EFFECTS OF REPETITIVE UNCONTROLLED TEMPERATURE EXPOSURE ON THE QUALITY OF PACKED RED BLOOD CELLS

BY

NOOR HAMIZAH BINTI MOHD HASSAN

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (TRANSFUSION SCIENCE)

ADVANCED MEDICAL AND DENTAL INSTITUTE
UNIVERSITI SAINS MALAYSIA

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DECLARATION

I hereby declare that this research has been sent to Universiti Sains Malaysia for the degree of Masters of Science (Transfusion Science). It is not to be send to any other universities. With that, this research might be used for the consultation and can be photocopied as reference.

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NOOR HAMIZAH BINTI MOHD HASSAN

IPM 047/18

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

%	Percent
+	Positive
<	Less than
>	Greater than
±	Plus, minus
≥	Greater than or equal
^{0}C	Degree celsius
g	Gram
g/dL	Gram/decilitre
g/L	Gram/Litre
g/mL	Gram/millilitre
L	Litre
min	Minute
mL	Millilitre
mL/kg	Millilitre/kilogram
mM	Milimolar
O_2	Oxygen
Qt	Quarts
rpm	Revolutions per minute
µL	Microliter

LIST OF ABBREVIATIONS

2,3-DPG	2,3-diphosphoglycerate
ADL	Advanced Diagnostic Lab
AMDI	Advanced Medical and Dental Institute
ATP	Adenosine triphosphate
CPDA	Citrate-phosphate-dextrose anticoagulant
CFC	Chlorofluorocarbons
CTC	Clinical Trail Complex
DC	Direct current
DDD	Desired detectable difference
EDTA	Ethylenediaminetetraacetic acid
FBC	Full blood count
Hb	Haemoglobin
HIV	Human immunodeficiency virus
IQR	Inter quartile range
JEPeM	Research Ethics Committee (Human)
LDH	Lactate dehydrogenase
PRBC	Packed red blood cell
RBC	Red blood cells
SOP	Standard operation procedures
TMU	Transfusion Medicine Unit
UK	United Kingdom
US	United States
USM	Universiti Sains Malaysia
WHO	World Health Organization

KESAN PENDEDAHAN SUHU TIDAK TERKAWAL SECARA BERULANG TERHADAP KUALITI SEL DARAH MERAH PEKAT

ABSTRAK

Sel darah merah pekat (SDMP) mesti ditransfusikan kepada pesakit dalam masa 30 minit selepas dikeluarkan dari peti sejuk darah dan SDMP tidak sesuai disimpan di wad di mana suhu penyimpanan tidak dikawal dan dipantau. SDMP yang tidak ditransfusi dan dikembalikan ke tabung darah akan dibuang melainkan SDMP tersebut telah disimpan dalam keadaan dan suhu yang bersesuaian. SDMP yang berulang kali terdedah kepada suhu di luar julat yang dibenarkan akan lebih cenderung untuk mengalami kerosakan dan tidak sesuai untuk digunakan. Kajian ini dilakukan secara keratan rentas untuk menentukan kadar kenaikan suhu SDMP dan kualiti SDMP apabila terdedah kepada suhu tidak terkawal secara berulang selama 30 minit dan empat jam. Dalam kajian ini empat unit SDMP (228 mL ± 37mL) diperolehi dari subjek sukarela yang layak dibahagikan kepada dua unit untuk menghasilkan lapan unit kecil SDMP (± 130mL) yang kemudian diasingkan kepada dua kumpulan yang berbeza; kawalan dan eksperimen. Lapan unit SDMP dari kedua-dua kumpulan kawalan dan eksperimen diuji tahap haemoglobin, hematokrit, kadar hemolisis, kalium, laktat dehidrogenase (LDH), pH dan kesterilan pada hari pertama, hari ke-7, 14 dan 35 penyimpanan. Kadar kenaikan suhu SDMP yang terdedah kepada suhu yang tidak terkawal dipantau dan direkodkan. Semua SDMP mencapai keperluan kualiti yang dikehendaki berdasarkan garis panduan kebangsaan sehingga hari ke-35 untuk parameter hematokrit, kadar hemolisis, dan kesterilan. Walau bagaimanapun, tiada unit SDMP memenuhi keperluan standard (> 45g/unit) untuk parameter hemoglobin bermula dari hari ke-14 sehingga hari ke-35. Tiada perubahan

