± 2.1	29.3 – 32.3	0.626
	Day 14	30.3 ± 2.1	28.8 – 31.8	30.6 ± 2.2	29.0 – 32.2	0.754
	Day 21	29.5 ± 2.1	28.0 – 31.0	30.0 ± 2.1	28.5 – 31.5	0.575
	Day 28	30.1 ± 2.2	28.6 – 31.7	30.4 ± 2.2	28.8 – 31.9	0.823
	Day 35	30.4 ± 2.1	28.9 – 31.8	30.9 ± 2.3	29.3 – 32.5	0.611
	Day 42	30.0 ± 2.4	28.4 – 31.5	30.4 ± 2.2	28.8 – 31.9	0.723
Mean Corpuscular Haemoglobin Concentrate (MCHC) Levels	Day 1	34.3 ± 0.9	33.6 – 34.9	34.2 ± 1.0	33.5 – 34.9	0.844
	Day 7	34.1 ± 0.9	33.5 – 34.7	33.9 ± 0.9	33.2 – 34.5	0.554
	Day 14	33.8 ± 0.9	33.1 – 34.4	33.6 ± 1.0	32. – 34.3	0.730
	Day 21	35.1 ± 1.1	34.3 – 35.9	36.0 ± 1.0	35.3 – 36.7	0.048*
	Day 28	33.6 ± 1.0	32.9 – 34.4	33.9 ± 0.9	33.2 – 34.5	0.548
	Day 35	33.5 ± 0.8	33.0 – 34.1	34.2 ± 0.9	33.5 – 34.8	0.159
	Day 42	32.7 ± 0.9	32.0 – 33.3	33.2 ± 0.8	32.7 – 33.8	0.182
Plasma Haemoglobin	Day 1	0.06 ± 0.02	0.05 – 0.07	0.05 ± 0.01	0.4 – 0.06	0.029*
	Day 7	0.10 ± 0.03	0.07 – 0.12	0.06 ± 0.04	0.04 – 0.09	0.065
	Day 14	0.22 ± 0.05	0.18 – 0.26	0.09 ± 0.02	0.08 – 0.10	<0.001*
	Day 21	0.32 ± 0.14	0.22 – 0.42	0.12 0.04±	0.10 – 0.15	<0.001*
	Day 28	0.37 ± 0.17	0.25 – 0.49	0.20 ± 0.04	0.17 – 0.22	0.002*
	Day 35	0.40 ± 0.15	0.29 – 0.50	0.21 ± 0.09	0.15 – 0.28	0.009*
	Day 42	0.57 ± 0.27	0.38 – 0.76	0.22 ± 0.09	0.16 – 0.29	0.019*
Percentage Plasma Haemolysis	Day 1	0.14 ± 0.03	0.12 – 0.16	0.10 ± 0.03	0.08 – 0.12	0.014*
	Day 7	0.19 ± 0.05	0.16 – 0.23	0.11 ± 0.06	0.07 – 0.15	0.003*
	Day 14	0.42 ± 0.09	0.36 – 0.49	0.18 ± 0.03	0.16 – 0.20	<0.001*
	Day 21	0.66 ± 0.19	0.52 – 0.79	0.25 ± 0.05	0.21 – 0.28	0.002*
	Day 28	0.71 ± 0.22	0.52 – 0.87	0.38 ± 0.05	0.35 – 0.41	0.002*
	Day 35	0.83 ± 0.19	0.69 – 0.96	0.43 ± 0.14	0.33 – 0.53	0.002*
	Day 42	1.12 ± 0.32	0.89 – 1.35	0.44 ± 0.14	0.34 – 0.54	0.002*

Key: CI - Confidence interval
SD - Standard deviation
p Value - Significant if < 0.05
N = 10

4.2.4 Paediatric Red Blood Cell Concentrate: Biochemistry and Haematology Results

The potassium ion results for the non-irradiated (NIRR) paediatric RBCC and irradiated (IRR) paediatric RBCC units indicate a significant increase ($p<0.05$) during the 42-day storage period except for Day 42 due to the 2 included low outliers. The 95% Confidence Interval (95% CI) for IRR paediatric RBCC potassium ion ranges from 41.2 – 48.3mmol/L on Day 1 and is similar to the IRR LRBCPS Day 1 result of 41.3 – 47.4mmol/L. The results for the 95% CI on Day 42 for potassium results ranged from 84.3 – 91.2mmol/L. The LDH indicators for IRR paediatric RBCC display significant differences ($p<0.05$) from Day 14 onwards and is similar to the IRR LRBCPS evaluations.

The comparison of the extracellular sodium (mmol/L) ion levels in the control and test groups from Day 1 to Day 42 is considered significant ($p< 0.05$) throughout the storage period. The phosphate levels show an increase while that of the glucose and pH concentrations indicate a decrease without any statistically significant differences observed throughout the 42-day storage period.

The results for the erythrocyte, leucocyte and thrombocyte counts as well as haemoglobin, HCT, MCV, MCH and MCHC indicate $p>0.05$ throughout the storage period and thus considered insignificant. The percentage plasma haemolysis results on Day 35 (1.01 ± 0.44%) and Day 42 (1.32 ± 0.67%) for IRR paediatric RBCC are higher than the 0.8% per recommendation by the Clinical Guidelines for use of blood products in South Africa but it should be noted that blood may be irradiated up to 14 days post collection date and then stored for an additional 14 days i.e. blood only kept until Day 28.

Table 4.9: Irradiated vs Non-irradiated paediatric Red Blood Cell Concentrate (PRBCC): Biochemistry Results

Biochemistry Indicators	Storage Period	PRBCC Irradiated (IRR)		PRBCC Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p-Value
Potassium Levels (mmol/L)	Day 1	44.7 ± 5.0	41.2 – 48.3	15.8 ± 2.6	13.9 – 17.7	0.002*
	Day 7	66.6 ± 6.7	61.8 – 71.4	25.5 ± 3.6	22.9 – 28.1	0.006*
	Day 14	81.4 ± 9.3	74.8 – 88.0	30.6 ± 7.6	25.2 – 36.0	0.002*
	Day 21	71.0 ± 4.2	68.0 – 74.0	84.4 ± 20.8	69.5 – 99.2	0.004*
	Day 28	79.2 ± 5.4	75.3 – 83.0	67.1 ± 7.1	62.0 – 72.1	0.002*
	Day 35	112.4 ± 9.6	105.6 – 119.3	75.5 ± 8.9	69.1 – 81.8	0.002*
	Day 42	71.0 ± 1.3	68.0 – 74.0	91.8 ± 34.8	67.0 – 116.7	0.131
Sodium Levels (mmol/L)	Day 1	125 ± 4	122 – 128	144 ± 2	143 – 146	0.006*
	Day 7	130 ± 4	127 – 133	155 ± 2	153 – 156	0.006*
	Day 14	117 ± 6	113 – 122	152 ± 5	148 – 156	0.006*
	Day 21	109 ± 4	106 – 112	133 ± 3	131 - 135	0.006*
	Day 28	120 ± 2	118 – 122	160 ± 7	155 – 165	0.006*
	Day 35	105 ± 3	103 – 108	122 ± 3	120 – 125	0.006*
	Day 42	111 ± 3	109 - 114	214 ± 85	154 - 275	0.006*
Phosphate Levels (mmol/L)	Day 1	1.7 ± 0.6	1.3 – 2.1	1.9 ± 0.5	1.5 – 2.2	0.432
	Day 7	3.0 ± 0.8	2.5 – 3.6	3.2 ± 0.5	2.8 – 3.5	0.625
	Day 14	3.8 ± 0.6	3.4 – 4.2	3.8 ± 0.6	3.4 – 4.2	1.000
	Day 21	5.8 ± 0.6	5.4 – 6.2	5.4 ± 0.6	5.0 – 5.8	0.027*
	Day 28	9.5 ± 1.1	8.7 – 10.3	8.4 ± 1.5	7.4 – 9.5	0.049*
	Day 35	6.9 ± 0.6	6.4 – 7.3	6.3 ± 0.7	5.8 – 6.8	0.037*
	Day 42	7.1 ± 0.9	6.5 – 7.7	5.2 ± 1.0	4.5 – 6.0	0.002*
Glucose Levels (mmol/L)	Day 1	31.2 ± 2.3	29.6 – 32.8	30.8 ± 0.9	30.2 – 31.4	0.625
	Day 7	28.9 ± 2.6	27.0 – 30.7	28.4 ± 0.9	27.8 – 29.0	0.734
	Day 14	24.1 ± 2.3	22.4 – 25.8	23.3 ± 1.1	22.5 – 24.0	0.375
	Day 21	23.0 ± 2.8	21.0 – 25.0	23.0 ± 0.9	22.3 – 23.6	0.922
	Day 28	20.7 ± 2.8	18.7- 22.8	20.9 ± 3.3	18.6 – 23.3	0.557
	Day 35	18.0 ± 2.9	16.0 – 20.1	17.7 ± 1.6	16.5 – 18.9	0.846
	Day 42	18.3 ± 3.3	15.9 – 20.6	17.7 ± 1.9	16.3 – 19.0	0.695
Lactate Dehydrogenase Levels (U/L	Day 1	156 ± 44	125 – 188	149 ± 74	96 – 202	0.557
	Day 7	236 ± 55	197 – 275	167 ± 86	106 – 229	0.064
	Day 14	310 ± 86	248 – 374	181 ± 105	106 – 256	0.027*
	Day 21	391 ± 108	314 – 468	284 ± 355	30 – 539	0.084
	Day 28	542 ± 109	400 – 68 4	262 ± 128	171 – 354	0.006*
	Day 35	608 ± 240	437 – 779	247 ± 127	156 – 338	0.002*
	Day 42	836 ± 333	597 - 1074	345 ± 175	220 - 470	0.002*
pH Levels	Day 1	7.25 ± 0.06	7.21 – 7.29	7.21 ± 0.07	7.16 – 7.25	0.169
	Day 7	6.83 ± 0.05	6.79 – 6.86	6.82 ± 0.08	6.76 – 6.87	0.919
	Day 14	6.83 ± 0.05	6.79 – 6.86	6.83 ± 0.04	6.80 – 6.85	0.866
	Day 21	6.75 ± 0.04	6.73 – 6.78	6.70 ± 0.04	6.66 – 6.73	0.024
	Day 28	6.63 ± 0.08	6.57 – 6.68	6.63 ± 0.04	6.60 – 6.66	0.878
	Day 35	6.56 ± 0.05	6.52 – 6 60	6.56 ± 0.05	6.53 – 6.60	0.575
	Day 42	6.51 ± 0.06	6.47 – 6.55	6.52 ± 0.05	6.48 – 6.56	0.609

