QUALITY OF PACKED RED CELLS AT REGIONAL BLOOD TRANSFUSION CENTER NAIROBI KENYA

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Quality of Packed Red Blood Cells at Regional Blood Transfusion Center Nairobi

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University

Signature…… Date……

Mary Wakarima Solomon

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

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UoN, Kenya

DEDICATION

I dedicate this thesis to my family my daughters, my sister and my brother for their moral, financial supports and encouragement during the course of this study.

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ABBREVIATIONS AND ACRONYMS

AABB	Association of American Blood Bank
ACD	Acid Citrate Dextrose
AKUH	Aga Khan University Hospital
A-P	Anticoagulant preservative
ATP	Adenosine Triphosphate
BC	Buffy coat
BTS	Blood Transfusion service
CBC	Complete blood count
CDC	Center of Disease Control
CMV	Cytomegalovirus
COHES	College of Health Sciences
CPDA-1	Citrate phosphate dextrose Adenine-1
CPDA-2-	Citrate phosphate dextrose Adenine 2
CPD	Citrate phosphate Dextrose
DCA	Drugs and Cosmetic Acts
DPG	2, 3-Diphosphoglycerate
EDTA	Ethylene Diamine Tetra Acetic Acid

FDA	Food and Drug Association
FFP	Fresh Frozen Plasma
g/dL	grams per decilitre
GVHD	Graft versus Host Disease
HCT	Haematocrit
HLA	Human Leucocyte Antigen
ISBT	International Standard for Blood Transfusion
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KNBTS	Kenya National Blood Transfusion Services
LR-RBC	Leuco-reduced red blood cell
NK	Natural Killer cells
NRAs	National Regulatory Authority
NRBTC	Nairobi Regional Blood Transfusion Center
NPHLS	National Public health Laboratories
O_2	Oxygen
PRBC	Packed Red Blood Cells
SAGM	Saline Adenine Glucose Mannitol
SCD	Sterile Connecting Device

WBC White blood cells

WHO World Health Organization

WB Whole blood

OPERATIONAL DEFINITIONS

Apheresis: A procedure whereby whole blood is separated by physical means into components and one or more of them returned to the donor

Blood collection: A procedure whereby a single donation of blood is collected in an anticoagulant solution.

Blood component: Any therapeutic constituent of blood that is separated by physical or mechanical means (e.g. red cells, platelets, plasma). It is not intended to capture plasma derived products.

Closed System: A system for collecting and processing blood in containers that have been connected together by the manufacturer before sterilization, so that there is no possibility of bacterial or viral contamination from outside after collection of blood from the donor.

Components: Parts of the whole blood that are separated by physical means

Haematocrit: The volume percentage (vol %) of red blood cells in blood.

Haemolysis: Breakdown or destruction of red blood cells, oxygen carrying pigment haemoglobin is freed to the surrounding.

Leukoreduction: Removal of white blood cells on blood

ABSTRACT

Blood is composed of red and white blood cells, platelets and the fluid plasma. Whole blood can be separated into different components for the purpose of transfusion namely: packed red blood cells (PRBC), platelet concentrate, white cells preparations, cryoprecipitate and fresh frozen plasma (FFP). Component use enables maximum utilization of donated blood and reduction of adverse events that may range from mild allergic manifestation to fatal reactions. Preparation of the packed red cells component is the most well-known function of blood transfusion services in Kenya. The aim of the study was to assess the quality of packed red blood cells (PRBC) using the following parameters: volume, haematocrit, white blood cell count, red blood cell count and platelets. This was a descriptive cross-sectional study conducted in Nairobi Regional Blood Transfusion Center (NRBTC). Eighty PRBCs prepared as per the standard operating procedure used at NRBTC, were sampled. For each sample, the volume of the packed red blood cells was first determined. A 5ml aliquot of each PRBC sample was collected in Ethylene Diamine Tetra Acetic Acid (EDTA) tubes. The samples were then analyzed using a Sysmex XT 2000I analyzer. For hematocrit, total white blood cell count, platelet count and red blood cell count were determined. The data was collected and entered in a MS Excel sheet which was secured with a confidential password. The hard copies of the print outs from the machine were kept under lock and key, only accessible to the principal investigator. The results were analyzed in relation to acceptable reference ranges. The total of PRBCs within the acceptable ranges were 79 (99%) for volume, 43 (54%) for residual WBC count, 69 (86%) for RBC count, 61 (76%) for platelet count and 64 (80%) for haematocrit. Overall, out of the 80 PRBCs units prepared, with exclusion of platelets and WBC, only 54 (68%) met the quality requirements assessed. This was in agreement with other studies done where WBC count and platelets were not factored in. This study showed that following the standard operation procedures set by NRBTC, most of the PRBCs prepared met the quality assessment. However, the high residual WBC count requires to be addressed. In general, PRBCs should have residual WBC count of less than 5 $x10^9$/L. High residual WBC count is caused by the technical challenge of removal of the buffy coat during preparation of PRBCs following centrifugation and separation of blood components. This may be remedied by provision of additional continuous training to maintain quality standards. In addition, inclusion of leukodepletion filters would further enhance the PRBC quality by maintaining a low residual WBC count.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Transfusion therapy depends on the availability of the various blood components. When available, these components, used separately or in a variety of combinations, can adequately meet most patient transfusion needs while keeping the risks of transfusion reaction to a minimum. Component transfusion therapy has the added benefit of using a limited natural resource more effectively to provide the needed therapeutic component to several patients from a single donation. In addition, this minimizes the burden on blood banks through use of appropriate components based on patient needs, reduced adverse transfusion related events and overall decline in wastage of donated blood compared to when whole blood is used for all transfusion procedures (Markus *et. al..,* 2015).

Packed red blood cells are prepared by removing approximately 80% of plasma from a unit of whole blood resulting in a product that is concentrated. Regulation requires that the final hematocrit of a packed red blood cell unit should not exceed 80%. Average hematocrit ranges between 65% and 80%. Red blood cells contain the same RBC mass with the whole blood and therefore the same O_2 –carrying capacity as whole blood, but with approximately half the volume. They can be used in any situation that requires increased O_2-carrying capacity. Red blood cells are particularly useful in a patient who requires the increased RBC mass but may be in a risk of circulatory overload (examples, patients with chronic severe anaemia with a compensated (normal) blood volume or those with anaemia together with cardiac failure). Other advantages of using RBCs rather than whole blood are: significant reduction in the level of plasma thus facilitating transfusion of group O cells to non O recipient, reduction in levels of acid, citrate and potassium and reduction of events associated with WBC, platelet and plasma volume (Roback *et. al..,* 2008).

Packed red blood cells are the component most commonly prepared and used in Kenyan health providing facilities. Each unit of whole blood has a volume of about 450mls and is mixed with 63mls of anticoagulant. In this single unit, red blood cells make up approximately 200ml. It is expected that one unit of packed red blood cells will usually raise hematocrit by almost 4%. The residual WBC and platelets are expected to be less than $1x10^{6}$ and $1x10^{6}$ respectively. When PRC preparation has or is suspected to have higher levels measures designed to reduce WBC and platelets are to be adopted so as to minimize unwanted events of transfusion. Such include severe leukoagglutinin reaction and platelets associated events. It may therefore be necessary to undertake procedure of reducing white blood cells

The transfusion events associated with white blood cells are usually enhanced in those who have been sensitized through previous transfusion or pregnancy. Most commonly develop fever and chills within 12 hours after transfusion. The hematocrit rises may also not attain the expected values. In reference to Nairobi Regional Blood Transfusion Center, blood component preparations is done using the AABB standard guidelines.

To determine the quality of prepared components, there are some parameters which have to be determined. For quality analysis of PRBCs, the determination of haematocrit, WBC, Hemoglobin and platelets is the recommended by AABB. These parameters analysis in this research were done using to XT 2000I Haematology Analyzer.

1.2 Statement of the problem

Quality of packed red blood cells for transfusion need to be done periodically to monitor their safety and efficacy. There has never been a study done on the quality of packed red cell prepared at Nairobi Regional Blood Transfusion Center. To determine the quality, the parameters used were Volume, Haematocrit, residual WBC count, RBC count and platelets count. The aim of this study was to find out whether the packed red blood cells prepared in the regional blood transfusion center met the used criteria. As the clinical manifestations of anemia are nonspecific, the indication for transfusion is based on

surrogate parameters, such as the hemoglobin concentration, in addition to clinical criteria. For patients with unimpaired cardiopulmonary and vascular function, transfusion is generally indicated at hemoglobin values of 6 g/dL (3.7 mmol/L) or less. Randomized controlled trials have shown that a restrictive transfusion strategy (trigger: haemoglobin 7–8 g/dL) in certain patient groups is as effective as a more liberal strategy (trigger haemoglobin about 10 g/dL).

1.3 Justification

Quality of the blood and blood component need to be assessed at regular intervals to check efficacy and safety of the transfusion operations and are part of good transfusion practice.

Every blood Transfusion center should have audits of quality outcomes as standard practice. In a large regional Transfusion centers like the Nairobi Regional Transfusion center and periodic data would be aptly relevant.

No study has been done to determine quality of packed red blood cells prepared at Nairobi regional blood transfusion center.

1.4 Research questions

1. Do the packed Red cells at Nairobi Regional Blood Transfusion Center met the quality?
2. Are the results obtained comparable to reference ranges?
3. Do set standard operation procedure require review?

1.5 Main Objective

Quality of packed red blood cells (PBRCs) at Regional Blood Transfusion Center Nairobi.

1.6 Specific objectives

1. To determine, volume and Complete Blood Count of prepared packed cells at Nairobi regional blood transfusion center.
2. To compare the results obtained with the reference range and find out their deviation from the expected CBC Standard Values.
3. To assess Regional blood transfusion center compliance to standard operation procedure.

1.7 Conceptual Frame work

The current study focused on assessing the quality of packed red blood cells by investigating five parameters: volume, residual WBC count, red blood cell count, platelet count and hematocrit (Fig 1.7).