signifikan bagi semua paramater kualiti SDMP dari kumpulan eksperimen yang terdedah suhu tidak terkawal secara berulang dalam 35 hari ($p < 0.05$). Selain itu, kajian mendapati tiada bukti pencemaran bakteria dalam semua unit SDMP pada hari terakhir tarikh luput. Pendedahan berulang kepada suhu yang tidak terkawal pada SDMP tidak menyebabkan perbezaan yang signifikan bagi parameter hematokrit, peratusan hemolisis, kalium, laktat dehidrogenase (LDH), dan pH apabila dibandingkan dengan unit kawalan. Walau bagaimanapun, terdapat perbezaan yang signifikan bagi tahap haemoglobin antara kumpulan kawalan dan kumpulan eksperimen (nilai $p = 0.021$ dan 0.021) bermula hari ke-14 dan hari ke-35. SDMP mula mencapai suhu $10^{\circ}C$ selepas kira-kira 4.5 jam di dalam kotak sejuk penyimpanan. Kesimpulannya, kajian ini menunjukkan tiada bukti perubahan kualiti yang signifikan terhadap SDMP selepas pendedahan kepada suhu yang tidak terkawal selama 30 minit dan empat jam untuk dua kali. Namun penurunan kadar haemoglobin dapat diperhatikan mulai hari ke 14 hingga hari akhir penyimpanan.

EFFECTS OF REPETITIVE UNCONTROLLED TEMPERATURE EXPOSURE ON THE QUALITY OF PACKED RED BLOOD CELLS

ABSTRACT

Packed Red Blood Cells (PRBC) must be transfused within 30 minutes upon removal from blood refrigerator and the PRBC should not be hold in the ward where the storage temperature is not controlled. Untransfused blood which returned to the blood bank shall be discarded unless it is kept in an appropriate condition and temperature. Intermittent storage outside allowable temperature range often leads to destruction of the PRBC unit. This was a cross sectional study performed to determine the PRBC warming rates and the quality of PRBC upon repetitive exposure to uncontrolled temperature after 30 minutes and 4 hours. In this study four PRBC units (228 mL ± 37mL) collected from eligible volunteer subjects were equivalently split into two units, producing eight smaller units PRBC (±130mL) which then assigned into two groups; control and experimental group. Eight units of PRBC from both control and experimental group were tested on day 1, day 7, day 14 and day 35 of storage for haemoglobin level, haematocrit, haemolysis rate, potassium, lactate dehydrogenase (LDH), pH and sterility. The PRBC warming rate upon exposure to uncontrolled temperature were monitored and recorded. All PRBCs achieved desired quality requirement from national guideline from day 1 to day 35 for haematocrit, haemolysis rate, and sterility. However, none of the PRBC units met the standard requirement (>45g/unit) for haemoglobin starting day 14 to day 35. No significant changes observed for all PRBC's quality over repetitive exposure to uncontrolled temperature in 35 days in experimental group ($P<0.05$). There was also no evidence of bacterial contamination in PRBC at the end of their shelf life.

Repetitive exposures to uncontrolled temperature did not cause any significant different in haematocrit, haemolysis rate, potassium, LDH, and pH when compared to control units. Nonetheless, there were significant different for haemoglobin level between control group and experimental group (p =0.021 and 0.021) starting day 14 and day 35. PRBC started to reach 10°C after about 4.5 hours in the cool box. After 24 hours, the temperature increased up to 20.2°C. In conclusion, this study suggests no evidence of significant quality changes to PRBC after exposure to uncontrolled temperature for two occasions of 30 minutes and four hours, but decreased haemoglobin level was observed starting day 14 till the end of storage.

CHAPTER 1

INTRODUCTION

1.1 Overview

Packed Red Blood Cells (PRBC) is a blood component obtained by removing most of the plasma from the whole blood collected from voluntary non-remunerated donors. PRBC do not contain viable platelets and clinically significant amounts of coagulation factors. PRBC can increase the oxygen carrying capacity and therefore is transfused into patient with indicated chronic anaemia and patient with acute blood loss resulting from surgery or trauma. In adults, one unit of PRBC able to increase haemoglobin (hb) levels by about 10 g/L (1 g/dL) and will raise the haematocrit by 3%. Nowadays, whole blood is not routinely transfused to maximise the efficiency of blood utilisation. But instead, patient is given the specific component which they need due to proven efficacy of component therapy. Thus, PRBC is more favour in patient who needs to restore oxygen carrying capacity.

Depending on types of preservative and anticoagulant used, PRBC has shelf life between 28 to 42 days. PRBC must be stored at controlled temperature of 2 to 6°C and transported in 2 to 10°C to maintain the viability of the red blood cells and to prevent bacteria growth. During storage throughout shelf life, PRBC undergo changes known as storage lesion. PRBC experience progressive damage during storage that may affect their viability, impact the ability of the vessels to facilitate blood flow, decrease the red

cell's ability to delivery oxygen, and increase the generation of potentially harmful waste particles.