Key: CI - Confidence interval
SD - Standard deviation
p Value - Significant if < 0.05
N = 10

Table 4.10: Irradiated vs Non-irradiated paediatric RBCC: Haematology Results

Haematology Indicators	Storage Period	PRBCC Irradiated (IRR)		PRBCC Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p-Value
Erythrocyte Count	Day 1	6.4 ± 0.5	6.1 – 6.8	6.3 ± 0.4	6.0 – 6.5	0.366
	Day 7	6.4 ±0.5	6.1 – 6.8	6.4 ± 0.5	6.0 – 6.7	0.670
	Day 14	6.5 ± 0.4	6.2 – 6.8	6.7 ± 0.5	6.3 – 7.0	0.504
	Day 21	6.8 ± 0.4	6.5 – 7.1	6.7 ± 0.5	6.4 – 7.1	0.708
	Day 28	6.5 ± 0.4	6.2 – 6.8	6.6 ± 0.5	6.2 – 6.9	0.666
	Day 35	6.4 ± 0.4	6.1 – 6.7	6.5 ± 0.4	6.2 – 6.7	0.920
	Day 42	6.4 ± 0.5	6.1 – 6.8	6.5 ± 0.4	6.2 – 6.8	1.000
Leucocyte Count	Day 1	0.2 ± 0.1	0.09 – 0.3	1.0 ± 1.0	0.3 – 1.8	0.021*
	Day 7	0.1 ± 0.05	0.1 – 0.2	0.5 ± 0.7	0.02 – 1.0	0.115
	Day 14	0.1 ± 0.0	0.1 – 0.1	0.3 ± 0.4	0.02 – 0.6	0.144
	Day 21	0.1 ± 0.05	0.02 – 0.1	0.2 ± 0.2	0.03 – 0.3	0.120
	Day 28	0.1 ± 0.04	0.05 – 0.1	0.1 ± 0.0	0.1 – 0.1	0.168
	Day 35	0.1 ± 0.05	0.02 – 0.1	0.1 ± 0.1	0.05 – 0.1	0.279
	Day 42	0.04 ± 0.1	0.0 – 0.08	0.1 ± 0.04	0.05 – 0.1	0.037*
Thrombocyte Count	Day 1	1.2 ± 0.9	0.5 – 1.9	3.3 ± 2.0	1.9 – 4.7	0.098
	Day 7	0.9 ±0.7	0.4 – 1.4	1.6 ± 1.5	0.5 – 2.7	0.285
	Day 14	0.7 ± 0.7	0.2 – 1.2	0.5 ± 0.7	0.1 – 1.0	0.591
	Day 21	0.5 ± 0.5	0.1 – 0.9	0.3 ± 0.5	0.05 – 0.7	0.168
	Day 28	0.2 ± 0.6	0.3 – 0.7	0.2 ± 0.4	0.1 – 0.5	1.000
	Day 35	0.2 ± 0.4	0.1 – 0.5	0.3 ± 0.5	0.05 – 0.7	0.591
	Day 42	0.0 ± 0.0	0.0 – 0.0	0.1 ± 0.3	0.1 – 0.3	0.343
Haemoglobin (Hb) Levels	Day 1	19.3 ± 1.2	18.5 – 20.1	18.9 ± 0.7	18.3 – 19.4	0.318
	Day 7	19.2 ± 1.4	18.2 – 20.2	18.8 ± 1.0	18.1 – 19.6	0.583
	Day 14	19.6 ± 1.3	18.7 – 20.5	20.0 ± 1.7	18.8 – 21.2	0.561
	Day 21	19.7 ± 1.2	18.8 – 20.5	19.4 ± 1.1	18.6 – 20.1	0.655
	Day 28	19.2 ± 1.1	18.8 – 20.0	19.6 ± 1.3	18.7 – 20.5	0.566
	Day 35	19.3 ± 1.2	18.5 – 20.1	19.4 ± 1.1	18.6 – 20.2	0.895
	Day 42	19.0 ± 1.2	18.2 – 19.9	19.2 ± 0.9	18.5 – 19.8	0.826
Haematocrit (HCT) Levels	Day 1	56.2 ± 3.1	54.0 – 58.3	55.3 ± 2.0	53.8 – 56.7	0.412
	Day 7	56.8 ± 3.8	54.1 – 59.5	56.3 ± 2.4	54.6 – 58.0	0.738
	Day 14	58.3 ± 3.3	55.9 – 60.7	59.3 ± 4.7	56.0 – 62.7	0.638
	Day 21	54.1 ± 2.5	52.4 – 55.9	51.6 ± 2.3	49.9 – 53.3	0.073
	Day 28	58.2 ± 2.8	56.2 – 60.2	57.6 ± 3.7	55.0 – 60.3	0.734
	Day 35	58.2 ± 2.9	56.1 - 60.2	56.9 ± 3.0	54.7 – 59.0	0.447
	Day 42	59.0 ± 2.8	57.0 – 61.0	56.1 ± 2.0	54.7 – 57.6	0.051

Table 4.10.1: Irradiated vs Non-irradiated paediatric RBCC (PRBCC): Haematology Results

Haematology Indicators	Storage Period	PRBCC Irradiated (IRR)		PRBCC Non – Irradiated (NIRR)		IRR vs NIRR
		Mean ± SD	95% CI	Mean ± SD	95% CI	p – Value
Mean Corpuscular Volume (MCV) Levels	Day 1	87.4 ± 4.3	84.3 – 90.5	88.3 ± 4.0	85.4 – 91.0	0.671
	Day 7	88.5 ± 4.3	85.5 – 91.5	88.8 ± 4.1	85.9 – 91.7	0.809
	Day 14	90.0 ± 4.37	86.9 – 93.1	89.0 ± 4.0	86.1 – 91.9	0.451
	Day 21	79.7 ± 4.3	76.6 – 82.8	78.5 ± 3.7	75.8 – 81.2	0.567
	Day 28	89.8 ± 4.2	86.7 – 92.8	87.7 ± 3.9	84.9 – 90.5	0.306
	Day 35	90.7 ± 4.3	87.6 – 93.8	88.5 ± 4.0	85.6 – 91.4	0.141
	Day 42	91.8 ± 4.5	88.6 – 95.0	89.1 ± 4.2	86.1 – 92.1	0.086
Mean Corpuscular Haemoglobin (MCH) Levels	Day 1	30.0 ± 1.6	28.9 – 31.2	30.1 ± 1.2	29.3 – 30.0	0.874
	Day 7	29.9 ± 1.7	28.7 – 31.1	29.7 ± 1.3	28.8 – 30.7	0.818
	Day 14	30.0 ± 1.6	28.9 – 31.4	30.1 ± 1.4	29.1 – 31.1	0.910
	Day 21	28.9 ± 1.6	27.8 – 30.0	29.3 ± 1.1	28.5 – 30.0	0.575
	Day 28	29.7 ± 1.5	28.7 – 30.8	29.8 ± 1.5	28.8 – 30.9	0.907
	Day 35	30.1 ± 1.6	29.0 – 31.2	30.2 ± 1.4	29.2 – 31.2	0.866
	Day 42	29.7 ± 1.6	28.5 – 30.9	29.7 ± 1.3	28.8 – 30.6	0.967
Mean Corpuscular Haemoglobin Concentrate (MCHC) Levels	Day 1	34.3 ± 0.43	34.0 – 34.6	34.1 ± 0.5	33.8 – 34.5	0.382
	Day 7	33.8 ± 0.6	33.4 – 34.2	33.4 ± 0.6	33.0 – 33.9	0.392
	Day 14	33.4 ± 0.6	33.0 – 33.8	33.7 ± 0.5	33.3 – 34.1	0.279
	Day 21	36.3 ± 1.1	35.5 – 37.1	37.0 ± 1.6	35.8 – 38.1	0.320
	Day 28	33.1 ± 0.6	32.6 – 33.5	34.0 ± 0.6	33.6 – 34.4	0.005*
	Day 35	33.2 ± 0.8	32.7 – 33.8	34.1 ± 0.5	33.8 – 35.5	0.022*
	Day 42	32.4 ± 0.8	31.8 – 30.0	33.4 ± 0.5	33.0 – 33.7	0.021*
Plasma Haemoglobin Levels	Day 1	0.04 ± 0.01	0.04 – 0.05	0.04 ± 0.01	0.03 – 0.05	0.832
	Day 7	0.10 ± 0.03	0.08 – 0.12	0.07 ± 0.08	0.02 – 0.13	0.437
	Day 14	0.18 ± 0.04	0.15 – 0.21	0.10 ± 0.05	0.06 – 0.13	0.359
	Day 21	0.27 ± 0.09	0.20 – 0.33	0.21 ± 0.14	0.11 – 0.31	0.250
	Day 28	0.39 ± 0.23	0.22 – 0.56	0.24 ± 0.19	0.10 – 0.38	0.177
	Day 35	0.46 ± 0.19	0.32 – 0.60	0.19 ± 0.11	0.11 – 0.27	0.005*
	Day 42	0.54 ± 0.22	0.38 – 0.70	0.30 ± 0.05	0.26 – 0.34	0.005*
Percentage Plasma Haemolysis Levels	Day 1	0.10 ± 0.02	0.08 – 0.11	0.10 ± 0.03	0.08 – 0.12	0.702
	Day 7	0.22 ± 0.05	0.18 – 0.26	0.16 ± 0.15	0.05 – 0.27	0.361
	Day 14	0.39 ± 0.11	0.31 – 0.46	0.18 ± 0.05	0.14 – 0.22	0.253
	Day 21	0.63 ± 0.27	0.44 – 0.82	0.53 ± 0.34	0.28 – 0.77	0.455
	Day 28	0.76 ± 0.28	0.55 – 0.96	0.53 ± 0.43	0.22 – 0.84	0.207
	Day 35	1.01 ± 0.44	0.69 – 1.32	0.41 ± 0.25	0.24 – 0.59	0.005*
	Day 42	1.32 ± 0.67	0.84 – 1.8	0.68 ± 0.13	0.59 – 0.77	0.034*