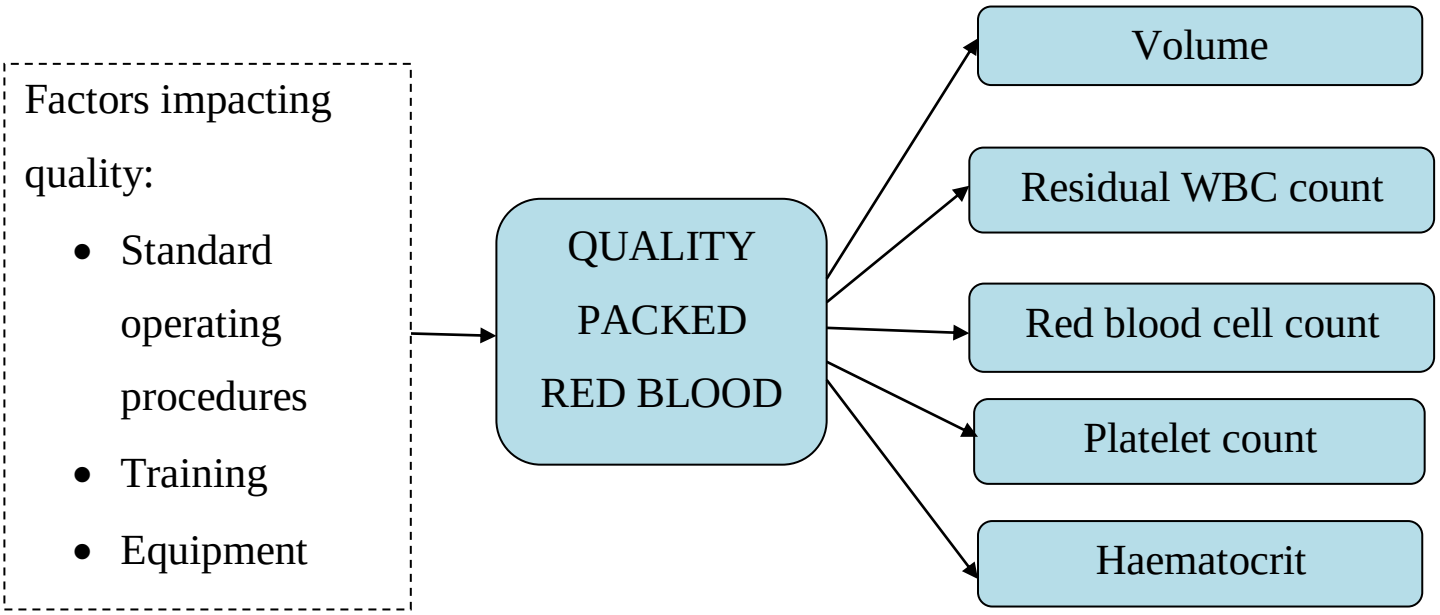

Figure 1.1: Assessment of quality of packed red blood cells.

The conceptual framework indicates the key aspects of packed red blood cell (PRBCs) assessed and the potential factors that may impact quality.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Blood transfusion services are required to provide blood and blood components which are safe and cost effective for transfusion into patients who require the blood products. In the past, whole blood was the only preparation that could be administered to replace red cells, platelets, and coagulation factors. This caused unnecessary administration of unwanted components. Significant advances in transfusion medicine however has made it possible to separate blood in a closed system. As such one recipient can benefit from transfusion of blood components (PRBCs, platelets and plasma components) from more than one donor after efficient component. (American Association of Blood Bank, 2005)

In 1818 James Blundell, a British obstetrician performed the first successful transfusion of human blood for the treatment of postpartum hemorrhage. This led him to be referred to as the father of transfusion. He first devised an instrument for performing transfusions. A few years later, in 1901 the Australian Karl Landsteiner documented the first three human blood groups A, B and O. One year later in 1902 the fourth blood type AB was found by A Decastello and A Sturli (Giangrande, 2000).

In 1914 long term anticoagulants among them, sodium citrate, were developed allowing longer preservation of blood. The British Red Cross then instituted the first human blood transfusion service in the world in 1926. Later, in 1950 Carl Walter and W.P Murphy introduced the plastic bags for whole blood. This was followed in 1979 with the introduction of CPDA-1 which extended the shelf life of PRBCs and whole blood to 35days (Giangrande, 2000).

2.2 Blood collection for transfusion

More than 75 million units of blood are collected annually worldwide (Red Cross Red Crescent – Blood Services). Of these, voluntary unpaid donations cover 92% in developed countries and 67% in developing countries (Blood transfusion, http://www.who.int/topics/blood_transfusion/en).

In the U.S., 15.3 million whole blood units were collected in 2001 (Sullivan *et. al.*., 2007). Donor recruitment and blood collection are the most critical parts of blood establishments. Blood donors have become less available compared to the growing demand for blood products. New recruitment techniques have been adapted to induce new donors, and donation criteria may need to be modified, for example, by extending the age limits, to retain regular donors (van der Pol & Cairns, 1998; Smith *et. al.*., 2002).

Multicomponent aphaeresis for deriving combinations of RBC, PLT, and plasma units (Waxman, 2002) and collection of double RBC units are increasingly used to alleviate blood shortages, especially in the U.S. (AuBuchon *et. al.*., 2007).

Recruiting donors and maintaining the donor base challenge blood establishments because these activities becomes costlier and require greater effort in the future. Blood establishments need to explore strategies that allow the collections to be performed at a lower cost and, at the same time, assure a sufficient supply of blood (van der Pol *et. al.*., 2000).

2.3 Preparation of blood components from whole blood

Until the late 1970s, most blood was transfused without being further processed to separate components. This was termed as ‘whole blood’. Current practice in most countries is to process most or all whole blood donations to components these are packed red blood cells, plasma, platelets concentrate and cryoprecipitate. In a typical blood establishment process, 450 -500mlof the donors’ blood is drawn into plastic pack containing 63 ml of an anticoagulant preservative solution such as citrate phosphate

dextrose (CPD) or CPD-Adenine. Citrate binds calcium and acts as anticoagulant and glucose and Adenine supports RBC metabolism during storage. (Schramm *et. al..*, 2009). The first study on the separation of whole blood to PRBCs and plasma using a hollow-fibre system was performed by Sekiguchi *et. al.*, (1990). By changing the filter systems, integrating leukocyte filters and using a storage medium for extended storage, different authors were able to show that it is possible to process PRBCs and plasma using gravity separation with comparable quality and stability data to those processed by classic centrifugation/apheresis methods. Nevertheless, the problem with these first attempts was the low concentration ability of the filter system with a resulting low HCT in the PRBCs and a reduced plasma recovery.

Various blood components have different relative density, sedimentation rate and size. They can be separated when centrifugal force is applied. In increasing order, the specific gravity of blood components is plasma, platelets, leucocytes (Buffy Coat [BC]) and packed red blood cells (PRBCs). Functional efficiency of each component is dependent on appropriate processing and proper storage. To utilize one blood unit appropriately and rationally, component therapy is to be adapted universally. Whole blood, packed red blood cells, fresh frozen plasma, platelets and cryoprecipitate (Hardwick *et. al..*, 2008).

Blood component preparation was developed in 1960 to separate blood products from one-unit whole blood, specialized equipment called refrigerated centrifuge was used. Preparation of only PRBC and fresh frozen plasma (FFP) was by single-step heavy spin centrifugation (Moog, 2006).

2.4 Blood Components preparation

The Whole blood is collected as 450 ml or 550 ml in double/triple/quadruple or penta bags with CPDA-1 or additive solution. After blood collection, components should be separated within 5 - 8 hours. Component room should be a separate sanitized room. All precautions to avoid red cell contamination have to be taken such as tapping the segment ends, proper balancing of opposite bags, following standard programs and protocols

described in the manual of refrigerated centrifuge manufacturer. The programme is run with mainly two spins-heavy spin (5000 G for 10-15 min) and light spin (1500 G for 5-7 min). The heavy and light spin centrifugation varies with manufacturer and model. Here 'G' is relative centrifugal force calculated using revolutions per minute and rotor length. Use of totally automated component separator instrument will allow for the preparation of low volume BCs with a recovery of 90% of whole blood platelets. (Vox Sang, 2014.)

A common goal of component preparation methods is to produce RBCs, PLTs, and FFP that contain maximum amounts of therapeutic blood elements and minimum amounts of unnecessary residual cells. The separation of blood into its components can be accelerated by centrifugation, which is based on different physicochemical properties, such as density of blood cells. Changing centrifugation parameters, such as time, temperature, and rotation speed can affect the composition of separated fractions. With the development of the processing technique, blood component preparation has become a routine procedure worldwide (Capraro *et. al..*, 2007).

Removal of white blood cells (WBCs) from blood components (leukocyte reduction) has been established to obtain less-contaminated blood concentrates to prevent febrile reactions following blood transfusions and to reduce alloimmunization. RBCs and PLTs can be filtered after the separation to obtain leukocyte-reduced components. (Uhlmann *et. al..*, 2001; Pruss *et. al..*, 2004).

The preparation process of packed red blood cells involves several steps to attain the best quality components. Figure 2.1 shows how the packed red blood cells and fresh frozen plasma are separated from whole blood.

Figure 2.1: Preparation of packed red blood cells and fresh frozen plasma.

Closed system of blood collection (A). The components of blood are spun down and separated into satellite bags (B). The components of blood (PRBCs, plasma) have been separated and transferred into satellite bags. Adopted from R Srihari (2015)

2.4.1 Buffy coat (BC) method

Concern about the contamination of RBCs, the quality of PLTs, and the need for plasma derivatives contributed to the switch from the PRP method to the BC method in Europe (Prins *et. al.*., 1980; Murphy, 2005). Technology for the removal of BC from a WB unit by manual techniques was initiated by the Finnish Red Cross Blood Service to use leukocytes in human interferon production. In the BC method, intense primary centrifugation of the WB unit allows separation of RBCs, the BC layer, and plasma. Further centrifugation of BC in PLT additive solution or plasma makes it possible to separate PLTs from WBCs and residual RBCs. If the aim is to prioritize obtaining pooled BCs, WB can be collected in a bag suitable for this purpose. Although PLTs can be obtained in sufficient amounts with current preparation methods, the short survival of PLTs is a challenge to their timely availability. Today, processing of BCs to clinical PLT products is performed by semi-automated, closed, and sterile systems accompanied by several technological improvements, such as sterile connecting devices, the bottom-and-top bag system, separation devices, and new synthetic storage media (Gulliksson *et. al.*., 2002; Larsson *et. al.*., 2005).

2.4.2 Centrifugation of blood components

The sedimentation of blood cells depends on their size as well as the difference of their density from that of the surrounding fluids. Other factors are viscosity of the medium and flexibility of the cells which is temperature-dependent.

In the first phase of centrifugation, the surrounding fluid is a mixture of plasma and anticoagulant solution. Leucocytes and red cells centrifuge out more rapidly than platelet as they both have bigger volume than platelets. Depending on the time and speed of centrifugation, most of the leucocytes and red cells settle on the lower half of the bag and the upper half contains platelet rich plasma (Table 2.1). There are two type of centrifugation options: high speed centrifugation and hard spin**.** The different components preparation requirement are summarized in Table 2.1...