Once the PRBC units are taken out from the blood bank refrigerator, it is said to be out of controlled temperature. Unlike domestic refrigerators, blood bank refrigerators feature stringent security measures to prevent tampering, and have special technical specifications such as high quality cabinet construction, with heavy walled, CFC-free insulation which helps to minimise energy use and ensure temperature stability. Besides, blood bank refrigerators have both directed and monitored forced air circulation that helps maintain uniform temperatures at all points in the cabinet and ensure quick temperature recovery after refrigerator door openings.

In blood bank, the PRBC exposure to the uncontrolled temperature during processing and testing are kept as minimal as possible and can easily close monitored by the laboratory personnel. However the concern is when the PRBC is taken to the ward or clinic for transfusion. The cool box used to transport the blood is rarely validated and the temperature is not monitored. The appropriateness of blood transportations and storage temperature in ward or clinic is depending on the individual practice and setting. Most of the wards and clinics in hospitals do not have designated blood bank refrigerator. Therefore it is crucial to start the transfusion as soon as possible after reaching the ward (Amin *et al.* 2016) and blood should not be taken out from the cool box until it is ready to be transfused. In the event when the transfusion is postponed or cancelled, the ward or clinic shall return all untransfused blood immediately to the hospital blood bank. This is to make sure the blood can be stored back into blood bank refrigerator with controlled temperature so that the quality of

PRBC is well preserved. Untransfused blood that is returned to the blood bank shall be discarded unless it is kept in an appropriate condition and temperature Amin *et al.* 2016).

In blood transfusion practice, the '30 minute rule' and the '4 hours rule' being practice worldwide where for 30 minute rule states that any red blood cell (RBC) units left out of controlled temperature storage for more than 30 minutes should not be returned to storage for reissue. While the '4 hours rule' state that transfusion of RBC units should be completed within four hours of their removal from controlled temperature storage (WHO 2005; BCSH 2012; ANZST 2018).

Meanwhile in Malaysia, according to National Blood Centre (2016) PRBC and whole blood should be transfused within 30 minutes of removal from the blood refrigerator and the transfusion of each unit shall not exceed four hours. These rules are set in order to maintain and control the quality of PRBC. There is significant risk of bacterial contamination if a unit of red cells is kept at room temperature for too long (Amin *et al.* 2016). Besides that, the effect of high temperature changes on the quality of RBC units is an important aspect to take into consideration.

1.2 Research Justification

The PRBC should not be hold in the ward or clinic where the storage temperature is not monitored and controlled. They transported and stored the PRBC in a cool box together with ice pack until the blood is ready to be transfused. The cool box and the ice pack have limitation and unable to maintain the blood temperature below

10°C for long time. Besides, the surrounding temperature in the ward which mostly do not equipped with air-condition also have to take into consideration. Therefore it is recommended in our national guideline that any untransfused blood must be return to the blood bank **immediately**. However, the guidelines do not state any cut off allowable time to safely accept and time to justifiably discard the returned untransfused blood.

Currently, most of the hospital blood bank practice that any returned untransfused blood which exceeds 10°C and/or being out from blood refrigerator for more than four hours should be discarded and cannot be put back into storage for reissue because it is believed that the blood is no longer suitable for transfusion. This practise is based on the maximum allowable transportation temperature for PRBC which is below 10°C and the four hours' time limit is based on the rule that the transfusion of each unit shall not exceed four hours. Apparently, the discarded blood due to this practice will create blood wastage problem. Blood is a very precious source, thus any kind of wastage and its causes must be taken care seriously. Statistical data from blood bank Hospital Sultanah Nur Zahirah Kuala Terengganu showed that 918 (6.9%) untransfused bloods was return back to blood bank in 2017. Out of that 918 pint, 716 (78.0%) return untransfused blood were re-issued with the assumption that they were still suitable to be transfuse (temperature <10ºC upon return). Another 202 (22%) was discarded due to various reasons where 115 pints (57%) from 202 were discarded due to high temperature (>10°C) upon return.

Therefore to ensure the quality and safety of blood for transfusion, this study is performed to determine and validate the PRBC warming rates and the quality of PRBC upon exposure to uncontrolled temperature after 30 minutes and four hours as per

guidelines. If the quality of PRBC is proven to be acceptable within minimum quality requirement upon exposure to uncontrolled temperature at certain timing from this study (30 minutes or four hours), it can be used as primary data to support and propose a standard guideline or procedure of allowable time to safely accept and time to justifiably discard the returned untransfused blood. This can later be implemented in blood transfusion service in Malaysia as it is may benefit the patient who receives the blood and in the same time reduce wastage.