Key: CI - Confidence interval
SD - Standard deviation
p Value - Significant if < 0.05
n = 10

CHAPTER FIVE

DISCUSSION

5.1 Discussion

While current literature indicates that gamma irradiation aggravates storage lesions in red blood cellular products, a study of this nature has not previously been done in Cape Town, South Africa. Also, the blood transfusion establishments in South Africa do not prescribe to the universal leucocyte-depletion (pre-storage) policy regarding leucocyte-reduced products as many of their global counterparts (Bellairs and Ingram, 2014). Instead, a filtered RBC product is usually provided upon request by a transfusion medicine clinician as only selected blood groups are processed due to the high cost involved.

The occurrence of storage lesions is dependent on the biochemical and physical properties of the RBC and can therefore be placed in different categories. There may be changes regarding energy metabolism where molecules such as ATP and 2, 3 DPG impacts on cellular respiration or the increased sodium concentration entering the cell when the Na^+ / K^+ pumps are inactive at 4 °C. Other changes which may occur during storage are the biomechanical alterations which affect rheological properties, for example, deformability, viscosity and shape of the RBC. These changes influence cellular diapedesis and neither a RBC nutritive additive nor added anticoagulant are able to prevent these storage lesions from appearing. The third change is oxidative lesions which may result in Band 3 clustering, oxidative alterations or the formation of hemichrome (produced by denatured haemoglobin). .Although some storage lesions may occur within days or weeks, elevated potassium and LDH levels may be detected within hours of storage and all these changes add to transfusion complications as rheology is ultimately affected (Delobel *et al.,* 2010).

Gamma irradiation damages red blood cells by increasing the RBC membrane permeability and thus the period between the products being irradiated to it been infused into the patient, is of prime importance. The rapid release of potassium, haemoglobin and LDH into the extracellular plasma is due to irradiation which also increases the extracellular potassium levels during the post-irradiation storage period. Irradiators are not available in many hospitals in developed countries due to the high cost involved and therefore these hospitals acquire the necessary irradiated products from a central blood transfusion establishment. This, however, becomes problematic as the demand for irradiated products are increasing and it is imperative that adequate records be maintained (Agarwal, Choudhury and Chaudhary, 2005).

In this study, the biochemical and haematological factors of gamma irradiated and non-irradiated red blood cellular products during the standard storage period of up to 42 days while at 1° to 6 °C were measured and compared. This was to determine the differences, if any, between non-irradiated and irradiated RBC products and to establish whether the South African policy regarding irradiated RBC products should be amended. The non-irradiated and irradiated blood products evaluated include whole blood, red blood cell concentrate with buffy-coat layer removed, leucocyte-reduced red blood cell concentrate and paediatric red blood cell concentrate.

The study indicates that the level of potassium as well as lactate dehydrogenase activity increase during the storage period and these results are confirmed by previous studies (Winter *et al*., 2015; Zimmermann *et al*., 2009). These previous studies also confirmed a reduction in sodium and glucose levels. The tabulated results presented in this study indicate a decrease in the sodium ion concentration for example, in non-irradiated WB (155 ± 2 mmol/L on Day 1 to 140 ± 2 mmol/L by Day 42) while that of the non-irradiated RBCC decreased from 147 ± 2 mmol/L on Day 1 to 122 ± 3 mmol/L by Day 42. The glucose concentrations in irradiated WB also declined from 19.8 ± 1.1mmol/L on Day 1 to 12.6 ± 1.1mmol/L on Day 42.

The universal leuco-reduction policy is practiced by most First World countries and our study has proven that leucocyte reduction moderates percentage plasma haemolysis. We observed a decrease in percentage plasma haemolysis in non-irradiated RBCC Day 42 (0.54 ± 0.21) when compared to non-irradiated LRBCPS Day 42 (42 to 0.44 ± 0.14) and will therefore improve blood safety and efficacy (Hess, 2006.

Although gamma irradiation exacerbates storage lesions, however, it is the accepted method to use in order to prevent transfusion-associated Graft vs Host disease (Mintz and Anderson, 1993). This is confirmed with an increase in plasma haemoglobin and subsequent percentage plasma haemolysis results observed in this study and this is in agreement other studies (Agarwal *et al*., 2005; Sawant *et al*., 2007; Makroo *et al*., 2011). The US Food and Drug Administration (FDA) recommended that percentage plasma haemolysis of RBC products nearing the expiry date, should not exceed 1% while the Council of Europe stipulates that the level should not be more than 0.8% by end of the storage period. However, the irradiated red blood cellular products in our study indicate a higher percentage plasma haemolysis level on Days 35 and 42, as confirmed by earlier studies (Leitner *et al*., 2001; Winter *et al*., 2015; Zimmermann *et al*, 2011). It should be noted that irradiated blood is only stored until day 28 or according to the original expiry date if it is sooner.

Blood transfusion establishments should be aware that there are many factors contributing to RBC haemolysis. Some of these factors include analyte differences between donors, diverse pre-transfusion and post-transfusion abilities of various donors, possible genetic inconsistencies leading to haemolysis as well as the pre-analytical factors and component processing strategies (D'Allessandro *et al.,* 2015).

5.2 Conclusion

The outcome of this study confirms that gamma irradiation exacerbates RBC storage lesions when blood is stored at 1°C to 6°C for up to 42 days. The study further indicates that significant results were observed during the evaluation of the irradiated WB and paediatric RBCC because the volume of plasma and RBC is greater than that of the paediatric RBCC. Significant differences were demonstrated between an adult irradiated LRBCPS and a paediatric RBCC on Day 1 for glucose, pH levels, plasma haemoglobin levels and percentage plasma haemolysis. Although both of these products are leucocyte-reduced, the irradiated paediatric RBCC undergo further product modification when the adult LRBCPS is divided into the 2 bags which may subject RBC to further oxidative stress. Despite the fact that South Africa does not prescribe to the ULR policy, the products tested yielded similar results to other studies in developed countries therefore, the South African transfusion medicine policy should remain unchanged.

5.3 Recommendation

Despite various and controversial debates occurring within the circle of transfusion medicine, the transfusion of the red blood cellular concentrate still remain a popular treatment resource. The published results of meta-analysis regarding storage lesions should be carefully reviewed before policies relating to transfusion medicine are amended. The scientific community should be contemplating improved storage strategies such as anaerobic storage, improving nutritive additives and proteomic-based biomarkers. An alternative to gamma irradiation where mitotic lymphocytes are inactivated without causing cellular degeneration should also be considered to ensure a safer blood product which may prevent transfusion-associated Graft-vs-Host disease from occurring. The damage of the red cell membrane and subsequent haemolysis that occur during the storage period of red blood cellular products may be due to oxidative stress and thus, the role of adding antioxidants to units of blood could decrease lipid peroxidation and therefore, also decrease leakage of potassium, LDH and haemoglobin.

5.4 Limitations

All the collected blood should have been tested for haematological and biochemical factors prior to component separation and subsequent irradiation as this would have provided baseline measurements. The collected blood donations used for this study should also initially have been centrifuged at 1 temperature only. Instead of producing RBCC, fresh frozen plasma and platelets from 1unit of whole blood which require a centrifugation temperature of 22 °C, only RBCC and fresh frozen plasma should have been produced at 4°C. An improved strategy would be to use 1 red blood cellular product and divide it into 2, where one bag is irradiated and the second bag used as the control. Also, not including the irradiation of blood on day 14 and keeping it stored until expiry (per USA guidelines), was an oversight.

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APPENDICES

Appendix A: Letter of consent from Dr. G.R.M. Bellairs

WP Blood Transfusion Service
Do something remarkable

www.wpblood.org.za / info@wpbts.org.za

Head Office
Old Mill Road, Pinelands, 7405 • PO Box 79, Howard Place, 7450
T: 021 507 6300 / F: 021 531 0322

17 July 2013

To whom it may concern

This is to confirm that Western Province Blood Transfusion Service grants Faieqa Adams permission to perform a research study in order to attain her M.Tech Biomedical Technology qualification.

Please do not hesitate to contact me should you have any queries in this regard.