Table 2.1: Temperatures, centrifugation speed and time for components preparations.

Blood component	Temperature (° C)	Speed (RPM)	Time (minutes)
Red blood cells (PRC)	**4**	**4200**	**9**
Platelets (1st spin)	**22**	**2100**	**6**
Platelets (2nd spin)	**22**	**3800**	**6**
FFP	**4**	**4200**	**9**
CRYO (1st spin)	**4**	**4200**	**5**
CRYO (2nd spin)	**4**	**4800**	**10**

Packed red blood cells are prepared by removing approximately 80% of plasma from a unit of whole blood. Regulation requires that the final haematocrit of a red blood cell unit not exceed 80% (Koch *et. al.* 2008). High speed centrifugation causes much trauma to cells and also the breakage of plastic containers.

For the preparation process of packed red blood cells after donation, the blood is weighed and two units of blood balanced to ensure equal weight before being centrifuged and expressed to remove the plasma (Figure 2.2).

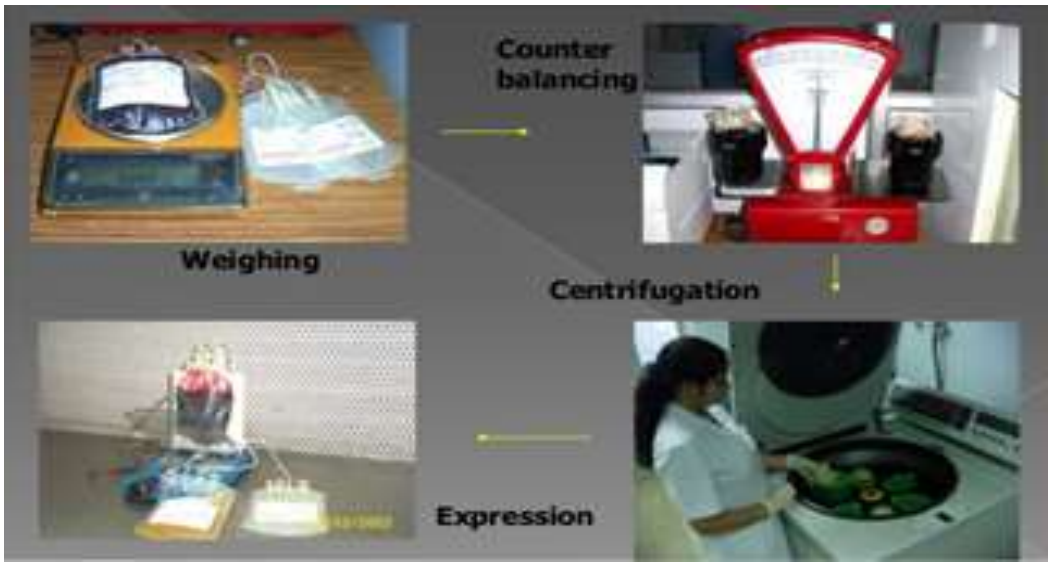

Figure 2.2: Packed red blood cells preparation procedure.

One PRBC unit is produced from one WB unit. The collection bag must contain the appropriate anticoagulant solution. Depending on the indication for use, PRBCs are modified to be suitable for clinical use by buffy coat removal and leukoreduction, washing, irradiation, or all of these procedures. A standard adult PRBCs unit should contain HB at minimum 43 g/unit (guide, 2007). double (2) units of PRBCs can be collected and processed at a time which allow transfusion using units of higher HB content (Snyder *et. al..*, 2003; Arsian *et. al..*, 2004).PRBCs can be stored before transfusion for 35-42 day at 2-8^{0}c depending on the storage solution.

Packed Red Blood Cells consist of erythrocytes concentrated from whole blood donations by centrifugation or collected by aphaeresis method. The component is anticoagulated with citrate and may have had one or more preservative solutions added (CDC, 2015). Depending on the preservative-anticoagulant system used, the haematocrit of Red Blood Cells ranges from about 50-65% (e.g., AS-1, AS-3, AS-5) to about 65-80% (e.g., Citrate Phosphate Dextrose Adenine-1(CPDA-1), Citrate Phosphate Dextrose (CPD). Red Blood cells contain an average of about 50 mL of donor plasma (range 20 mL to 150 mL), in addition to the added preservative and anticoagulant solutions.

Since 1916 red cells were recognized to be stored for brief periods of time in a citrate-glucose solution, it was not until the 1930s that blood was transfused after storage, in 1937 the first blood "bank" was established at Cook County Hospital in Chicago. Early 1940s, a major breakthrough came with the development of the first effective anticoagulant-preservative solution (A-P) ACD (acid citrate dextrose), which allowed blood to be stored for up to 21 days (Synder *et. al..,* 2003).

In the UK, there is a requirement that the red cell unit be discarded if it has been outside of controlled storage conditions for longer than 30 minutes. This requirement is based on historical data from the 1970s related to red cell quality, and on concerns about bacterial proliferation in the very uncommon circumstance where the stored red cell unit contains viable organisms (Dzik *et. al..,* 2013) However, studies have shown that red cell units can be maintained outside controlled temperature conditions for up to 60 minutes without either of these adverse effects. For those hospital transfusion services that are accredited by AABB, an AABB Standard requires that each hospital establish and validate procedures to ensure that units eligible for reissue have been maintained under conditions that ensure an appropriate temperature while outside of the hospital blood bank (i.e., between 1 to 6°C or 1 to 10°C depending on local hospital policy) (Hess *et. al..,* 2009)

Packed red blood cells (PRBCs) are indicated for normovolemic anemia, such as neonatal isoerythrolysis, erythropoietic failure, and chronic blood loss. In cases of chronic or hemolytic anemia, markers of tissue oxygenation, such as lactate and oxygen extraction are still useful. Packed Cell Volume is a better “transfusion trigger” for chronic anemia compared to acute hemorrhage, with transfusions suggested recipient with evidence of tissue hypoxia and a PCV less than 21%.

At RBTC, whole blood collections are routinely separated into components (including PRBC fresh frozen plasma, platelets, and cryoprecipitate); this provides the ability to support the transfusion needs of multiple patients from a single donation and has led to the virtual disappearance of the availability of whole blood from blood banks.

2.5 Quality of blood components

Quality concepts depend on quality control, quality assurance, quality management and their maintenance. Quality control involves initiatives to ensure quality of blood component for its varied usage. Improved quality testing over the periods of the study results in safer transfusion practices and decreased adverse outcomes.

A study was done in India of quality analysis of blood component (Upadhyay *et. al.* 2016). In preparation of blood and blood components, the following parameters were assessed whole blood of CPD, haematocrit, platelet count WBC count and Red blood cell count. (PRBC) and whole blood unit quality was assessed. Whole blood quality in the data was found to be satisfactory for range of haematocrit level of 43.7% to 63 were noted. Cell counts (WBC, platelet and RBC) were also noted to be within the acceptable range, by Upadhyay *et. al.* (2016). These study shows that the quality control for PRBC-has no established guidelines as per the Drug and Cosmetics Act available.

PRBC-QC in that study was considered satisfactory because. The haemoglobin level of 92.9% and haematocrit of 85.1% units met European council criteria. A mean haematocrit of 54% and a mean haemoglobin of 13.5 g/dl were also noted in the same study. More than 50% blood donors had Haemoglobin >15 gm/dL. WBC depletion in 48% units satisfied EC criteria (Upadhyay *et. al..*, 2016).

Periodic quality control should be performed on the final component to ensure that the manufacturing process is consistent. Parameters measured depend on the type of red-cell concentrate product obtained. At a minimum, the following critical parameters should be checked during the quality control assays: volume, haemoglobin or haematocrit, the end of storage, residual leukocytes, if leukocyte reduction is performed. (World Health Organization WHO Technical Report Series, No. 961, 2011)

2.5.1 Quality monitoring of blood and blood components

Quality control data should demonstrate that critical manufacturing processes are under control. Blood and blood components should comply with specifications and their testing should be performed using test methods approved by the AABB. All processes, including data transfers and computerized systems that have an influence on the quality of the products in the area of collection, preparation or testing of blood and blood components should be validated.

For critical processes such as rapid freezing of plasma, the need for revalidation should be defined. Quality control of blood and blood components should be carried out according to a defined sampling plan based on statistical methods.

The sampling plan should take into account different collection and production sites, transport, methods of preparation and equipment used. Acceptance criteria should be based on a defined specification for each type of blood component (World Health Organization, 2010).

2.5.2 Quality of Packed red blood cells

The red cells should be filtered as close to the collection time as possible, in order to remove intact leukocytes that would otherwise fragment over time, releasing cytokines that are responsible for the febrile response. This requires that leukoreduction filters be used in the blood collecting facility rather than in the hospital blood bank or at the bedside. Leukoreduction is an effective method to reduce the risk of transfusion-transmitted cytomegalovirus infection (Renzette *et. al..,* 2011) CMV resides in the leukocytes and is removed during leukoreduction. Leukoreduction eliminates leukocyte debris and leukocyte-generated cytokines which would be performed on all cellular components intended for transfusion.

This is a standard practice in many developed countries, while a minority of other countries (including the United States) has yet to require universal leukoreduction as the official standard of care (Drew *et al.,* 2007).

2.6 Packed red cell volume

Volume generally is calculated by dividing the component weight by its specific gravity. The following conventions should apply in order to ensure some element of standardization. To provide quality monitoring data that demonstrate the capability of the blood collection process, the weight of the anticoagulant is indicated before converting to volume.

For red cell components, volume is calculated by weighing the pack, deducting the weight of the pack assembly only, and dividing the resultant weight by the nominal specific gravity 1.06. The weight of anticoagulant and, if relevant, additive solution are not deducted when calculating the volume of red cell components " Guidelines for the Blood Transfusion Services in the United Kingdom" 8th Edition, 2013.

2.7 Types of Packed Red Blood Cells

2.7.1 Red blood cell concentrate:

Blood component obtained by removing part of the plasma from whole blood by centrifugation, without further manipulation or addition of additive solutions. This product contains all the RBCs initially present, most of the leucocytes ($2.5 – 3 \times 10^{9}$) and a variable number of platelets (related to the method of centrifugation used). The HTC is between 65 and 75%, the minimum haemoglobin content is 45 g. The volume of a RBC concentrate is 280 ± 50 ml. The concentrated RBC, prepared without interrupting the closed circuit, must be stored at + 4 °C (± 2 °C); the storage period depends on the type of anticoagulant used (Nguyen *et. al...* 2016). The shelf-life for red blood cell units anticoagulated with CPDA-1 solution is 35 days.