1.3 Research Objectives

1.3.1 General Objective:

To study the effects of repetitive uncontrolled temperature exposure on the quality of packed red blood cells.

1.3.2 Specific Objective:

a. To evaluate the changes of PRBC's quality (haemoglobin, haematocrit, haemolysis rate, pH, potassium, lactate dehydrogenase (LDH) & sterility) over repetitive exposure to uncontrolled temperature over 35 days storage.
b. To compare the quality of PRBC in control group and experimental group (exposed to uncontrolled temperature for 30 minutes and 4 hours).
c. To determine the warming rate of PRBC exposed to uncontrolled temperature.

1.4 Hypothesis

1.4.1 Null Hypothesis

a. There is no significant difference in PRBC's quality (haemoglobin, haematocrit, haemolysis rate, pH, potassium, lactate dehydrogenase (LDH) & sterility) over repetitive exposure to uncontrolled temperature over 35 days storage.

b. There is no significant difference between quality of PRBC in control group and experimental group (exposed to uncontrolled temperature for 30 minutes and 4 hours).

1.4.2 Alternative Hypothesis

a. There is significant difference in PRBC's quality haemoglobin, haematocrit, haemolysis rate, pH, potassium, lactate dehydrogenase (LDH) & sterility) over repetitive exposure to uncontrolled temperature over 35 days storage.

b. There is significant difference between quality of PRBC in control group and experimental group (exposed to uncontrolled temperature for 30 minutes and 4 hours).

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Blood transfusion is practiced everywhere around the world but the general status of medical care in that area will determine the availability, safety and quality of blood and component supplied to the patient. In 1818, Dr. James Blundell has successfully handled the first human-to-human transfusion in post-partum haemorrhage patients in England (Lotterman & Sharma 2019). Ever since, rapid progression of knowledge and practice of blood typing, blood components, storage and blood transfusion has become a relatively common procedure for patient treatment. It was reported by Lotterman & Sharma (2019) that about 85 million blood units are transfused worldwide and 15 million blood units transfused yearly in the United States alone. Meanwhile it is estimated that an average of 2000 red blood cells units are needed daily to be transfused to patients throughout Malaysia (Lim Mei Ling et al., 2018).

2.2 Red Blood Cells.

Blood is highly specialised tissue which circulates in cardiovascular system. It contributes 7- 8% of total body weight with the average of 5L in adult men (Sharma & Sharma, 2018). The blood functions are to transport oxygen from lungs to body cells & tissues, transport carbon dioxide to lungs where it is excreted, transport nutrients from *gastrointestinal* tract to cells, nitrogenous wastes from body cells to

kidneys and transport hormones from glands cells. It also helps to maintain normal body pH by blood proteins (albumin) and bicarbonate, maintain circulatory/interstitial fluid by electrolytes that aid blood proteins (albumin) as well as maintaining body temperature. Besides, blood provides protection from foreign material and infections by leukocytes, antibodies & complement proteins. From whole blood, 55% are plasma, 45% are red blood cells and less than 1% are leukocytes and platelets.

Red blood cells (RBC) or erythrocytes matures in the bone marrow where it have evolved to optimally shedding nuclei and other organelles and packing high haemoglobin concentrations in their cytosol (Yoshida *et al.*, 2019). The haemoglobin is crucial for gaseous exchanged. Immature erythrocyte, known as a reticulocyte takes one day to become matured. Erythrocytes are lack of endoplasmic reticula and mitochondria. Thus they do not synthesise proteins and rely on anaerobic respiration which means they do not utilised the oxygen molecule they carry. On the other hand, RBC contains some structural proteins such as protein spectrin, a cytoskeletal protein element that enable them to change their shape to squeeze through capillaries and maintain their unique structure. Red blood cells is a flexible biconcave disc shape cell, 7-8 μm in diameter which allowing them to pass through the microcirculation with minimum diameter of 3.5 μm. It has 120 days lifespan with estimated total journey around 480 km (Hoffbrand & Moss, 2011).