Yours faithfully

Dr GRM Bellairs
CEO/Medical Director

Paarl
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T: 021 871 1030 / F: 021 872 5945

Worcester
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T: 023 342 2450 / F: 023 342 7556

George
Medical Centre, Courtenay Street
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WP Blood Transfusion Service NPC
Pr. No: 7800045 / Reg. No: 1943/016692/08

Directors: GRM Bellairs, AR Bird, GR Bosman, MR Burton, NB du Toit, F Essop, Bdl Figaji, I Kaprey, N Parker, R Ramsbottom, PK Slack (Chairman), E Steyn

Appendix B: Donor Questionnaire

WP Blood Transfusion Service
Do something remarkable

www.wpblood.org.za / info@wpbts.org.za
Head Office
Old Mill Road, Pinelands, 7405 • PO Box 79, Howard Place 7450
t:021 507 6300 / f:021 531 0322

CONFIDENTIAL DONOR QUESTIONNAIRE

Please make sure that you complete all required sections carefully and honestly.

SECTION 1
Lifestyle Questionnaire:
Though personal, these questions don't aim to offend, but rather to identify potential risk to the recipient.

SECTION 2
Health Questionnaire:
Your safety is as important to us as the safety of the recipient. Therefore, you might not be able to donate if you answer 'yes' to any of these questions. The qualified nurse will discuss your answers with you.

SECTION 3
Contact Details and Donor Enrolment Form:
New donors must complete all sections of the questionnaire.
Regular blood donors should only complete section 3 if any personal information has changed.

PLEASE DO NOT DONATE BLOOD IF YOU MAY HAVE BEEN EXPOSED TO HIV/AIDS.

You may be endangering someone's life.
Never donate blood for personal health screening purposes.

Thank you for donating blood today!

Your donation could save at least three lives. Remarkable, isn't it? As a Service, we provide safe blood and blood products to those who need them. Please continue to make a difference by remaining a regular blood donor.

CLH 12 (01 Nov 13)

DONOR LABEL

BAR CODE

ection 1 | LIFESTYLE QUESTIONNAIRE

Please circle your answers.

lease read all questions carefully and answer honestly. Your answers will be treated confidentially.

1	In the past 6 months have you: Had a tattoo, body piercing, ear piercing or permanent make-up applied?	No	Yes	STAFF SECTION
	Had Raatib, ritual scarring, ritual piercing, ritual circumcision, blood sharing or been stabbed?	No	Yes	
2	For Health Care Workers and their partners only: In the past 6 months: Have you or your sexual partner had a needle stick or skin penetrating injury; or had skin, eye or mouth contact with another person's blood?	No	Yes	
3	In the past 6 months: Have you taken antiretroviral medication?	No	Yes	
The following questions are of a sexual nature. We ask these questions as sexual contact may cause infectious diseases like HIV/AIDS. "Sexual contact" refers to vaginal sex (contact between penis and vagina); oral sex (mouth or tongue contact with vagina, penis or anus) and anal sex (contact between penis and anus). Where applicable, please answer "Yes" to the following questions even if a condom was used:				
4	Do you have AIDS or are you HIV positive?	No	Yes	
	Have you ever had sexual contact with anyone who has AIDS or is HIV positive?	No	Yes	
	Are you only giving blood for an HIV test?	No	Yes	
5	In the past 6 months (with or without a condom): - Have you started having sexual contact with a new sexual partner?	No	Yes	
	- Have you had sexual contact with more than one person?	No	Yes	
	- To the best of your knowledge has your sexual partner had sexual contact with more than one person?	No	Yes	
	- Have you had sexual contact with someone whose sexual history you do not know?	No	Yes	
	- Have you had sexual contact with anyone who takes money, drugs or other favours for sex?	No	Yes	
	- Have you received money, drugs or other payment for sex?	No	Yes	
	- Are you a sex worker?	No	Yes	
	- Have you been sexually assaulted?	No	Yes	
6	In the past 6 months: Have you or your sexual partner had any sexually transmitted disease (STD) including genital herpes, syphilis, gonorrhoea (drop) or human papilloma virus?	No	Yes	
7	Have you or your sexual partner ever used recreational/street drugs by nose, mouth or injection needle?	No	Yes	
8	Do you consider your blood safe to be transfused to a patient?	No	Yes	

DECLARATION : Please read and sign before donating blood.

1. I have read and understood the pamphlet "Important Information for Blood Donors".
2. To the best of my knowledge all the information supplied is the truth.
3. I understand that if I have not answered these questions truthfully this could endanger the patient and lead to legal proceedings against me. I undertake that should I for any reason deem my blood not safe for use, I will immediately inform WPBTS.
4. I consent to my blood being tested for Syphilis, Hepatitis B, Hepatitis C and HIV.
5. I understand that I will be informed of any test results that are important to my health or affect my ability to donate blood.
6. I accept that samples of my blood and / or donation data may be used on occasion for scientific research, the objective of which is to improve the safety of the blood supply to patient and donor health and well being. On occasion the Service may permit researchers to request additional samples from me with my consent.
7. I confirm that I am 16 years of age or older.
8. I understand that the information on this form will be kept in a secure facility indefinitely under my donor code, not my name.
9. I understand the donation process and the possible risks involved as explained.
10. I consent to the administration of such fluids and medications as deemed necessary in the management of an untoward donor reaction.
11. I consent to the infusion of fluids, medications and re-infusion of my own blood components during apheresis collection procedures.

Please do not sign until you have answered all the questions and read the declaration.

Cell number: Tel number:	
Name and surname:	
Date of birth:	
Donor's signature:	

FOR OFFICE USE:				
Interview done	No		Yes	
Signature: Phlebotomist				
Signature: Interviewer (only if interview was done)				

DONOR LABEL

BAR CODE

Section 1 | LIFESTYLE QUESTIONNAIRE

Please circle your answers.

Please read all questions carefully and answer honestly. Your answers will be treated confidentially.

1	**In the past 6 months have you:** Had a tattoo, body piercing, ear piercing or permanent make-up applied?	No	Yes	STAFF SECTION
	Had Raatib, ritual scarring, ritual piercing, ritual circumcision, blood sharing or been stabbed?	No	Yes	
2	**For Health Care Workers and their partners only:** **In the past 6 months:** Have you or your sexual partner had a needle stick or skin penetrating injury; or had skin, eye or mouth contact with another person's blood?	No	Yes	
3	**In the past 6 months:** Have you taken antiretroviral medication?	No	Yes	
	The following questions are of a sexual nature. We ask these questions as sexual contact may cause infectious diseases like HIV/AIDS. "Sexual contact" refers to vaginal sex (contact between penis and vagina); oral sex (mouth or tongue contact with vagina, penis or anus) and anal sex (contact between penis and anus). Where applicable, please answer "Yes" to the following questions even if a condom was used:			
4	Do you have AIDS or are you HIV positive?	No	Yes	
	Have you ever had sexual contact with anyone who has AIDS or is HIV positive?	No	Yes	
	Are you only giving blood for an HIV test?	No	Yes	
5	**In the past 6 months (with or without a condom):** - Have you started having sexual contact with a new sexual partner?	No	Yes	
	- Have you had sexual contact with more than one person?	No	Yes	
	- To the best of your knowledge has your sexual partner had sexual contact with more than one person?	No	Yes	
	- Have you had sexual contact with someone whose sexual history you do not know?	No	Yes	
	- Have you had sexual contact with anyone who takes money, drugs or other favours for sex?	No	Yes	
	- Have you received money, drugs or other payment for sex?	No	Yes	
	- Are you a sex worker?	No	Yes	
	- Have you been sexually assaulted?	No	Yes	
6	**In the past 6 months:** Have you or your sexual partner had any sexually transmitted disease (STD) including genital herpes, syphilis, gonorrhoea (drop) or human papilloma virus?	No	Yes	
7	Have you or your sexual partner ever used recreational/street drugs by nose, mouth or injection needle?	No	Yes	
8	Do you consider your blood safe to be transfused to a patient?	No	Yes	

DECLARATION : Please read and sign before donating blood.

1. I have read and understood the pamphlet "Important Information for Blood Donors".
2. To the best of my knowledge all the information supplied is the truth.
3. I understand that if I have not answered these questions truthfully this could endanger the patient and lead to legal proceedings against me. I undertake that should I for any reason deem my blood not safe for use, I will immediately inform WPBTS.
4. I consent to my blood being tested for Syphilis, Hepatitis B, Hepatitis C and HIV.
5. I understand that I will be informed of any test results that are important to my health or affect my ability to donate blood.
6. I accept that samples of my blood and / or donation data may be used on occasion for scientific research, the objective of which is to improve the safety of the blood supply to patient and donor health and well being. On occasion the Service may permit researchers to request additional samples from me with my consent.
7. I confirm that I am 16 years of age or older.
8. I understand that the information on this form will be kept in a secure facility indefinitely under my donor code, not my name.
9. I understand the donation process and the possible risks involved as explained.
10. I consent to the administration of such fluids and medications as deemed necessary in the management of an untoward donor reaction.
11. I consent to the infusion of fluids, medications and re-infusion of my own blood components during apheresis collection procedures.

Please do not sign until you have answered all the questions and read the declaration.

Cell number: Tel number:	
Name and surname:	
Date of birth:	
Donor's signature:	

FOR OFFICE USE:				
Interview done	No		Yes	
Signature: Phlebotomist				
Signature: Interviewer (only if interview was done)				

CLN 12 (01 Nov 13)

Section 2 | HEALTH QUESTIONNAIRE

Please read all questions carefully and answer honestly. Your answers will be treated confidentially.

Please circle your answers.