2.7.2 Red blood cell concentrate deprived of the buffy-coat

Blood component obtained by using centrifugation to separate part of the plasma and the leucocyte-platelet layer (buffy-coat - 20 – 60 mL volume) from the RBCs. The haematocrit of this blood component is between 65 and 75%. The unit must contain the original amount of RBCs, except 10 – 30 ml. The white cell content must be below 1.2 x 10^9 and the mean platelet count < 20 x 10^9 per unit. The minimum content of haemoglobin in each unit is 43 g; the volume is 250 ± 50 ml (Nguyen *et. al.*, 2016). The potential duration of storage is the same as that indicated for the RBC concentrates.

2.7.3 Red blood cell concentrate with additive solution:

Blood component obtained from whole blood after centrifugation and removal of the plasma, with subsequent addition of appropriate nutrient solutions to the RBC concentrate. The volume of the additive solution is between 80 and 110 ml. The haematocrit depends on the quantity of the additive solution, the method of centrifugation and the amount of residual plasma, and must be between 50 and 70%. Each unit must have a minimum haemoglobin content of 45 g. The product contains all the initial starting RBCs and, unless removed, most of the leucocytes (from 2.5 to 3 x 10^9) as well as a variable number of platelets, depending on the method of centrifugation used. The volume differs according to the method of preparation used. The shelf-life is related to the type of additive solution used (Saline Adenine-Glucose-Mannitol: 42 days).

2.7.4 Red blood cell concentrate deprived of the buffy-coat and re-suspended in additive solution.

Blood component obtained from whole blood by centrifugation and removal of both the plasma and buffy-coat, with subsequent resuspension of the RBCs in appropriate nutrient solutions. The volume of the additive solution is between 80 and 110 ml. The haematocrit of this blood component depends on the volume of the additive solution, on

the method of centrifugation used and on the volume of residual plasma, and must be between 50 and 70%. Each unit must contain at least 43 g haemoglobin at the end of the preparation procedures. The unit must contain all the initial RBCs, except a portion of no more than 30 ml. The leucocyte and platelet counts must be < 1.2 x 10^9/unit and < 20 x 10^9/unit, respectively (Nguyen *et. al.*, 2016). The volume differs in relation to the method of preparation used. The shelf-life depends on the additive solution used (SAG-M: 42 days).

2.7.5 Washed red blood cells

Blood component obtained from whole blood after centrifugation, removal of the plasma and subsequent washing with isotonic solutions at + 4 °C. This is a suspension of RBCs from which most of the plasma, leucocytes and platelets have been removed. The haematocrit can vary according to clinical needs, but should remain between 65 and 75%. At the end of the washing procedure, each unit must contain a minimum of 40 g haemoglobin and no more than 0.3 g of protein. The product must be stored at + 4 °C (± 2 °C) for as short a period as possible, but, in any case, no more than 24 hours, unless methods ensuring the integrity of the closed circuit are used.

2.7.6 Leuco-depleted red blood cells

Blood component obtained by removing most of the leucocytes from a RCC by in-line pre-storage filtration or post-storage filtration in the laboratory or at the bedside. The white blood cell count must be between < 1 x 10^6/unit, but preferably < 0.5 x 10^6. The haematocrit must be between 50 and 70%. The haemoglobin content must be at least 40 g. If the system is opened in order to prepare the product, the storage period must not exceed 24 hours at + 4 °C (± 2 °C).

2.7.7 Frozen red blood cells

Blood component obtained by freezing RCCs (within 7 days of collection) with an appropriate cryoprotectant and storing at a temperature between − 60 °C and −80 °C in a

mechanical freezer, if using a method involving a high concentration of glycerol, or at lower temperatures in liquid nitrogen, if using a method involving a low concentration of glycerol. The frozen RBCs can be preserved for up to 10 years; their use for transfusion purposes is dependent on them fulfilling the criteria for suitability laid out by existing legislation and that they have been stored at all times at the correct temperature. The indications for freezing are: storage of units of rare groups and phenotypes and, in special cases, autologous blood. Before being used, the RBCs are thawed, deglycerolised, washed, re-suspended in physiological saline or additive solution and used as soon as possible; they can be stored at + 4 °C (± 2 °C) for no more than 24 hours, unless methods ensuring the integrity of the closed circuit are used. The reconstituted unit of frozen RBCs effectively does not contain proteins, leucocytes and platelets. The haematocrit must be between 65 and 75%. Each unit must have a haemoglobin content of at least 36 g.

2.7.8 Apheretic red blood cells

Blood component obtained by collecting red blood cells using an automatic cell separator. With the cell separators currently in use, the apheresis units are usually leucodepleted. Each unit must contain a minimum of 40 g haemoglobin and have haematocrit of 65 – 70%, reduced to 50 – 70% if the RBC are re-suspended in additive solution. The duration and methods of storage are the same as those for RBC concentrates.

2.7.9 Irradiated red blood cells

Blood component obtained by irradiating a RCC with between 25 and 50 Gy radiation. The irradiation has the purpose of decreasing lymphocyte viability and is the only method currently available for preventing transfusion-related Graft versus Host Disease. The product should be irradiated within 14 days of collection and irradiated units must be transfused within 28 days of collection. In cases of intrauterine or neonatal transfusion, or transfusions in patients with or at risk of hyperkalaemia, the transfusion

should be given within 48 hours of irradiation or the excess potassium removed from the unit. (Blaney & Howard, 2013).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was conducted at the National Blood Transfusion Center (NBTC) located within the Kenyatta National Hospital grounds. This center serves the county of Nairobi which has a population of approximately 5million people (Muga & Mohammed, 2001). This center also serve other regions as need arises.

Nairobi Regional Blood Transfusion Center (Figure 3.1) receives over 200 blood donations per week. Majority of these blood donations (70%) are separated into the various blood components with the predominant ones being packed red blood cells, FFP and platelet preparations. The institution provides the most sophisticated health services and provides facilities such as the Kenyatta National Hospital and the many hospitals within and outside Nairobi with blood and blood components for use in transfusions.

Figure 3.1: National Blood Transfusion Centre

The National Blood Transfusion Center is located near Kenyatta National Hospital and neighbours the National Aids and STIs Control program (Figure 3.2)

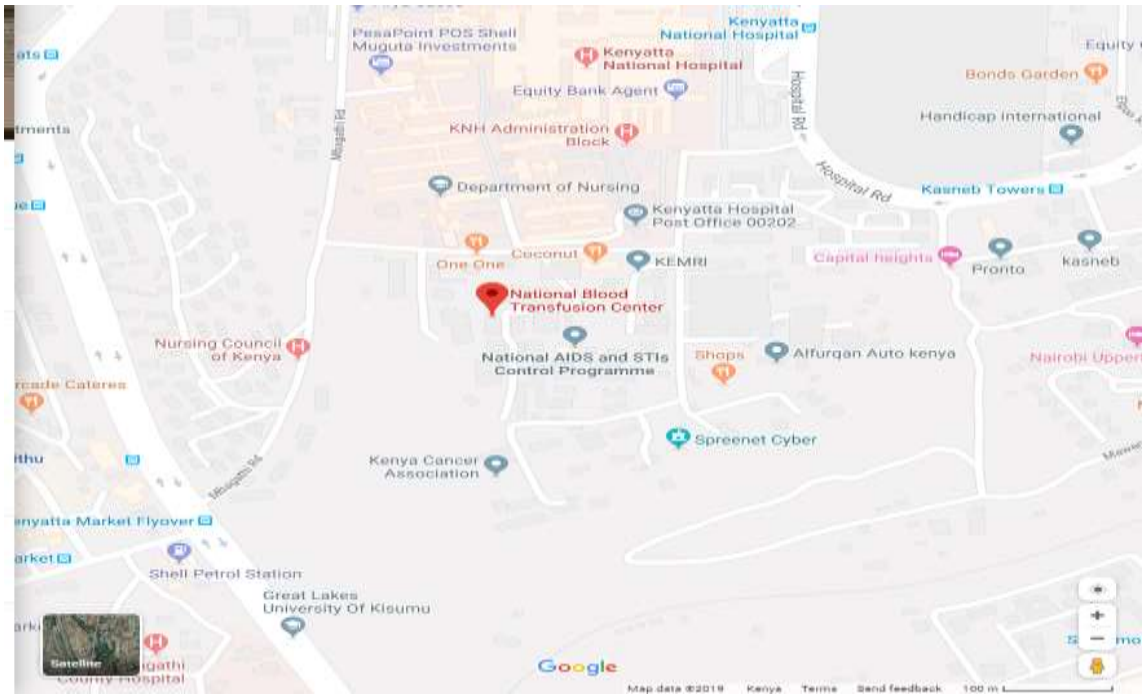

Figure 3.2: Location of National Blood Transfusion Center Nairobi (NBTC)

(From Google Maps, 18 November 2018)

3.2 Study design

This was descriptive Cross-section study for the assessment of quality of packed red blood cells prepared at National Blood Transfusion Center Nairobi (NBTC). Eighty packed red blood cells were assessed after meeting the inclusion criteria. This study was carried out between January 2017 and June 2017.

3.3 Study population

The study population consisted of qualified donors aged between 18 years and 55 years as recommended by Kenya National Blood Transfusion Services guidelines.

3.3.1 Inclusion criteria

Packed red blood cells (PRBCs) prepared at NBTC were used for the current study. The following criteria were used to include the appropriate samples. First the PRBCs were from whole blood that was tested and found to be negative for transfusion transmissible infections (TTIs). Furthermore, the PRBCs must have been freshly prepared or stored for not more than two day at between 2-6^{o}C.

3.3.2 Exclusion criteria

Any PRBCs that appeared under filled due to insufficient blood collection or overfilled which may result in micro-clot formation were not used in the study.

3.4 Sample size determination

The appropriate samples size for the study was calculated using formula; $n=Z^2 P (1-P)/d^2$ (Daniel *et. al..*, 2013). The following values were applied using this formula to determine the appropriate number of PRBC samples required:

n = Sample size

z = Value set at 1.96 which corresponds to 95% confidence interval

P = Estimated proportion of good quality (0.95)

D =precision 5%

$$n =1.96^2 x0.05 (1-0.95)/0.0025$$

$$n =3.8416x0.95 (0.05)/0.0025$$

$$n =80 \text{ Subject}$$

After substituting the above values, the samples size for the study was 80 PRBCs.

3.5 Sampling of PRBCs from the National Blood Transfusion Centre

A convenient sample was selected to obtain the total of eighty packed red cells from PRBCs prepared from the NBTC. Sample collection was conducted between January and June 2017.