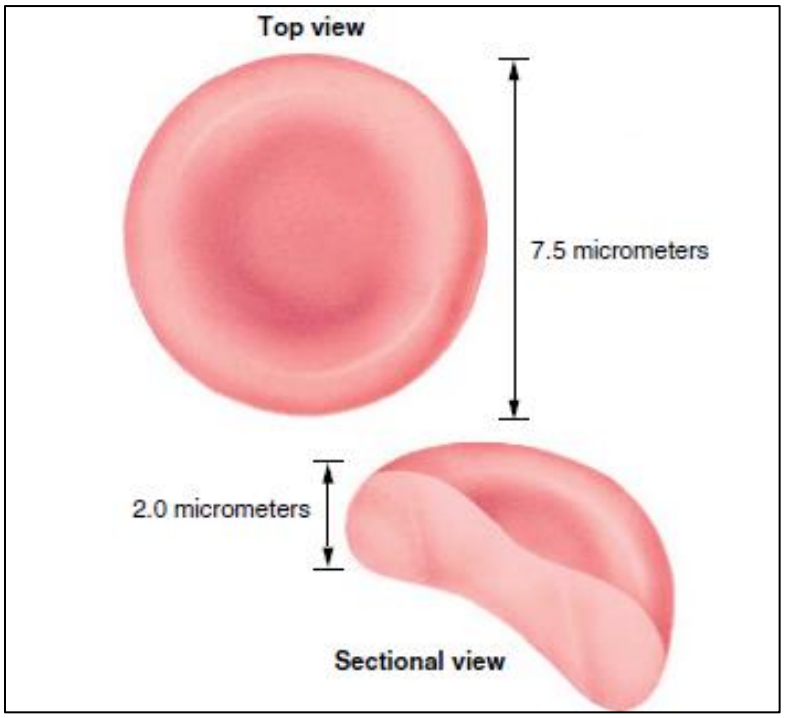


Figure 2.1 Discoid shape of RBC (Sonker, 2018)

2.3 Packed Red Blood Cells (PRBC)

The separation of whole blood into its components were started since the plastic blood bags was introduced in blood transfusion service and patented in 1950's (McCullough, 2012). From a unit of whole blood, PRBC, platelet concentrates and fresh frozen plasma can be obtained. Whole blood is rarely used because some coagulation factors especially factor V and VIII, and platelets lose viability and reduced in quantity within few hours or days in stored whole blood (Minatoguchi *et al.*, 2016). Consequently, whole blood is separated into its components and each of the components then can be stored at their optimal storage condition. PRBC are the most commonly used blood component in blood transfusion services. It is a blood component obtained by removing most of the plasma from the whole blood (Amin *et al.*, 2016).

The PRBC is prepared by centrifugation from whole blood with volume within 10% range from specified bag type used. For preparation of PRBC, double blood bag system is usually used. The blood components are separated by their mean density where the red blood cells with mean density of 1.100 g/mL are sedimented at the bottom part and the plasma with mean density of 1.026 g/mL will be at the top (Europe, 2015). About three-quarter of plasma is removed from whole blood to transfer bag after centrifugation leaving only about 50mL of plasma in one unit of PRBC. The volume for one pint of PRBC is normally between 191 – 245 mL if prepare from 350mL whole blood or 230 mL – 330 mL if prepared from 450mL whole blood (Europe, 2015). PRBC do not contain viable platelets and clinically significant amounts of coagulation factors. According to World Health Organization (WHO), the haemoglobin (Hb) concentration of PRBC shall be relatively 20 g/100 mL (not less than 45 g /unit) and the haematocrit is between 55-75%.

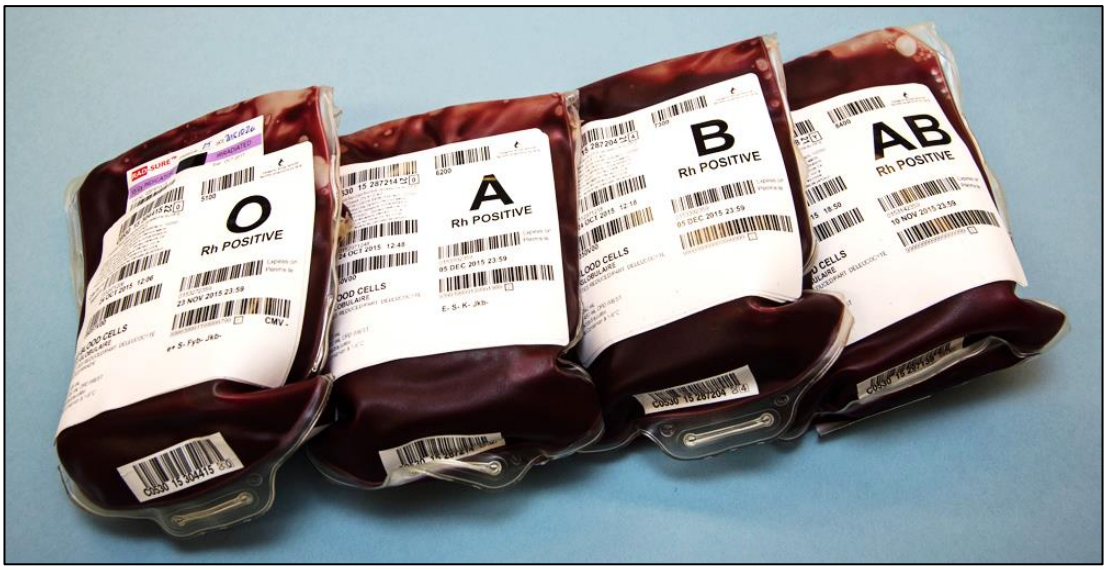


Figure 2.2 Packed Red Blood Cells

(Adapted from http://saskblood.ca/blood-products/red-blood-cells/)