1	Are you feeling well today?	No	Yes
	In the last 4 hours have you had something to eat or drink?	No	Yes
2	**Are you involved in any of the following:** Driving a public or heavy-duty vehicle, flying an aeroplane, working on scaffolding or using power tools?	No	Yes
	Sky diving, deep-sea diving or mountaineering?	No	Yes
3	**In the past 3 days:** Have you been to the dentist?	No	Yes
	Have you taken any painkillers, anti-inflammatories or aspirin (Ecotrin)?	No	Yes
	In the past 7 days: Have you had a cold, flu, sore throat, fever, infection or allergies?	No	Yes
	In the past 30 days: Have you had diarrhoea or vomiting?	No	Yes
	Have you taken Androcur, Proscar, Propecia, Roaccutane, Warfarin or Dabigtran Etexilate (Pradaxa)?	No	Yes
4	**In the past 3 months:** Have you taken any medication (including traditional medication), injections or tablets?	No	Yes
5	**In the past 6 months:** Have you or your sexual partner had a blood transfusion or received blood products or clotting factors?	No	Yes
	Have you had acupuncture, botox or dry-needling?	No	Yes
6	**In the past 6 months:** Have you had a vaccination or immunization (inoculation)?	No	Yes
	Have you taken part in a drug trial, vaccine trial, or clinical research?	No	Yes
7	**In the past 6 months:** Have you had a surgical procedure or been admitted to hospital?	No	Yes
	Are you scheduled to have surgery in the next 6 weeks?	No	Yes
8	**In the past 2 years:** Have you taken (Neo) Tigason for skin problems?	No	Yes
9	**Have you ever had:** High blood pressure?	No	Yes
	Heart, lung or circulatory problems?	No	Yes
	Epilepsy, convulsions or strokes?	No	Yes
	Cancer, skin cancer or leukaemia?	No	Yes
	Diabetes, asthma, TB or kidney disease?	No	Yes
	Haemochromatosis, polycythaemia or a bleeding disorder?	No	Yes
10	**HEPATITIS:** Have you ever had yellow jaundice, hepatitis, liver disease or a positive test for hepatitis?	No	Yes
	In the past 6 months have you been in contact or lived with anyone who has hepatitis (jaundice)?	No	Yes
11	**MALARIA:** Did you grow up in a malaria prevalent area?	No	Yes
	Have you been in a malaria area in the last 3 months?	No	Yes
	Have you had malaria in the last 3 years?	No	Yes
12	**VARIANT CREUTZFELD-JAKOB DISEASE: (also known as Mad Cow disease)** Have you ever had neuro surgery, received a dura mater (brain covering) graft or taken pituitary growth hormone?	No	Yes
	Have you or your sexual partner ever received a tissue, cornea or organ transplant?	No	Yes
	Were you residing in the United Kingdom for a total period of 12 months or longer between January 1980 and December 1996?	No	Yes
13	Have you ever had any other serious illnesses, tropical diseases or medication not mentioned in above questions?	No	Yes
14	Are you participating in a regular training or athletic programme?	No	Yes
15	Have you ever injected yourself or been injected with illegal steriods (body building drugs)?	No	Yes
16	**FOR WOMEN ONLY:** Are you pregnant or undergoing fertility treatment?	No	Yes
	In the last 3 months have you had a baby, miscarriage or abortion?	No	Yes
	Are you breastfeeding?	No	Yes

STAFF SECTION

STAFF SECTION

FOR OFFICE USE:		
Blood pressure:	Pre	Post
Donor's pulse:	Pre	Post
HemoCue:	g/dL	Sign

SAMPLE TAKEN BY: _____

NEEDLE REMOVED BY: _____

BAR CODE

CLN 12 (01 Nov 13)

WP Blood Transfusion Service
Do something remarkable

Section 3 | DONOR ENROLMENT FORM

First time donors: Complete fully
Regular donors: Only complete this page if your personal information has changed.

Surname:																				Title:					
Firstname:													Other initials:							Female:			Male:		
Date of birth:	D	D	M	M	Y	Y	Y	Y		ID no:															
Home address:																									
																				Postal code:					
Postal address:																									
																				Postal code:					
Telephone no's:	H:	C	O	D	E								W:	C	O	D	E								
Cellphone no:																									
Workplace name & address:																									
																				Postal code:					
E-mail address:																									

Please circle your answers.

Language:	Eng		Afr		Other					Ethnic Group:		Asian				Black			Coloured			White			
Have you ever attended a blood donor clinic or donated blood before?												Yes			No			No. of previous donations:						
If you have donated blood before, where:																			When:	Y	Y	Y	Y		
Have you ever used a different name to donate blood?										Yes			No			If yes, state name used:									
Preferred place of donation:																									
I hereby agree to receive notifications and reminders from WPBTS via:												E-mail			SMS			Written material				Phone calls			

I understand that all calls received from WPBTS will be recorded for quality purposes.

I hereby declare that I would like to enrol as a blood donor.	Donor Signature: _____ Date: _____

OFFICE USE: Gift received: _____ Donor signature: _____	BAR CODE

FOR OFFICE USE ONLY STATS CODE: PANEL CODE: RESIGNATION CODE: COMMENTS:	DONOR LABEL

DEFERRAL REASON:											
RECEPTIONIST SIGNATURE:								DATE:			
MEDICAL COMMENTS:											
ATTACH MALARIA STICKER TO BLOOD PACK UNTIL (DATE):											
DONOR CODE:											

CLN 12 (01 Nov 13)

Appendix C: Ethical clearance certificate 1

Cape Peninsula
University of Technology

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

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
•Tel: +27 21 959 6352 • Fax +27 21 953 8490
Email: danielso@cput.ac.za

3 October 2013
CPUT/HW-REC 2013/H30

Faculty of Health and Wellness Sciences – Biomedical Sciences Department

Dear Ms Faieqa Adams

APPLICATION TO THE HW-REC FOR ETHICAL CLEARANCE

Approval was granted on 20 September 2013 by the Health and Wellness Sciences-REC to Faieqa Adams for your application. This approval is for research activities related to an MTech: Biomedical Technology at this Institution.

Title: An *in vitro* comparison of cellular destruction and metabolic effects occurring in stored, leuco-reduced and irradiated red blood cells.

INTERNAL SUPERVISOR: **Prof O Oguntibeju**

EXTERNAL CO-SUPERVISOR: **Dr GRM Bellairs & Dr A Bird**

Comment:
Approval will not extend beyond 3 October 2014. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

Note:
The investigator(s) should understand the conditions under which they are authorized to carry out this study and they should be compliant to these conditions. **It is required that the investigator(s) complete an annual progress report that should be submitted to the HW-REC in December of that particular year, for the HW-REC to be kept informed of the progress and of any problems you may encounter.**

Kind Regards

Zuleika Nortjé
CHAIRPERSON – ETHICS RESEARCH COMMITTEE
FACULTY OF HEALTH AND WELLNESS SCIENCES

Appendix D: Ethical clearance certificate 2

Cape Peninsula
University of Technology

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

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
•Tel: +27 21 959 6917 • Fax +27 21 953 8490
Email: lebenyat@cput.ac.za

06 October 2014
CPUT/HW-REC 2014/H10

Faculty of Health and Wellness Sciences – Biomedical Sciences Department

Dear Ms. Adams

YOUR APPLICATION TO THE HW-REC FOR EXTENSION

Approval was granted by the Health and Wellness Sciences-REC on 02 October 2014 to Ms. Faieqa Adams for ethical clearance. This approval is for research activities related to your MTech Biomedical Technology at CPUT.

TITLE: An *in vitro* comparison of cellular destruction and metabolic effects occurring in stored, leuco-reduced and irradiated red blood cells

SUPERVISOR: Prof. OO Oguntibeju
CO-SUPERVISER: Dr. DRM Bellairs and Dr. A Bird

Comment:
Approval will not extend beyond 07 October 2015. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the conditions under which they are authorized to carry out this study and they should be compliant to these conditions. **It is required that the investigator(s) complete an annual progress report that should be submitted to the HW-REC in December of that particular year, for the HW-REC to be kept informed of the progress and of any problems you may encounter.**

Kind Regards

MR. NAVINDHRA NAIDOO
CHAIRPERSON – ETHICS RESEARCH COMMITTEE
FACULTY OF HEALTH AND WELLNESS SCIENCES

Appendix E: Published journal article: Biochemical storage lesions occurring in nonirradiated and irradiated red blood cells: A brief review

Hindawi Publishing Corporation
BioMed Research International
Volume 2015, Article ID 968302, 8 pages
http://dx.doi.org/10.1155/2015/968302

Hindawi

Review Article

Biochemical Storage Lesions Occurring in Nonirradiated and Irradiated Red Blood Cells: A Brief Review

F. Adams,[1] G. Bellairs,[2] A. R. Bird,[2] and O. O. Oguntibeju[3]

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[3]*Oxidative Stress Research Centre, Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville, Cape Town 7535, South Africa*

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Received 19 November 2014; Revised 6 January 2015; Accepted 6 January 2015

Academic Editor: Khalid M. Khawar

Red blood cells undergo a series of biochemical fluctuations during 35–42-day storage period at 1°C to 6°C. The sodium/potassium pump is immobilised causing a decrease in intracellular potassium with an increase in cytoplasmic sodium levels, glucose levels decline, and acidosis occurs as a result of low pH levels. The frailty of stored erythrocytes triggers the formation of haemoglobin-containing microparticles and the release of cell-free haemoglobin which may add to transfusion difficulties. Lipid peroxidation, oxidative stress to band 3 structures, and other morphological and structural molecular changes also occur leading to spheroechinocytes and osmotic fragility. These changes that transpire in the red cells during the storage period are referred to as "storage lesions." It is well documented that gamma irradiation exacerbates storage lesions and the reports of increased potassium levels leading to adverse reactions observed in neonates and infants have been of particular concern. There are, however, remarkably few systematic studies comparing the *in vitro* storage lesions of irradiated and nonirradiated red cell concentrates and it has been suggested that the impact of storage lesions on leucocyte reduced red blood cell concentrate (RBCC) is incomplete. The review examines storage lesions in red blood cells and their adverse effects in reference to blood transfusion.