3.6 Quality analysis of sampled PRBCs

The quality of each selected PRBC samples was assessed using five parameters: volume, residual WBC count, red blood cell count, platelet count and hematocrit. The Volume was taken for each packed red blood cell and recorded on the raw data log. The expected reference ranges is 150-200mls in Citrate Phosphate Dextrose Adenine-1(CPDA-1) and 220 to 340mls in Saline Adenine Glucose Mannitol (SAGM) From each of the selected PRBCs. Seventy nine of the assessed PRBCs was within .only one PRBC had 212mls in SAGM. 5ml aliquot was transferred into an EDTA tube for analysis of residual white blood cells count, red blood cell count, platelet count and hematocrit. Each sample was allocated a unique identification number. These aliquots were tightly closed, put in a spill prove container then transported to the Haematology laboratory for analysis.

The PRBC aliquots collected were gently mixed on a blood mixer and analyzed for red blood cell count, residual white blood cell count, platelet count and hematocrit. This was carried out following the procedure as described in Appendix 3 using a Sysmex Xt 2000i Haematology Analyzer (Figure 3.3). The results were then printed and recorded.

Figure 3.3: Sysmex XT 2000i Haematology Analyzer Quality Control

3.6.1 Quality controls for PRBC sample analyses

Quality controls were used to monitor the Sysmex 2000i analyzer performance. These were the Check Cells Controls, which are commercial cell controls provided by the analyzer manufacturer, Sysmex. They included Low, Normal and High levels of cell controls. The Check Cells Controls were run before analysis of the PRBC aliquots. Controls were examined prior to every run to ensure that they were not expired. XT CHECK kit insert.

3.7 Data management

For each packed red blood cells, a unique laboratory number was assigned and data was collected for quality parameters assessed namely, volume, haematocrit, WBC, RBC, platelet as counts and percentages. All collected data were entered into computer in MS Excel. Hard copies were printed and kept under lock and key the principle investigator

was the custodian. The data in the computer was saved using a password to avoid interference.

3.8 Data Analysis

Descriptive statistics were used to summarize the data. The data type for red cell count, white blood cell count, platelet count, hematocrit level and volume were continuous variables. Therefore means and SDs were reported. These complete blood counts and volumes were categorized based on the reference ranges as either within acceptable range, below or above the acceptable range. Frequencies and proportions were reported to summarize the variables. Pie charts and stack bar plots were plotted to compare the complete blood counts and volume of packed cells. Spearman rank correlation test was used to evaluate the relationship between the volume and complete blood count in packed cells. Rho statistic and p-value were reported

3.9 Ethical Consideration

Approval to carry out the study was obtained from the Kenyatta National Hospital/University of Nairobi Ethics and Research Committee (Ref: KNH – ERC/P691/10/2016 (Appendix 4). Consent was obtained from the Regional Director of NBTC prior to enrolment in the study. Confidentiality was maintained by coding the participating donor unit rather than using their names.

CHAPTER FOUR

RESULTS

4.1 Parameters Affecting Quality of Packed Red Blood Cells

Eighty units of packed red blood cell were assessed on whether they met the quality criteria. The standard quality assessment criteria utilized measured the volume, the white blood cell count, the red blood cell count, the platelet count and the haematocrit level of the packed red blood cells (PRBCs) prepared. Table 4.1 shows the summary of parameters assessed and their acceptable reference ranges.

Table 4.1: PRBC quality parameters and the acceptable reference ranges

Unit	Parameter	Category
Packed Red Blood cells (PRBCs)	**Volume (ml)**	220-340
	White blood cells	Less than 5×10^9
	Red blood cells	$4\text{-}8 \times 10^{12}$
	Platelets	$150\text{-}450 \times 10^9$
	Haematocrit (%)	45-75

4.2 Packed Red Blood Cell Quality Assessment

4.2.1 Summary of PRBC quality assessment

Examination of the 80 PRBC units using five quality parameters revealed that a substantial number of the units did met the required individual quality thresholds (Table 4.2). In combining the all the parameters, the number of PRBCs that attained the quality criteria was 26%. This was due to a high residual white blood cell count across many PRBC units. When platelet and residual WBC counts were excluded, 68% of the PRBCs were within the acceptable reference ranges. The complete data for the eighty PRBC for each quality parameter is shown in appendix 7.

Table 4.2: Summary of quality assessment of 80 packed red blood cells units

Quality Assessment by individual parameters						OVERALL Quality Assessment	
Packed Red Blood Cells	VOLUME ml *(220-340)*	RESIDUAL WBC COUNT $X10^9$ *(<5)*	RED CELL COUNT $x10^{12}$ *(4-8)*	PLATELET $x10^9$ *(150 - 450)*	HAEMATOCRIT % *(45 - 75)*	All parameters	excl WBC and platelet counts
No. within ref. range	79	43	69	61	64	21	54
No. outside ref. range	1	37	11	19	16	59	26
% within ref. range	99%	54%	86%	76%	80%	26%	68%
% outside ref. range	1%	46%	14%	24%	20%	74%	33%

4.2.2 Descriptive Statistics of assessed PRBCs

For each of the parameters analyzed, the mean, standard deviation, median and range (minimum and maximum values) were determined as presented in Table 4.3.

Table 4.3: Distribution of quality parameters in packed red blood cells

Unit	Parameter	Mean	SD	Median	Min	Max
Packed Red Blood Cells	**Volume (ml)**	266.1	24.9	257.0	212.0	340.0
	White blood cells (x 10^9cells/dl)	5.4	2.6	5.2	1.3	14.1
	Red blood cells	6.4	1.4	5.3-7.3	4.1	10.0
	Platelets	204.5	78.8	182.5	102.0	484.0
	Haematocrit	55.8	10.8	54.7	32.7	84.9

4.2.3 Quality Assessment of Volume of packed red cells

The first quality parameter assessed was the total volume of the PRBCs following processing. The administration of high volumes of transfusion products can lead to circulatory overload. In order to avoid these adverse effects but still administer enough red blood cells to achieve the action of restoring the oxygen carrying capacity of the recipient, it is important to have consistent products with a high red blood cell mass to volume ratio (i.e., hematocrit). Physicians may prescribe a specific volume of a RBC product, the labeled volume should be an accurate representation of the volume of the product in the bag. Most (79/80) of the prepared packed red cells in NBTC had volumes

within the acceptable range of between 212 and 340ml. Only one PRBC had a volume of 212ml and did not meet the assessment criteria. (Figure 4.1).

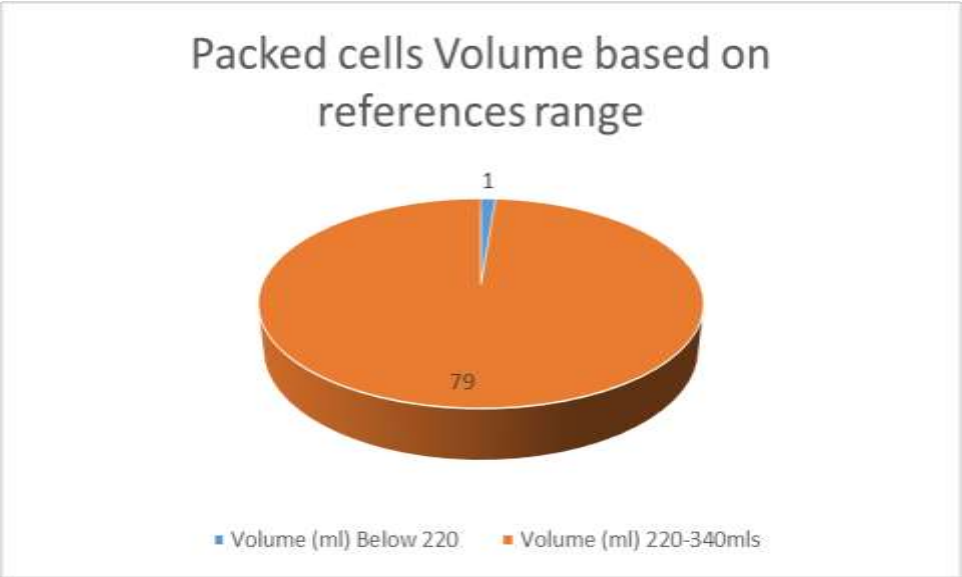

Figure 4.1: Volume of packed red blood cells

4.2.4 Quality Assessment of Residual white blood cells count in PRBCs

The white blood cells count in the research was high compared to the recommended counts by American association of blood banks. Thirty six units of the assessed eighty packed red blood cells were within the reference range for residue WBC count, while 44 were higher than required. Residual white cells are known to be the cause of febrile transfusion events. Figure 4.2 shows the number of unit packed red cells that were assessed which were within the reference range and those that failed

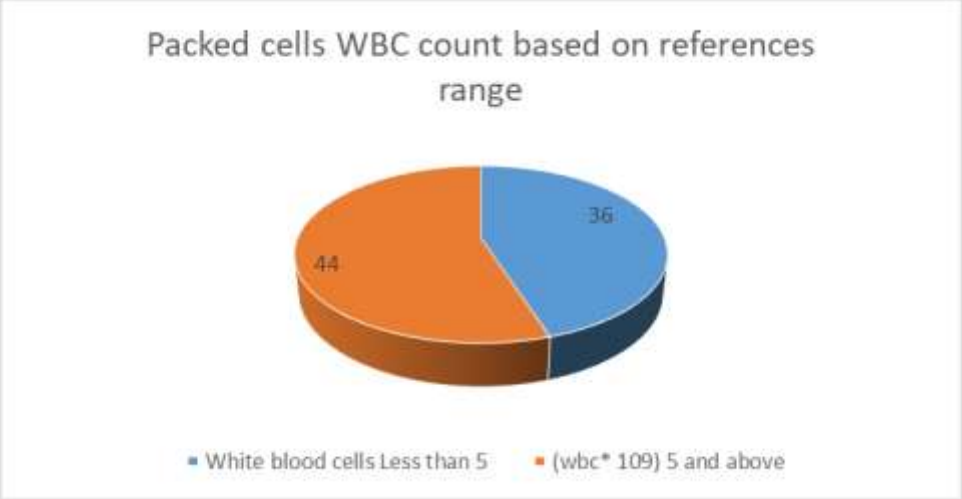

Figure 4.2: Residual WBC count in packed red cells

4.2.5 Quality Assessment of Red Blood cell count in PRBCs

The red blood cell count varied from 4.1 x 10^9cells/dl to 8.0 x 10^9cells/dl among packed cells. The mean red blood cell count was estimated at 6.4 x 10^9cells/dl with SD 1.4 x 10^9cells/dl in packed cells. Sixty nine; (86.3%) of the prepared packed red blood cell had the expected red blood cells count (Figure 4.3)