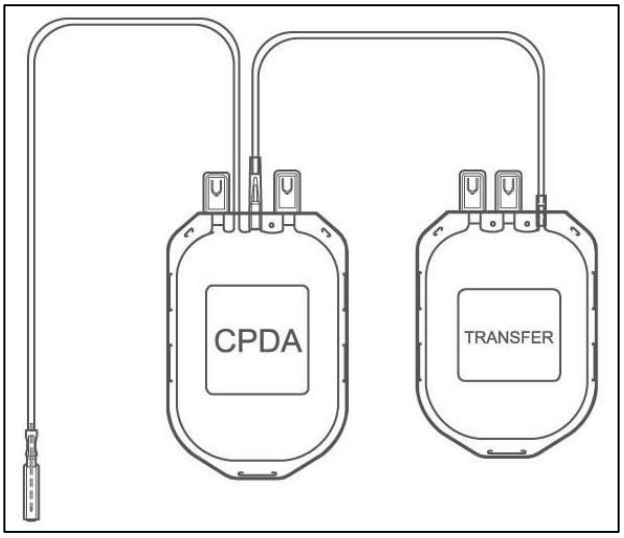


Figure 2.3 Double blood bag system.
(Adapted from http://www.inlabinstruments.com)

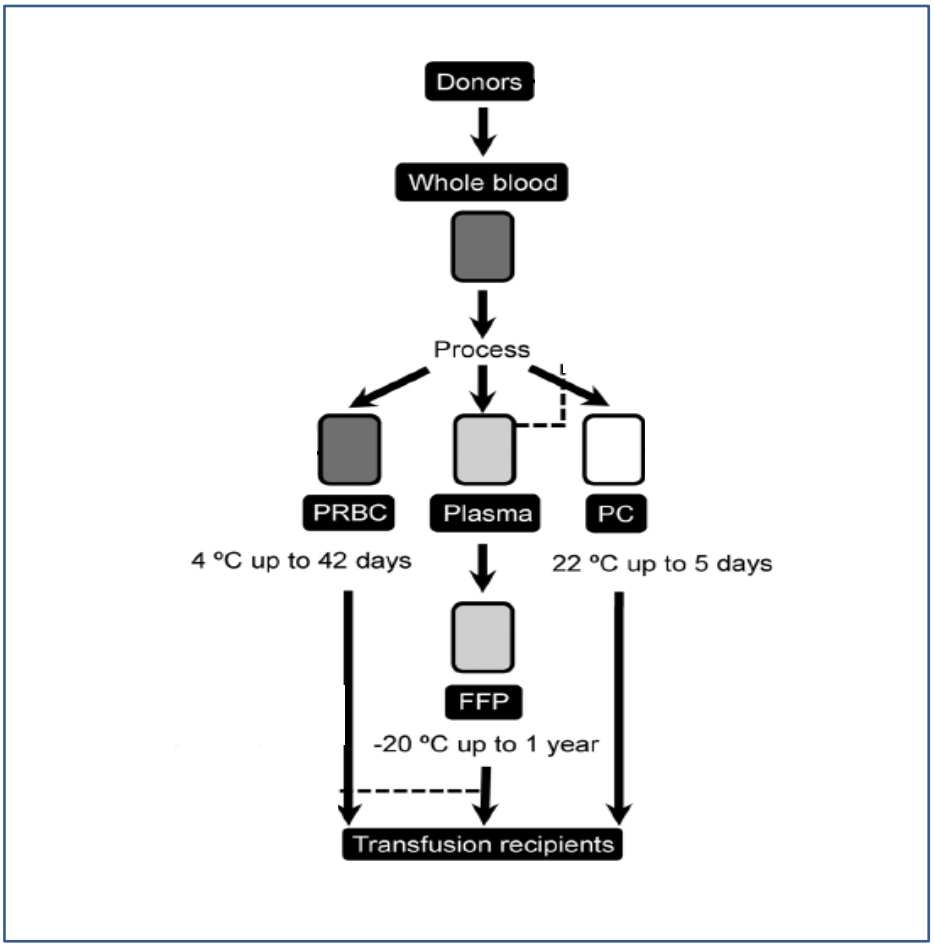


Figure 2.4 Schematic diagram showing blood component processing

(Noulsri & Palasuwan, 2018)