1. Introduction

Red blood cell transfusions are essential in the treatment of anaemia triggered by various pathologies or due to haemorrhage caused by trauma or surgery [1]. Globally, approximately 107 million units of whole blood were collected in 2013 of which 65% of blood transfusions in low-income countries were administered to children under 5 years of age while patients over 65 years comprised approximately 76% of transfusions in high-income countries [2]. While millions of whole blood and red blood cellular products are transfused annually, red blood cell concentrates (RBCCs) are still the most commonly transfused component.

There is a constant debate in the scientific community between individuals seeking to increase the time frame between donation and transfusion and those who are apprehensive regarding the efficacy and safety of stored blood. Although most clinicians agree that degeneration in blood and cellular components occur as soon as it is withdrawn from the donor's arm, patients requiring transfusions depend on the safety and efficacy of blood and blood components [3].

Since the First World War (1914–1918), technology has been available to store red cells under refrigerated conditions for short periods of time using sodium citrate as an anticoagulant. The advent of the Second World War (1939–1945) and the development of an anticoagulant containing an acid-citrate-dextrose (ACD) solution which significantly decreased the volume of anticoagulant required led to refrigerated blood being stored for 21 days and blood banking becoming a reality [4]. This allowed for increased volumes of blood to be transfused, a longer storage period, and a reduction in patients' experiencing citrate toxicity. Subsequently, further advances in the storage of red cell donations were made possible with the introduction of phosphates and adenine which allowed for a longer storage period of whole blood units. These advances encouraged the scientific

community to develop additive solutions which would not only extend the storage period but also preserve the quality of the red cell concentrate during storage.

The introduction of component therapy where red cells are separated from plasma by centrifugation and the development of preservative solutions containing saline, mannitol, glucose, and adenine (e.g., SAGM) which were added to the separated erythrocytes, increased the storage period of red cell concentrates to 42 days when stored at 1°C to 6°C [5]. The addition of saline and mannitol decreases the haemolysis rate, and glucose provides an energy pathway substrate while adenine maintains the ATP levels. The standard RBC additive solution used in Europe is SAGM. In South Africa, the western coastal region uses SAGM, whereas the inland areas use ADSOL (a solution consisting of adenine, dextrose, sodium chloride, and mannitol). The blood transfusion establishments in America use AS-1 and AS-5 as additive solutions while the third additive solution to be licensed is AS-3. Although the additive solution AS-3, is a saline-adenine-glucose solution, it also contains phosphate and citrate as it is a SAGM variant. It should be noted that neither of the additive solutions has a major advantage over the other as fragmentation or vesiculation of the red cells still occurs in both solutions, although it has been reported that the membrane protein profile of RBCC stored in AS-3 seems to be better than that stored in SAGM [6].

Most customary blood bank practices involve the collection of approximately 450–575 mL of whole blood into a collection bag containing citrate-phosphate-dextrose (CPD) solution as the anticoagulant. A whole blood unit is not a commonly transfused product as the clotting factors and thrombocytes depreciate within hours of donation and therefore the expiry date is 35 days compared to the 42-day expiry date of other red blood cellular products. Whole blood is mainly used for patients suffering with massive haemorrhage or when neonatal exchange transfusions are needed [7]. Adult massive haemorrhage is defined as transfusion of more than 10 RBCC units within 24 hours which is approximately the total blood volume or replacement of more than 50% total blood volume using blood products within a 3-hour period.

Regardless of aetiology (surgical, trauma, or obstetrical aetiology), a hospital emergency patient undergoing massive haemorrhage requires the administration of a large volume of blood products within a short time to maintain haemostasis and satisfactory circulation as they are often admitted to hospital with multifactorial early trauma-induced coagulopathy (ETIC) which is associated with mortality. The hyperfibrinolysis and systemic anticoagulation observed in ETIC may be due to the tissue injury from surgery or trauma which leads to the local or systemic release of tissue factorcausing the activation of the coagulation pathways and it is this activation that causes a disseminated intravascular coagulation-like syndrome [8]. The resulting anaemia due to massive haemorrhage causes a reduction in primary haemostasis leading to platelet adhesion impairment and aggregation. The current management protocol used for hemorrhaging trauma patients is equal parts packed red blood cell concentrate, plasma, and platelets (i.e., 1:1:1 transfusion ratio), but this mixture of components is not whole blood as it also contains ±180 mL of added preservative solutions such as mannitol, dextrose, sodium phosphate, adenine, sodium bicarbonate, sodium chloride, and citric acid. While fresh whole blood also contains a preservative solution, the amount is less and the advantage of using this product compared to component therapy is the decreased total volume being transfused as well as the conservation of platelet function, all in one single unit [9].

In the processing laboratory, the unit of whole blood is centrifuged using a closed sterile system which results in maximal plasma removal. The major components are separated so that the different products may benefit multiple recipients. The additive solution, SAGM, is contained in an attached satellite bag and is added to the packed red blood cells (a red blood cell concentrate unit). The removal of the buffy layer reduces the presence of leucocytes by 70–80% from the original collection pack. The volume of the RBCC is approximately 300 mL, including the anticoagulant. In South Africa, RBCCs are normally prepared by removing the buffy layer. Clinical indications for transfusion of RBCC include acute blood loss of more than 30% of blood volume, anaemia, obstetric haemorrhage, or patients undergoing surgery. According to clinical trials examining the transfusion of red blood cell products, an RBCC transfusion is recommended when the haemoglobin level of the adult patient is less than 7 g/dL with a maintenance haemoglobin of between 7 to 9 g/dL, but this restrictive transfusion trigger is not necessarily applicable to all cardiac patients [10].

A guide regarding the indications for blood transfusions has constantly been under review since the advent of component therapy where the "10/30" (haemoglobin level maintained at 10 g/dL or haematocrit levels ≥30%) is maybe one of the oldest indications. Shander et al. provide a summary of current transfusion guidelines according to the American Association of Blood Banks, Society of Critical Care Medicine, the College of American Pathologists, and American Society of Anaesthesiologists, including the Society of Cardiovascular Anaesthesiologists, Society of Thoracic Surgeons, and the Italian Society of Transfusion Medicine and Immunohaematology. The similarities, albeit some of them questionable, indicate that transfusion should rather be implemented to escape ischaemia and improve patient general outcomes by limiting the number of allogeneic donor exposure instead of focusing on the maintenance of patient haemoglobin concentration levels to improve transfusion practices [11]. However, it has been reported that although these guidelines promotes best transfusion practice, it does not eliminate transfusion complications such as infectious and immunologic problems or adverse transfusion reactions causing mortality, for example, haemolytic transfusion reactions, transfusion circulatory overload, and transfusion related lung injury. Also, early reoperation due to extreme blood loss is associated with acute kidney failure, thrombotic embolism, myocardial infarction, and increased mortality and therefore best transfusion practice on its own may not be the best clinical practice [12].

The plasma and buffy layer (rich in thrombocytes, leucocytes, and reticulocytes) are subsequently extracted from the RBCC and separated into different, yet attached satellite bags.

The buffy layer may be pooled and used to produce random donor platelet concentrates while the separated plasma may be used to produce fresh frozen plasma and cryoprecipitate or sent for fractionation to be processed into coagulation factor concentrates and plasma colloids such as albumin, stabilised human serum, or immunoglobulins [7].

It has been observed that ill infants requiring surgery or due to haemorrhage may have to be transfused with large quantities of stored red blood cell and transfusions of RBC containing increased levels of potassium have been associated with myocardial hyperkalaemia and neonatal arrhythmia [13, 14]. To reduce cost and wastage and to provide optimum benefit to neonates and infants, blood transfusion establishments produce the paediatric red blood cell concentrates (PRBCCs) and the infant red blood cell concentrates (IRBCCs). PRBCC is produced when a unit of red blood cells in additive solution is filtered through a leucocyte removal filter and equally divided between the SAGM bag and a transfer pack with a volume of 130 mL per unit while the IRBCCs are equally divided between 4 transfer bags after filtration with a volume of approximately 55 mL per unit. The additional transfer bag and leucocyte filter are sterile docked into the pack configuration. The IRBCC units are processed for neonates placed on the limited donor exposure program (LDEP) where multiple transfusions are expected in premature infants with low birth weights of less than 1500 g [15, 16].

The red blood cellular product may remain in the pack and used as RBCC or it may be further refined via leucocyte reduction using filtration methodology (a prestorage filtered RBCC unit). Many first world countries have adopted a universal leucocyte reduction (ULR) policy as filtered RBC products are believed to limit febrile nonhaemolytic transfusion reactions, prevent cytomegalovirus (CMV) transmission via transfusion, reduce postoperative infections, and reduce plasma haemolysis concentrations. In South Africa, however, selective use of these concentrates is recommended, as implementing the ULR policy would add significantly to the costs and there is still some controversy regarding some of the claimed benefits. South Africa has other main health concerns such as the escalating human immunodeficiency virus (HIV) pandemic and while the authors acknowledge the benefits of implementing a ULR policy, the cost of individual donation nucleic acid testing for HIV is immense, compared to the 4-, 8-, 16-, or 96-sample minipool NAT testing used in the United States. Also, leucocyte depletion may not inhibit the transmission of variant Creutzfeldt-Jakob disease to patients nor has the reactivation of viral infections (HIV and CMV) in standard nonleucocyte components been demonstrated [17]. Many of the filters used to deplete leucocytes from RBCC remove 3-4 $\log^{10}$ of white blood cells, but studies using rodents indicate that only 40–70% of the infectivity is removed and, as adequate infectivity remains, it may therefore become transmissible to a recipient [18]. A randomised clinical trial involving trauma patients demonstrated that there was no difference in infectious morbidity or mortality when transfused with prestorage leucocyte reduced RBCC compared to nonleucocyte reduced RBCC [19].