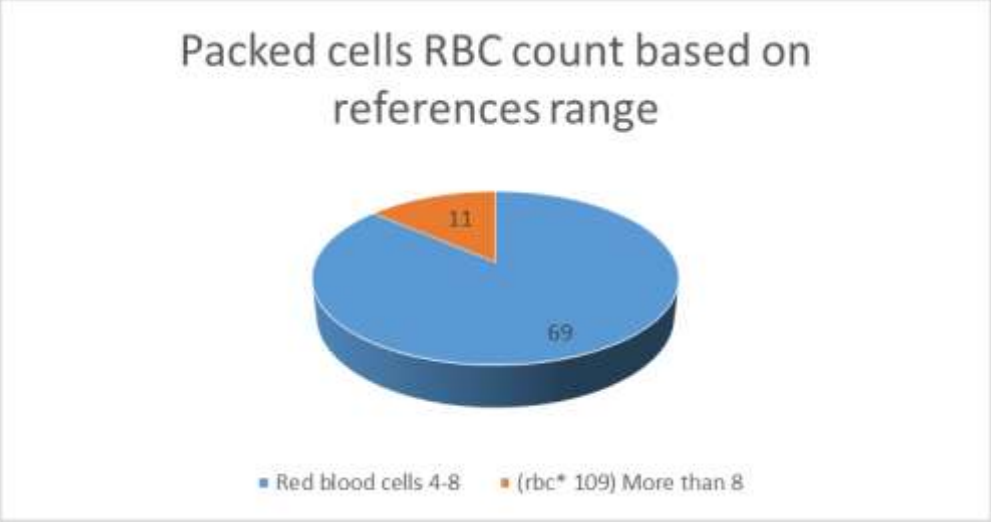

Figure 4.3: Red blood cell count in packed red cells

4.2.6 Quality Assessment of Platelet count in PRBCs

The PRBC platelet count range was 102 x 10^9 cells/ml to 484.0 x 10^9 cells/ml. In 18 (22.5%) PRBCs the platelet count was lower than 150. 61 (76.3%) PRBCs were within the acceptable range and 1 (1.2%) PRBC unit was elevated. Platelet shelf life is only 5 days. Platelets in PRBCs stored at 4°C beyond 5 days are not considered viable.

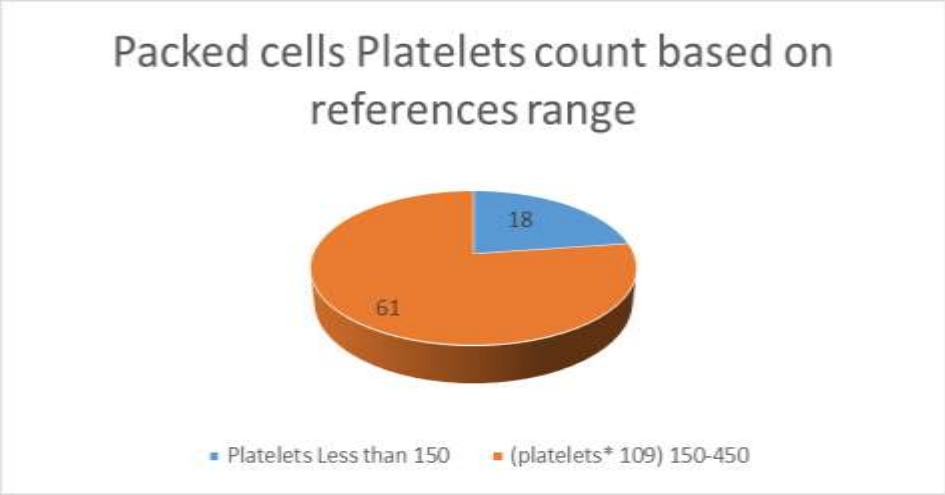

Figure 4.4: Platelets count in the packed red blood cells

4.2.7 Quality Assessment of Haematocrit level in PRBCs

Haematocrit was compared with the recommended range of between 45%-75% according to the Nairobi Regional Blood Transfusion Center and American Association of Blood Bank standards. Figure 4.5 shows that 66 of the 80 packed red blood cells that were within the acceptable reference range.

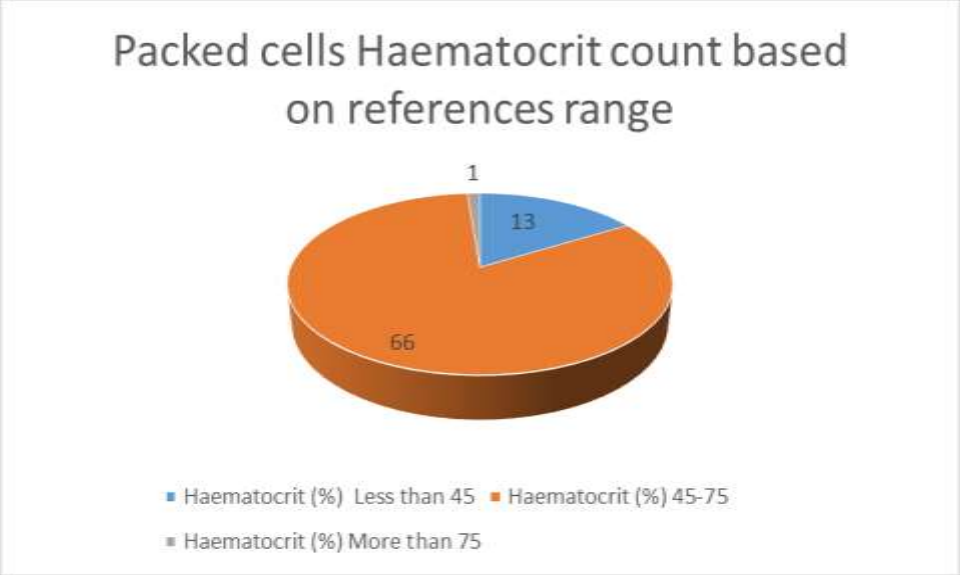

Figure 4.5: Haematocrit in the packed red cells

4.2.8 Overall assessment of quality parameters of PRBCs

The quality parameters were analyzed on aliquots immediately after PRBC preparation. There are additional changes that occur during storage and would affects these parameters if assessed later. For instance, the shelf life of platelets is only five days at 22^{oC} while on a functional agitator. As a result platelet count is not a critical quality parameter for PRBCs. At least 68% of the PRBCs attained the required quality threshold (once platelet and WBC counts were excluded). The quality parameter measurements for PRBC units were further classified using the standard reference ranges into three groups those within the acceptable reference ranges and those below and above the acceptable range. (Figure 4.6).

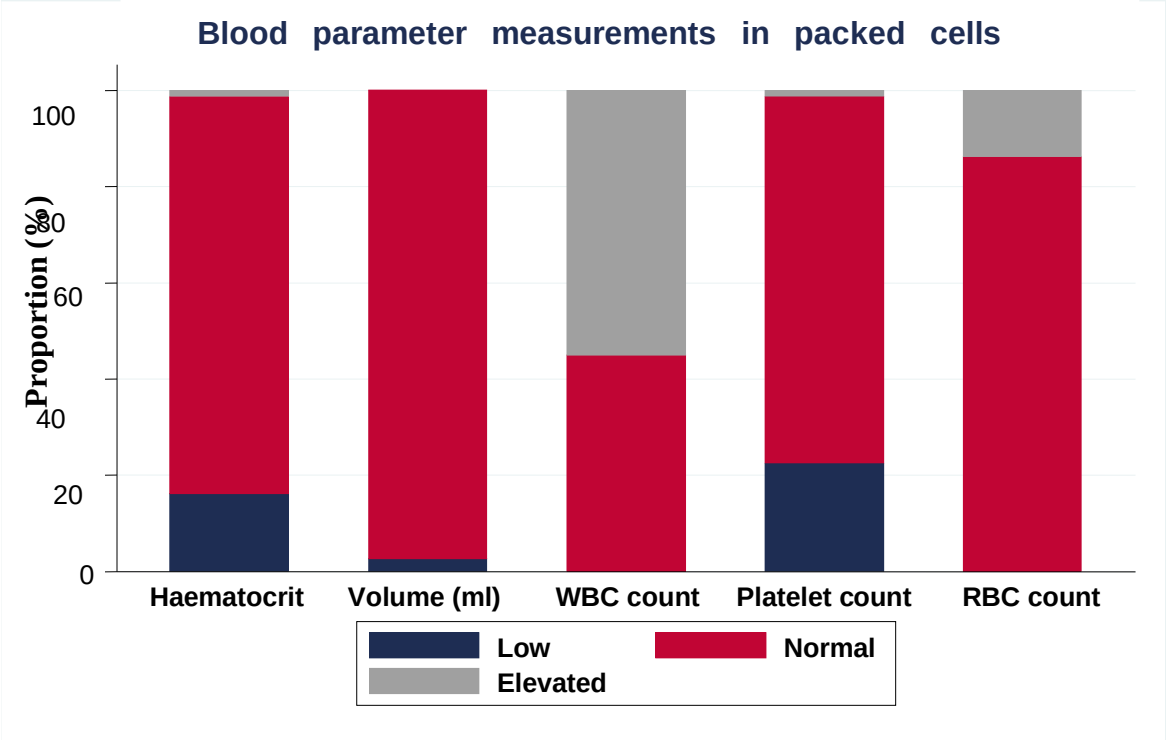

Figure 4.6: Quality parameters based on standard reference ranges

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMEDATION

5.1 Discussion

The current study set out to assess the quality of PRBCs prepared at the Kenya National Blood Transfusion Centre (KNBTS). A total of 80 PRBC samples were collected and examined using the following critical quality parameters: volume, residual white blood cell count, red blood cell count, platelet count and haematocrit.

Typically, one unit of PRBC is approximately 350 mL in volume, of which RBC volume is 200–250 ml. The remaining volume is due to plasma (typically 50-100 mL), white blood cells (WBCs), platelets and anticoagulants. The volume in 79 PRBCs assessed met quality criteria by having volume of 220 to 340 ml. Only one PRBC unit had volume of 212 which was below the reference range. This was in agreement with manual packed red blood cell collection by Upadhyay S and Pangtey (2016) which had volume of 198 to 350 mL per packed red blood cells. The volume of packed red blood cell components in this study were satisfactory in accordance to the "components "Guidelines for the Blood Transfusion Services in the United Kingdom" 8th Edition, 2013.

Low PRBC volume results in a high concentration of the anticoagulant. This may lead to transfusion of more anticoagulant which chelates the patient's calcium. On the other hand high volumes may cause clot formation due to low anticoagulant to blood ratio. Clots may have difficulty passing through the transfusion giving set filter, leading to a lot of wastage. Assessment of PRBC volume therefor would minimize the likelihood of these harmful effects on recipients.