PRBC is indicated for patients with chronic anaemia and patient with acute blood loss resulting from surgery or trauma. It is transfused to prevent tissue hypoxia. However, the indication for PRBC transfusion is based on multifactorial parameters, such as the Hb concentration, together with the clinical criteria, patient preferences, and whether there are alternative treatments (Müller *et al.*, 2015). In adults, one unit of PRBC can increase Hb levels by about 1 g/dL and will raise the haematocrit by 3%. Where as in children, the transfusion of 5 mL/kg increases the Hb concentration by about 1 g/dL (Liumbruno *et al.*, 2009). Liumbruno G. and his colleague also reported that the transfused RBCs life span is about 50–60 days shorter than 120 days and can be significantly shorter in the presence of factors reducing their survival.

Unless patients have anaemia due to a hematinic deficiency, PRBC transfusion is typically recommended when their Hb levels reach 7 g/dL in those who have stable vital signs and non-symptomatic (Carson *et al.*, 2016). There is no evidence that a restrictive transfusion strategy with haemoglobin threshold of between 7 g/dL to 8 g/dL affects death or major adverse events such as cardiac events, myocardial infarction, stroke, pneumonia and thromboembolism compared with a liberal transfusion strategy. According to Carson J et al. (2016), a decreased by 43% of PRBC transfusion are observed across a broad range of clinical specialities including those people who are critically ill when using restrictive transfusion strategy.

With advanced technologies and proper knowledge, PRBC transfusions are relatively safe. However, some complications and adverse reaction still had a chance

to occur even rare, but can be life threatening. Common reactions related transfusion are allergic reactions, fever, urticarial and itchiness. Other serious reactions include acute immune haemolytic reaction, delayed haemolytic reaction and transfusion associated graft versus host disease (TA-GVHD) (Savage *et al.*, 2013; Sahu *et al.*, 2014). Each unit of PRBC are tested and screened for transfusion transmitted infection disease such as HIV, hepatitis B, hepatitis C and syphilis. The infectious complications have reducing to almost negligible levels with the implementation of better and newer blood screening methods (Sahu *et al.*, 2014).

2.4 Storage

PRBC has shelf life between 28 to 42 days depending on types of preservative and anticoagulant used. Anticoagulant and preservatives used are to prevent clotting as well as to supply nutrients for RBC viability and functionality during storage. The most commonly used anticoagulant is CPDA-1 (citrate, phosphate, dextrose and adenine) which allows for 35 days of shelf-life (Nguyen *et al.*, 2016). Citrate prevents coagulation by chelating calcium that will interferes the calcium-dependent steps in the clotting cascade. Dextrose aiding the generation of adenosine triphosphate (ATP) whereas adenine is added to improves ATP synthesis (Hardwick, 2008).

Blood oxygen-carrying ability is greatly reduced if it is not stored between 2°C to 6°C (WHO, 2005). Thus, PRBC must be stored at controlled temperature of 2°C to 6°C and transported in 2°C to 10°C to maintain the viability of the red blood cells and to prevent bacteria growth (Amin *et al.* 2016). Red blood cells are very sensitive to freezing where their membranes may rupture and the haemoglobin is

released (haemolysed). The transfusion of bacterial contaminated and haemolysed blood can be fatal.

Dedicated blood refrigerator shall be used to store the PRBC to ensure its integrity and quality. Unlike domestic refrigerator, the compressor of blood refrigerator is mechanically isolated from its body, thus there will be minimal or no vibration from the compressor that will unfavorly affects red blood cells. In addition, blood refrigerator has internal cooling fan in the cabinet, which will ensure the temperature is evenly distributed. It also has all around heavy insulation all to maintain temperatures between 2°C and 6°C and enable a longer holdover time in the event of power failure (WHO, 2002). More importantly, blood refrigerator usually comes with temperature-monitoring devices such as alarms for proper monitoring and safety. WHO does not advocate the use of domestic equipment for the storage of blood and blood components because of their design is not fit and does not guarantee the safe storage of blood components (WHO, 2005).

2.5 Storage Lesion

During storage throughout shelf life, PRBC experience progressive damage changes during storage. All the destructive biochemical and biomechanical changes in RBC during storage known as storage lesion (Almizraq *et al.*, 2012). This storage lesion is as consequences of storage under ex vivo, immobilisation, plasma environment, low temperature conditions, and low presentation of natural cellular (Antonelou & Seghatchian, 2016). As proposed by Vani *et al.*, (2016), RBC storage lesion can be categorised in three that are biochemical changes, biomechanical changes and oxidative damage.