While there is extensive literature available regarding leucocyte reduced RBCC, there is less data available regarding the effects of gamma irradiation on prestorage leucocyte reduced red blood cellular concentrates. The American Food and Drug Association (FDA) recommends that a minimum of 75% recovery of transfused red blood cells must be present in the blood system 24 hours after transfusion [15]. Gamma irradiation exacerbates the storage lesion and the increased potassium levels over and above those seen in nonirradiated red blood cellular products due to the seepage of lactate dehydrogenase and potassium ions caused by irradiation exposure have been of particular concern. Gamma irradiation is indicated when a patient is at risk of developing transfusion-associated graft versus host disease (TA-GVHD) from being exposed to red blood cell components containing viable lymphocytes via transfusion [18]. TA-GVHD is a rare complication of transfusion and although it may be fatal, using gamma irradiation protects the vulnerable patients. The damaging effects of gamma irradiation on blood components are largely limited to red blood cells and do not significantly affect granulocyte and platelet function.

2. Biochemical Storage Lesions

It is well documented that certain biochemical changes occur during the 35 to 42 days of storing red blood cells at temperatures between 1°C and 6°C. The biochemical structure of the red blood cell (RBC) changes due to anaerobic glycolysis (cellular metabolism) and these changes are relative to the storage period.

2.1. pH. Ongoing glycolysis occurs when blood is stored in a plastic bag. Adenosine deaminase causes the breakdown of adenosine resulting in the formation of inosine and ammonia but is not regarded as clinically significant. An increase in protons causes the pH level to decrease and subsequently changes glycolytic metabolism. The decrease in pH causes the 2,3-diphosphoglycerate levels to decline with a simultaneous surge in adenotriphosphate (ATP) production. Glycolysis is slowed down and, as acid accumulates, the levels of ATP decrease and the shape of the red cell is gradually altered from discoid to echinocytic formations. This alteration in erythrocyte formation fades when stored blood is rejuvenated and is reversed when blood is warmed. The process of rejuvenation is when red blood cells are stored in a nutrient solution having a neutral pH [20]. The accumulation of lactic acid and proteins appear in the red cells after 14 days of storage due to glycolytic metabolism. It has been reported that a decrease in pH level and increases in lactate and potassium concentrations may occur within a few hours of storage while other changes may take weeks to appear [21].

2.2. 2,3 DPG. 2,3-Diphosphoglycerate (2,3 DPG) is the enzyme regulator of haemoglobin and aids in oxygen transportation to the tissues of the body. The decrease in pH levels leads to an increase in 2,3 DPG degradation. This causes an increase in the oxygen affinity of haemoglobin leading to the oxygen dissociation curve shifting to the left, resulting in a reduction in oxygen to the peripheral tissues. In cases of hypoxia, the oxygen dissociation curve shifts the delivery to

the right, thereby increasing oxygen transport to the tissues. After the 42-day storage period, a red blood cell unit may lose more than 90% of its 2,3 DPG concentration [5, 19, 22]. While 2,3 DPG levels may become undetectable within 2 weeks of storage, levels normalize within 72 hours after transfusion without any irreversible outcome observed and it is not considered clinically significant [21, 23].

Although multicountry guidelines recommend that RBC less than 5 days old be issued to patients undergoing massive transfusions, patients having RBC exchange procedures done, or patients in shock who are unable to increase their cardiac output, [7, 16, 17, 24] many retrospective trials demonstrate various conflicting outcomes including transfusion of older RBCC to be safer than fresh blood, no difference between transfusing fresh or older RBCC, or abnormal clinical conditions resulting from transfusing older blood. While it has been established that there is a definite difference between fresh and older blood, the clinical implication remains uncertain and therefore initiating prospective double-blind randomised clinical trials may resolve the ongoing debate [25]. A recent double-blind randomised clinical trial (the Age of Red Cells in Premature Infants {ARIPI}) compared the transfusion of fresh blood (mean storage of 5, 1 days) versus using older blood (mean storage of 14, 6 days) in premature neonates with birth weights less than 1250 g, to demonstrate the reduction of neonatal morbidities associated with organ failure or organ dysfunction as well as major nosocomial infections. The investigators concluded that there was no difference in clinical outcomes when transfusing premature, very low birth weight neonates with fresh blood compared to using older blood [26]. This study has, however, raised a few concerns regarding the implication that using older blood for transfusion does not affect necrotising enterocolitis, a common morbidity in premature infants. Also, a liberal transfusion practice was followed as haemoglobin levels were not stated prior to transfusion even though each infant received approximately 5 RBCC aliquots of about 14 mL per aliquot and thus the results of the trial may be challenging to establishments using a more conservative transfusion practice. Furthermore, the older blood used for neonatal transfusion had a mean storage time of 14, 6 days, whereas the average storage period of RBCC in American centres is about 18 days [27]. The aim of another randomised clinical trial was to determine whether the age of stored blood, used for transfusion, influences clinical outcomes in patients undergoing cardiac surgery. The results of the Red Cell Storage Duration Study (RECESS) demonstrated similar results to those of the ARIPI clinical trial. No differences regarding adverse transfusion reactions, changes in multiple-organ dysfunction scores, or mortality at day 28 were observed when using either fresh leucocyte reduced RBCC (stored for 10 days or less) or transfusing older RBCC (stored at 21 days or more) to cardiac patients requiring surgery [28].

2.3. ATP. The progressive loss of adenosine triphosphate (ATP) is well documented regarding morphological changes and RBC deformability during the storage period. ATP is not only an intracellular energy source but when ATP is released from the erythrocyte, it stimulates the production of nitric oxide leading to vasodilation during hypoxic conditions. The decrease of ATP concentration during storage causes the cellular reactions requiring energy, for example, phospholipid membrane distribution, active transport, and antioxidant reactions, to also decrease. It has been indicated that there is a 60% decrease in ATP levels after more than 5 weeks of storage [19]. The continuous reduction in ATP concentrations and acidification results in irreversible shape alteration of the RBC as echinocytic surface protrusions appear. The phospholipid bilayer loses its asymmetry and the shedding of microvesicles occur [20].

2.4. Potassium and Sodium Ions. Blood stored at 1° to 6°C decreases the rate of cellular metabolism and energy demand which allows blood to be stored for 35 to 42 days. This makes the sodium–potassium pump inoperative and consequently allows potassium ions to exit the cell and sodium ions to enter via the semipermeable membrane. It was demonstrated in critically ill patients that the sodium levels will revert to their normal levels within 24 hours after transfusion, whereas the potassium levels take about 4 days to stabilize [21, 22]. The extracellular potassium levels of stored blood increase daily at approximately 1 mEq/L. with the higher concentrations observed during the early days of storage [20]. Increased potassium levels in red blood cells may lead to arrhythmia when neonates or infants are transfused with large volumes of stored blood [14, 22].

2.5. Plasma Haemolysis. Due to a longer storage period, the red cell membrane experiences both biochemical and morphological alterations. These changes are referred to as storage lesions and such a biochemical indicator is plasma haemolysis or percentage haemolysis. Haemolysis of red blood cells (RBC) may occur during collection due to bacterial contamination, transportation, storage, donor red cell membrane deficiencies, presence of leucocytes in unfiltered RBC, mechanical injury during filtration process, or because of increased levels of vitamin C or penicillin in the donor [29].

The interaction of plasma haemoglobin with nitric oxide has been shown to cause endothelial dysfunction and is a risk factor for vasoconstriction, leucocyte adhesion, and intravascular thrombosis [30]. The release of hydrogen peroxidase and proteases by the leucocytes present in unfiltered blood may cause lysis of red blood cells during the storage period. Signs of haemolysis in the plasma or suspending fluid may suggest that the red blood cells have been either ruptured or it may be due to the loss of membrane-bound haemoglobin in microvesicles found on the cell's surface of intact cells. The addition of membrane stabilizers, for example, mannitol and citrate, may decrease haemolysis. It has been reported that, although the mean percentage haemolysis of RBCC stored in ADSOL (AS-1) was lower than its counterparts stored in SAGM, the difference was not statistically significant [31].

The easiest approach to assess the presence of haemolysis in a RBC unit before the unit is issued from the blood bank or prior to transfusion is by observation, but this visual inspection is often deceptive as it leads to an overestimation of haemolysis levels [32]. It has also been reported that

pink/red discoloration due to haemolysis observed in either plasma or suspending fluid may often be due to plasma haemolysis levels being as low as 25 g/dL (±0, 09% plasma haemolysis) and under normal conditions these units are discarded unnecessarily. It is therefore advisable to incorporate a measure of quality control to determine plasma haemolysis accuracy by using either photometric or spectrophotometric methods on random units or prior to discarding the RBC unit [33].

The clinical implication of RBC haemolysis for the transfused individual is very serious and may lead to redox injury of the tissues, endothelium, or the proximal tubules of the kidneys while procoagulant and proinflammatory surfaces appear due to the infusion of microvesicles which affects the microcirculation and consequently impacts systemic haemodynamics [34]. Previous studies have indicated that patients with cardiovascular or circulatory pathologies should carefully consider using rheologically compromised RBC due to the haemodynamic risk [1, 23, 25, 34].

As the presence of haemolysis is a cause for concern, the guidelines dictated by the Council of Europe stipulate that the mean haemolysis level should be less than 0, 8%. The FDA has amended their standard regarding the mean haemolysis concentration by adding the "95/95 rule." This rule states that, in addition to attaining the standard plasma haemolysis concentration of less than 1%, blood transfusion establishments must now demonstrate that 95% of their red blood cellular products meet the standard, statistically achieving 95% of the time [21, 35]. It is well documented that the concentration of haemolysis escalates during the storage period, but due to rigid quality control standards before, during, and after processing, together with trained personnel, the percentage haemolysis levels of most RBCCs do not exceed the prescribed limits [33, 34].