Leukocyte reduction of blood component is widely practiced to achieve the required residue white blood cell count in the components. The American association of blood bank recommends residue white blood cell count of less or equal to 5.0 $x10^9$. The

packed red blood cells in this study had high white blood cell counts. This could be attributed to lack of filtration on collection. Forty three out of the eighty PRBC units had white blood cell counts above 5 X 10^9. The leucocytes present in the packed red blood red cells are implicated in several important immunological and infective complications of blood transfusion. They also cause febrile haemolytic reaction. Using filters or removal of buffy coat and suspension of the packed red blood cells reduces the residue white blood cells. Leukoreduction is an effective method to prevent or markedly reduce the risk of transfusion-transmitted cytomegalovirus infection. CMV resides in the leukocytes and is removed during leukoreduction. (B Bicalho *et. al.* 2015).

Quality assessment of packed red blood cells using RBC count as a quality parameter takes into consideration the 10/30 rule as noted by Carson *et. al.* (2012). The rule states that the RBC count multiplied by 3 gives the haemoglobin level and that the haemoglobin level multiplied by 3 gives the haematocrit. The rule of "10/30" for RBC, haemoglobin and haematocrit was met. In this study, 69 (86%)of the packed red blood cells that had red blood cell count within the reference range were and thus achieved this quality criteria.

Platelet counts that met quality criteria in this study were 61/80 (76%). According to American Association of Blood Bank, platelet levels in the packed cells do not have much significance since after storage their shelf life is less than 8hrs in the fridge at 2-6^{o}C. This allowed for the exclusion of the platelet count in the determination of the overall quality of PRBCs across the quality parameters.

Regulation requires that the final haematocrit of a red blood cell unit not exceed 80% (Markus *et. al., 2015).* The PRBCs in the present study were considered satisfactory because haematocrit of 54/80 (80%) met the European council criteria. This is comparable with a haematocrit of 84% determined by Upadhyay *et. al.* (2016) in their study where they assessed PRBC quality. The study had a mean haematocrit of 55.8% which was also comparable to the mean haematocrit of 54% by Upadhyay *et. al.* (2016) on quality analysis of blood component.

Another study done by Eiman Hussen (2014) on clinical and quality evaluation of red blood cell obtained manually found that 70% of the packed red blood cells had haematocrit values of 66% to 80%. Very few studies have been done in relation to assessment of quality of packed red cells but the few done gave results similar to the current investigation.

Overall, 54 (68%) of PRBCs met the quality criteria when WBC and platelet counts were excluded. With the observation that majority of the poor PRBC quality was due to high WBC counts, further study is required to establish the best procedure to produce packed red blood cells that meet this quality criteria.

5.2 Conclusion

At Nairobi Regional Blood Transfusion Center, most of the packed cells met criteria of quality assessment on volume, haematocrict and RBC counts. According to the study WBC count was very high in comparison to the standard. High residual WBC count is caused by the technical challenge of removal of the buffy coat during preparation of PRBCs following centrifugation and separation of blood components. This may be remedied by provision of additional continuous training to maintain quality standards. Thus continuous medical education training is inevitable. Furthermore, introduction of leukodepletion could be considered. In addition, to achieve and maintain the best quality PRBCs, periodic quality checks would be beneficial to promote effective use of the Standard Operation Procedures and improve on the quality production of PRBCs.

5.3 Recommendations

1. Consider leukodepletion to meet the Standard of $<5*10^9$l residual WBC
2. Further studies on PRBC preparation process and sustainability of quality including aspects on bleeding of the donors and component preparation
3. Consider review of the Standard Operating Procedure and sensitization of technical staffs to ensure compliance.

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APPENDICES

Appendix I: Data Collection Tool B Packed red cell

Data collection sheet parameters

Date of Sampling Date of processing

Sample number KNBTS no

Red Cell count Volume

Platelets

Haemoglobin

White Blood Cells Haematocrit

Comments

Signed: **Signed:**

NBTC Technologist **Investigator**

Date **Date**

Appendix II: Procedure for preparation of Packed Red Cells concentrates at KNBTS

1. Determine weight of whole blood
2. Record weight in KNBTS component register
3. Place in centrifuge buckets
4. Balance before placing in the refrigerated centrifuge
5. Place balanced blood buckets in the refrigerated centrifuge
6. Ensure all sides are balanced before closing the refrigerated centrifuge door
7. Programme refrigerated centrifuge according to required blood component and press start button
8. Gently remove centrifuged blood from centrifuged
9. Place centrifuged blood unit on plasma extractor.
10. Place clips to the satellite bags except the plasma bag and express plasma to suspend red cells if CPDA for Additive solution (SAGM)PRBCs, Express all the plasma up to the pot and add the Additive solution to suspend the red cells and prepare desired component
11. Weight and label prepared component
12. Using the tube sealer heat seal and make segments on pack tube
13. Detach component and record in KNBTS blood component register
14. Place blood components into transport tray and quarantine at appropriate temperature.

Appendix III: Procedure for Automated blood count Sysmex XT 2000i Haematology Analyzer

Procedure:

1. Make sure the button on the right edge of the control menu is “MANUAL”
2. When the mode is set to “SAMPLER” press the mode switch to the analysis that you want.
3. Press “OK”.
4. Touch on the item to set the condition
    - Sample Number
    - Discrete
    - Sample information
    - Aspiration sensor
5. Open sampler cover(manual unit)
6. Place the sample tube in the sample tube holder
7. Press the start button
8. Remove the sample tube
9. Close the sampler cover.

Appendix IV: Published Article

East African Journal of Pathology

Evaluation of quality of packed red blood cells prepared at Regional Blood Transfusion Center Nairobi

Solomon MW, MSc (Med Lab Sci - Haematol & Blood Trans)[1], **Mbugua MA**, PhD[1], **Maturi PM**, MBChB, MMed (Path), FC Path ECSA[2]

ABSTRACT

Background: Preparation of packed red cell components is the one most well-known functions of the blood transfusion service in Kenya. Therefore its of great importance to assess the quality of the prepared packed red cells blood components.
Objective: To assess quality of Packed Red Blood Cells (PRBCs) prepared at Regional Blood Transfusion Center Nairobi (RBTC).
Design: Descriptive cross-sectional study.
Setting: The study was conducted at Nairobi Regional Blood Transfusion Center.
Sample size: Eighty packed red cells were selected for the study as the size was convenient and cost effective
Results: Out of 80 packed red blood cells assessed, 86% met the criteria for acceptable red cell count, 76.3% for acceptable platelet count, 98.7% for acceptable volume, 45% for white blood cell count and 82.5% for haematocrit level.
Conclusion and recommendation: Among the prepared packed red blood cells, not all met the quality criteria. Based on these findings, there is need to enhance the quality assurance protocol and focus on the processes used to prepare packed red blood cells namely centrifugation, separation and storage to attain quality standards set by the Kenya National Blood Transfusion Services (KNBTS), European Directorate for the Quality of Medicine (EDQM), American Association of Blood Banks (AABB).

Key words: Quality, Packed Red Cells Components, Regional Blood Transfusion Center Nairobi

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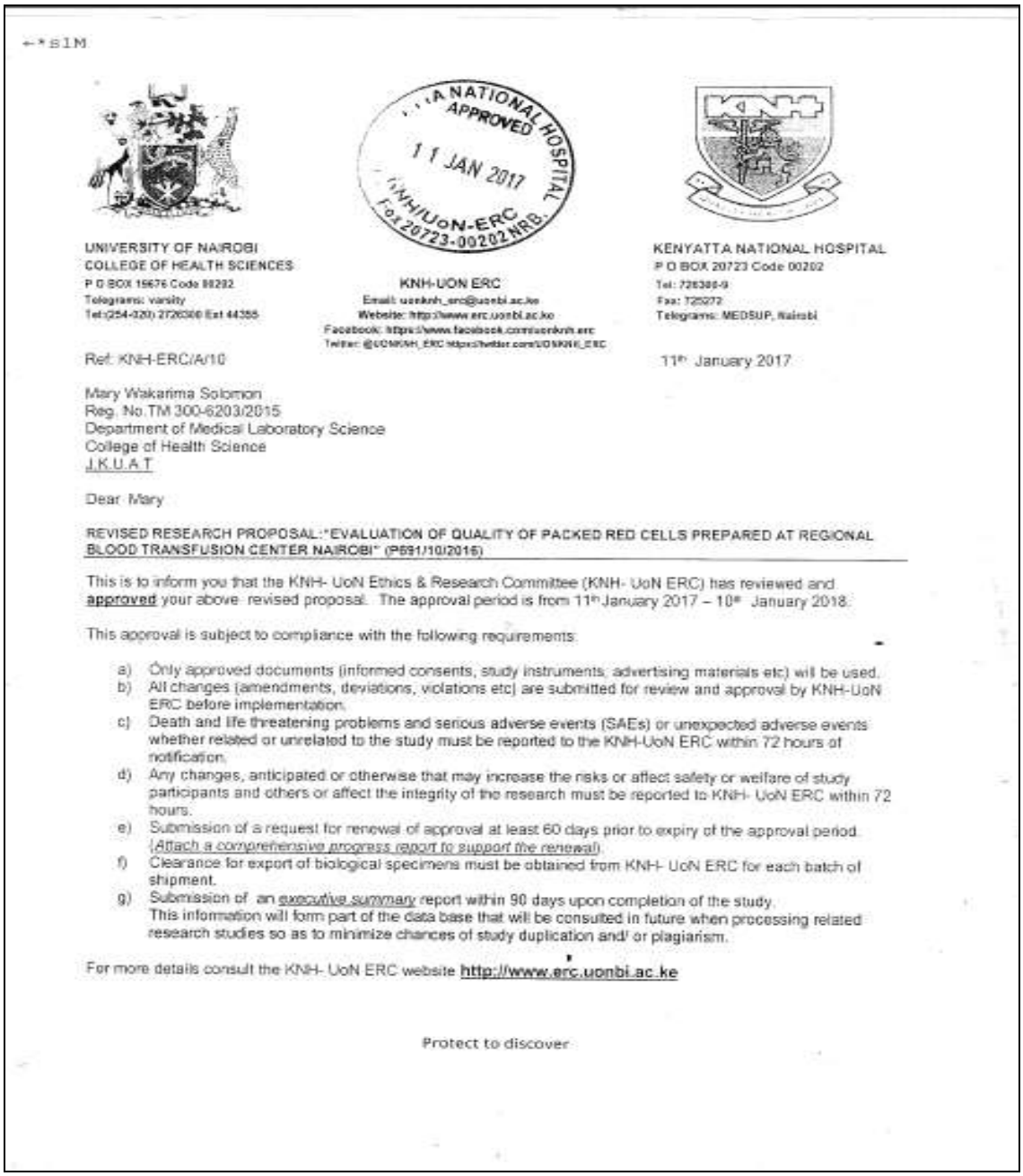
INTRODUCTION

Blood transfusion services are required to provide blood and components which are safe[1], affordable and accessible for transfusion into patients who require the blood products[2]. Therefore, one donated unit can benefit more than one recipient after components preparation[3]. The current transfusion therapy depends on the availability of blood components[4]. These components, used separately or in various combinations, can adequately meet most patient transfusion needs while keeping the risks of transfusion to a minimum. Component transfusion therapy has the added benefit of using a limited natural resource more effectively by providing the needed therapeutic component to several patients from a single donation. In addition there is reduced burden on blood banks because of reduced transfusion related events and overall wastage when whole blood is transfused[5].