2.6. Leucocyte Reduced Red Blood Cells. Leucocytes found in red blood cellular allogeneic products are seldom of therapeutic benefit to the patient but are known to escalate the rate of cellular damage and to cause adverse transfusion reactions in recipients. These adverse reactions include alloimmunization to human leucocyte antigens (HLA), nonhaemolytic febrile transfusion reaction (NHFTR), transfusion-associated lung injury (TRALI), and immunomodulatory effects which include possible postoperation infection, postoperative mortality, or cancer recurrence [36, 37].

Leucocytes may also be regarded as the vector of infectious pathogens for instance Epstein Barr virus, cytomegalovirus and human T-lymphotropic virus I/II. It has been established that B-lymphocytes are vectors for the prions causing variant Creutzfeldt-Jakob disease [37]. It has been reported that using leucocyte reduced RBC reduces the incidence of multiorgan failure in patients having vascular or oncological surgery and decreases hospital stay by 2, 4 days as well as mortality in patients having gastrointestinal oncological surgery. The average reduction of 2, 4 days per patient would significantly cut costs of a national hospital [38]. British haemovigilance evidence demonstrates that using filtered RBC components reduce the frequency of transfusion-associated graft versus host disease.

It should be noted, however, that only using leucoreduced RBC to prevent TA-GvHD is not recommended as the RBC used for transfusion should be filtered and irradiated to prevent this serious and often fatal disease [39].

The filters used for leucocyte depletion are readily available and filtration of RBCC may be prepared at the patient's bedside during transfusion, before storage (in-line filtration) or after the buffy-coat layer and plasma have been removed (prestorage or 24-hour expiry product). Leucocyte depletion by filtration is best performed in the processing laboratory of the transfusion services as this maintains better quality assurance. It is advisable to filter the blood soon after collection and/or processing as granulocytes fragment and degranulate during storage, which may cause a NHFTR or the antigen-presenting cells presenting major histocompatibility complex (MHC) classes I and II antigens, leading to alloimmunization. It has been reported that leucocyte antibodies associated with TRALI are possibly targeted at HLA antigens (class II) and neutrophil alloantigens. In antibody-mediated TRALI, the antibody causing TRALI in a patient is usually recognised in multiparous female donors, but these donors cannot be excluded as this would reduce the donor-pool substantially [40]. The FDA recommends that a filtered unit of blood contains less than 5×10^6 of white blood cells (WBC) and a retention of approximately 85% of the original RBC. Patients are stimulated to produce antibodies against the transfused histocompatibility antigens when the WBC exceed the 5-log count and thus to prevent primary alloimmunization, the FDA has stipulated this rule. They also suggest that quality control testing be done on 1% of filtered units, of which 100% should not have more than 5×10^6 WBC.

Bedside filtration should be the last option to use as adequate quality control procedures cannot be completed on bedside leucocyte reduction filters. Bedside filtration requires a slow flow rate which reduces the filter performance. The filters that are currently used in most blood transfusion establishments provide a 3-log leucocyte depletion [41].

An increase in lactate dehydrogenase (LDH) concentration, glucose depletion, and haemolysis with a decrease in pH has been documented when buffy-coat-poor RBCC stored in SAGM was compared with its leucocyte depleted RBCC counterpart. This result shows that the presence of leucocytes in an RBCC unit was the source of the higher rate of haemolysis [37]. The reduction of leucocytes via filtration in red blood cellular products has not only minimized transfusion complications in patients exposed to allogeneic blood but has also decreased the occurrence of bacterial contamination, for example, *Yersinia enterocolitica* in red blood cellular components [23, 42], and a decrease in postoperational infections has been observed [35, 41].

As a result of the benefits, but despite the additional expense, leucocyte reduced RBCC has become the standard component for transfusion in many countries except in developing countries where it is not cost-effective. Germany had introduced filtered RBC as a primary component since 2001, whereas, in South Africa, selective use of these concentrates is recommended [17].

2.7. Irradiated Red Blood Cells. Despite reduced leucocyte concentration in the RBC units, a small amount of leucocytes remain in the pack and this usually does not pose a problem for patients with a healthy immune system unless they are receiving designated/directed donations. Red blood cellular products are irradiated to decrease the risk of transfusion-associated graft versus host disease (TA-GvHD) [43, 44].

TA-GvHD is a rare but fatal adverse transfusion reaction resulting from clonal proliferation and engraftment of viable donor T-lymphocytes and may occur in immunocompromised patients or in patients transfused with blood from donors who are homozygous for shared human leucocyte antigen (HLA) haplotypes. Gamma irradiation targets lymphocytic nucleic acids but also damages nonlymphoid cells in the process. There is a noticeable change in the properties of the RBC when they are irradiated. These alterations include lipid peroxidation due to reactive oxygen species (ROS), integrity of cell membrane is affected, seepage of potassium ions is accelerated, intracellular nucleotides are altered, and cell elasticity and deformability are decreased [39]. The literature reports increased plasma potassium, lactate dehydrogenase, and haemoglobin concentrations in irradiated red blood cellular products [43, 44]. Due to the storage lesions occurring during irradiation, *in vitro* haemolysis increases and, consequently, when irradiated RBC is transfused, the *in vivo* recovery is decreased, but this is not regarded as being clinically significant unless patients present with renal failure or the onset of hyperkalaemia. It has been reported that children are more susceptible to cardiovascular pathology due to hyperkalaemia than adults [45]. Neonatal hyperkalaemia may be prevented by using a cell saver to wash the irradiated RBC product for neonates undergoing cardiopulmonary bypass surgery [43]. It is also recommended that neonates at risk of hyperkalaemia requiring intrauterine or exchange transfusions be transfused within 24 hours after irradiation of RBC. Gamma irradiation of products is recommended for intrauterine transfusion, exchange transfusions, transfusions of first-degree relatives, premature neonates weighing less than 1200 g, patients with congenital immunodeficiency pathologies such as DiGeorge syndrome, Wiskott-Aldrich syndrome, all recipients of allogeneic bone marrow transplants, those undergoing stem cell harvesting for later autologous reinfusion, and patients who have received aggressive chemotherapy. Currently, gamma irradiation is the endorsed method for the prevention of TA-GvHD [46].

The American FDA and the Canadian guidelines have stipulated that the maximum storage period for irradiated RBC is 28 days; the Chinese State Food and Drug Administration (SFDA) has a ruling of 35 days [47], while the Council of Europerecommends that irradiation of nonleucocyte reduced RBC should not be completed for more than 14 days after donation and should not be stored for longer than 14 days after irradiation. Regarding irradiation dosage, the Council of Europe stipulates 25 Gy to 40 Gy on any given place on the bag, whereas the American FDA recommends not less than 15 Gy at any given place of the bag while 25 Gy must be delivered in the middle of the bag [39]. South Africa abides by the guidelines stipulated by the Council of Europe regarding expiry of irradiated RBC, but the irradiation dosage is between 25 and 50 Gy where the centre of the container is targeted [47].

The available data on the effects of irradiated red blood cellular products is limited as few systematic studies have been done, quality control testing is not performed on these units, and there is a variety of available guidelines regarding irradiation. Therefore, irradiated RBC storage lesions cannot be predicted.

3. Discussion

While blood transfusion establishments have certain guidelines specifying acceptable parameters regarding metabolic changes occurring in red blood cellular products during storage, the literature regarding its gamma irradiated counterparts is less extensive.

Many differences in the studies that evaluated the biochemical storage lesions and clinical impact of storage lesions in critically ill adult or paediatric patients were detected, for example, heterogeneous distribution (using fresh and old blood), preparation of blood products, study design, sample population, discrepancy in the differentiation of fresh and old blood, variety in irradiation guidelines, and different storage media used or preserving solutions not documented, small sample size and mostly limited to retrospective observations. It should also be noted that while it is common practice to use leucoreduced red blood cellular products in many first world countries, it is not a global practice.

4. Conclusion

The clinical impact that storage lesions have on RBC survival in transfused patients is debatable and therefore the evidence to substantiate the need for a shorter storage period is unsatisfactory. There are relatively few systematic studies comparing the *in vitro* metabolic changes occurring in irradiated red blood cellular concentrates and there is a need for randomised controlled trials to study the effect that storage lesions have on mortality and morbidity. Once these are underway, then perhaps this much debated question will be answered.

5. Recommendations

While the results of the RECESS and ARIPI clinical trials indicate that there are no differences regarding morbidity or mortality of transfusing patients using fresh blood compared to older blood, the occurrence of red blood cellular storage lesions may be influenced by the preparation of cellular components separated from the whole blood donation. Counties and transfusion establishments in different areas do not use the same anticoagulant solution such as SAGM, AS-3, CPD, or CPDA-1 and this may also influence the occurrence of storage lesions. Thus, the results attained from these trials should be carefully considered prior to implementing it as transfusion policy. Another randomised controlled trial of standard transfusion versus fresher red blood cell use in

intensive care (TRANSFUSE) is currently ongoing and will determine whether using the freshest blood available for transfusion instead of using the standard older blood will reduce mortality in critically ill patients in intensive care units.

Considering the above reservations and inconsistencies observed regarding storage lesions occurring in nonirradiated and irradiated red blood cell components, it is recommended that more systematic research studies be initiated. Although prospective, randomised controlled trials may prove challenging due to storage lesion intricacy, diversity of patient pathophysiologies, and donor to donor variation, these studies should follow a similar pattern when investigating the *in vivo* effect of storage lesions in patient mortality and morbidity.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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