Packed red blood cells are prepared by removing approximately 80% of plasma from a unit of whole blood. Standards require that the final haematocrit of a red blood cell unit not exceed 80%[6]. Multicomponent aphaeresis for deriving combinations of Red Blood Cells (RBCs), Platelets (PLT) and plasma units[7] and collection of double RBC units are increasingly used to alleviate blood shortages, especially in the U.S[8]. Average haematocrit is between 65% and 80%.

Packed Red Blood Cells Units (PRBCS) contains the same RBC mass and therefore the same oxygen –carrying capacity as whole blood, but with approximately one-half the volume. Other advantages of using PRBCs rather than whole blood are: significant reduction in the level of plasma thus facilitating transfusion of group O cells to non O recipient, reduction of potassium and reduction of adverse events associated with WBC, platelet and plasma volume[9].

Appendix V: Approval Letter



←*slM

KNH APPROVED 11 JAN 2017 KNH/UoN-ERC P.O. Box 20723-00202 NRB

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Ref: KNH-ERC/A/10

11th January 2017

Mary Wakarima Solomon
Reg. No.TM 300-6203/2015
Department of Medical Laboratory Science
College of Health Science
J.K.U.A.T

Dear Mary

REVISED RESEARCH PROPOSAL:"EVALUATION OF QUALITY OF PACKED RED CELLS PREPARED AT REGIONAL BLOOD TRANSFUSION CENTER NAIROBI" (P691/10/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 11th January 2017 – 10th January 2018.

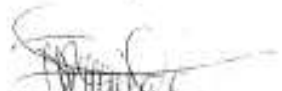
This approval is subject to compliance with the following requirements:

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

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Protect to discover

Yours sincerely,



PROF M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Deputy Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chair, KNH- UoN ERC
Supervisors: Dr. Amos Mbugua(J.K.U.A.T), Dr. Peter Maturi(UoN), Prof.Walter Mwanda(UoN)

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Appendix VI: KNBTS Consent Letter

MINISTRY OF HEALTH

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

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

JOMO KENYATTA UNIVERSITY

OF AGRICULTURE AND TECHNOLOGY

COLLEGE OF HEALTH SCIENCE

DEPARTMENT OF MEDICAL LABORATORY SCIENCE

Date 23rd Feb 2017

RE: CONSENT TO UNDERTAKE A STUDY AT KNBTS- RBTC NAIROBI

This is to confirm that we have consented for the study entitled **"Evaluation of quality of packed red cells prepared at regional blood transfusion center Nairobi"** by Mary Wakarima Solomon TM 300-6203/2015 at RBTC Nairobi

She is required to make her own arrangements with the RBTC staff on how to conduct the study using samples from the segments already prepared without undue manipulation of the blood pack. She is also expected to share her findings with this office.

Thank you

MOduor.

Dr Margaret Oduor

Head KNBTS

CC. Mary Wakarima Solomon

Mr Abdi Dahir Head RBTC Nairobi

Appendix VII: Quality assessment of packed red blood cells at RBTC

	Quality Assessment by individual parameters						OVERALL Quality Assessment	
	SERIAL NUMBER	**VOLUME ml *(220-340)***	**RESIDUAL WBC COUNT $X10^9$ *(<5)***	**RED CELL COUNT $x10^{12}$ *(4-8)***	**PLATELET $x10^9$ *(150 - 450)***	**HAEMATOCRIT % *(45 - 75)***	**All parameters (0 =Poor, 1 = Good)**	**excl WBC and platelet counts (0 =Poor, 1 = Good)**
1	104102	**240**	**5.05**	**5.81**	**131**	**49.7**	0	1
2	104103	**226**	**8.12**	**8.59**	**191**	**70.7**	0	0
3	104104	**258**	**5**	**7.42**	**297**	**62**	0	1
4	104105	**243**	**4.57**	**8.16**	**123**	**64.6**	0	0
5	104109	**251**	**6.94**	**4.13**	**134**	**48.1**	0	1
6	104108	**236**	**8.73**	**7.78**	**262**	**66.4**	0	1
7	104110	**258**	**8.03**	**8.61**	**221**	**73.3**	0	0
8	104111	**283**	**4.27**	**9.18**	**253**	**68.9**	0	0
9	104112	**276**	**4**	**7.23**	**177**	**64.3**	1	1
10	104113	**290**	**10.08**	**8.24**	**180**	**71.4**	0	0
11	104115	**254**	**9.21**	**7.63**	**165**	**67.7**	1	1
12	104116	**258**	**4.11**	**5.37**	**426**	**48.3**	1	1
13	104119	**240**	**6.26**	**6.15**	**164**	**49.2**	0	1
14	104122	**275**	**7.16**	**5.65**	**123**	**51.4**	0	1
15	104123	**265**	**4.61**	**6.12**	**104**	**54.5**	0	1
16	104124	**290**	**8.99**	**6.34**	**103**	**53.6**	0	1
17	104126	**259**	**6.29**	**4.21**	**102**	**32.7**	0	0
18	104127	**300**	**6.75**	**7.33**	**149**	**66.1**	0	1
19	104128	**310**	**14.12**	**9.96**	**278**	**84.9**	0	0
20	104131	**298**	**4.33**	**9.12**	**127**	**73.3**	0	0
21	104132	**330**	**10.64**	**7.26**	**196**	**64.2**	0	1
22	104133	**322**	**2.86**	**10**	**157**	**81.3**	0	0
23	104134	**310**	**13**	**7.78**	**347**	**59.9**	0	1
24	104135	**302**	**5.44**	**7.67**	**160**	**67.6**	0	1
25	104136	**300**	**6.55**	**8.55**	**166**	**72.7**	0	0
26	104138	**212**	**5.25**	**7.86**	**199**	**70.2**	0	0
27	104696	**285**	**2.55**	**5.04**	**194**	**41.9**	0	0
28	104697	**297**	**6.72**	**5.06**	**158**	**50.7**	0	1
29	104698	**265**	**5.18**	**4.99**	**264**	**46**	0	1
30	104700	**250**	**4.94**	**5.15**	**145**	**51.3**	0	1

31	104703	250	2.68	5.27	159	50.1	1	1
32	104704	250	2.32	5.49	190	48.7	1	1
33	104738	250	3.03	4.59	265	43	0	0
34	104739	250	1.84	6.64	235	62	1	1
35	104742	250	3.23	5.77	185	53.7	1	1
36	104743	250	4	4.98	115	44.7	0	0
37	104744	250	3.1	6.59	148	45.4	0	0
38	104745	250	3.15	4.28	170	39.4	0	0
39	104747	250	3.17	4.93	212	43.7	0	0
40	104749	250	2.41	4.51	234	39.9	0	0
41	104751	250	4.65	4.74	342	42.8	0	0
42	104752	250	3.21	5.58	301	43.8	0	0
43	104753	250	3.07	4.85	126	47.3	0	1
44	104754	250	3.01	5.32	114	43.5	0	0
45	104756	250	4.04	5.27	260	47.4	1	1
46	104757	250	3.22	5.18	215	50.2	1	1
47	104758	250	3	5.77	214	52.9	1	1
48	104760	250	2.54	5.52	182	55.7	1	1
49	104761	250	2.97	5.05	154	48.1	1	1
50	104809	250	1.34	5.53	215	47.9	1	1
51	104810	250	4.23	4.35	156	36.3	0	0
52	106300	267	4.24	5.71	236	52.4	1	1
53	106301	273	9.47	5.32	212	49	0	1
54	106302	297	4.67	6.63	158	58.5	1	1
55	106303	235	11.07	6.13	336	50.3	0	1
56	106305	238	4.8	4.95	182	45	1	1
57	106312	291	4.9	6.33	190	57.8	1	1
58	106313	240	6.28	7.11	161	59.2	0	1
59	106314	239	6.68	6.27	245	55.8	0	1
60	106315	238	6.92	6.44	167	56.5	0	1
61	106316	292	5.7	7.19	213	63.8	0	1
62	106317	240	5.99	6.39	190	54.7	0	1
63	106321	248	4.09	4.69	249	40.2	0	0
64	106326	296	7.83	6.87	484	56.9	0	1
65	106332	313	5.15	7.24	145	57.7	0	1
66	106334	258	6.26	5.48	183	49.2	0	1
67	106336	287	4.11	9.23	160	77.3	0	0
68	106347	292	4.12	5.83	182	49.5	1	1
69	106352	250	5.58	4.82	194	42.1	0	0
70	106356	256	4.76	6.3	166	58.1	1	1

71	106363	**252**	**4.97**	**5.86**	**151**	**54.5**	1	1
72	106367	**285**	**3.57**	**6.59**	**140**	**55**	0	1
73	106372	**276**	**5.4**	**6.54**	**114**	**59.1**	0	1
74	106377	**290**	**8**	**7.94**	**319**	**65**	0	1
75	106378	**301**	**3.93**	**6.26**	**148**	**58.1**	0	1
76	106384	**276**	**7.68**	**6.93**	**373**	**60.1**	0	1
77	106389	**300**	**8.78**	**7.13**	**449**	**63.6**	0	1
78	106391	**272**	**8.46**	**7.72**	**294**	**61**	0	1
79	106394	**283**	**4.27**	**9.18**	**253**	**68.9**	0	0
80	106398	**276**	**4**	**7.23**	**177**	**64.3**	1	1
No. within ref. range		79	43	69	61	64	21	54
No. outside ref. range		1	37	11	19	16	59	26
% within ref. range		99%	54%	86%	76%	80%	26%	68%
% outside ref. range		1%	46%	14%	24%	20%	74%	33%