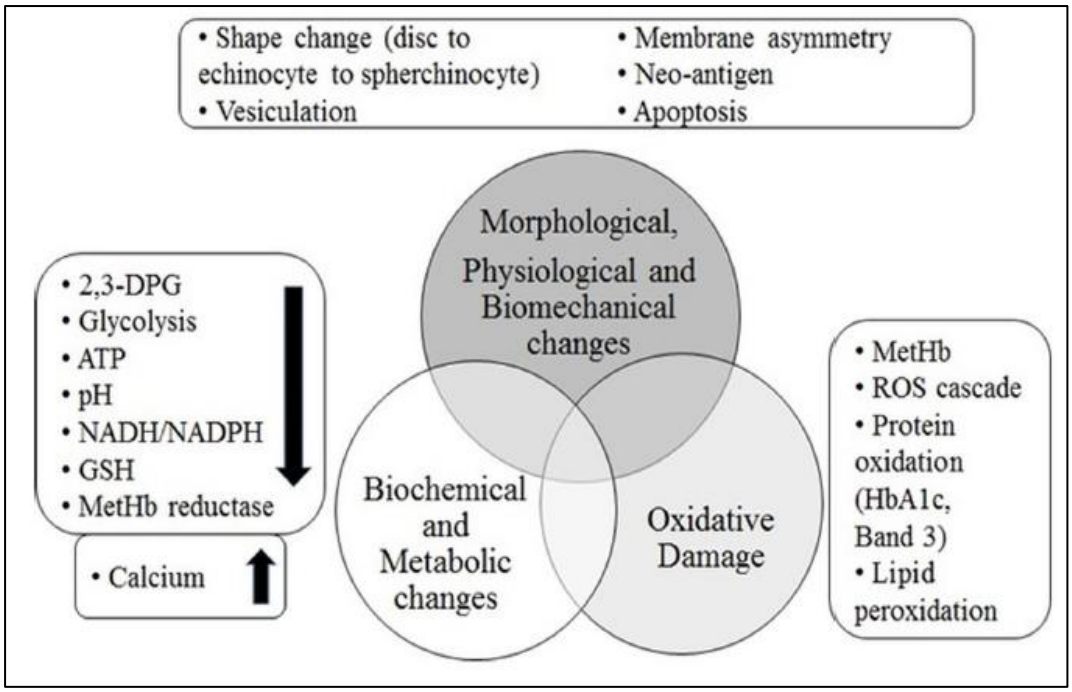


Figure 2.5 RBC storage lesion (Vani *et al.*, 2016)

2.5.1 Metabolic effects

RBC depends solely on adenosine triphosphate (ATP) generated from glycolysis for energy supply. The rate of glycolysis depends on temperature and more importantly on pH. Low storage temperature of 2ºC-6ºC will slow down the metabolism and reduce ATP production and RBC energy required functions (Flatt F. *et al.*, 2014). RBC breaks down one glucose molecule to produce two molecules of lactate, and at the same time two ATP molecules are generated. Accumulation of 2 protons produced from ATP hydrolysis then will leading to a decrease in pH and cause acidosis over time. The acidosis reduced the production of ATP as the glycolysis rate become slower (Chen *et al.*, 2016).

According to Chen *et al.*, (2016), RBC in vitro quality is affected by the gradual ATP decline in during storage. This is because RBC shape changes are correlated to the ATP concentration. Moreover, pH affects the level of 2,3-

diphosphoglycerate (2,3-DPG) which then will affects the haemoglobin oxygen carrying capacity. The decrease in pH value in stored RBC promote the breakdown of 2,3-diphosphoglycerate (2,3-DPG). The 2,3-DPG molecule enhance RBC O_2 unloading ability by enhancing haemoglobin to easily release oxygen to tissue. The oxygen dissociation curve will shift to the left in respond to low level 2,3-DPG. Patients undergo massive transfusion can be affected in this situation because of transfusion-induced acid–base abnormalities (Chen *et al.* 2016).

At low PRBC storage temperature, the major membrane sodium potassium pump (Na+/K+ ATPase) is inhibited leading to continuous intracellular potassium leakage and thus the potassium accumulate in the PRBC unit (Zimrin & Hess, 2009). The transfusion of high potassium PRBC will increase the risk of hyperkalaemia induced arrhythmia or even death in vulnerable patients, like those receiving massive transfusions and neonates (Müller *et al.*, 2015). These changes begin their evolution immediately as the blood is taken by the phlebotomist; however clinically relevant storage lesions only appear after a few days of refrigeration as shown in Figure 2.6. (Yartsev., 2016)

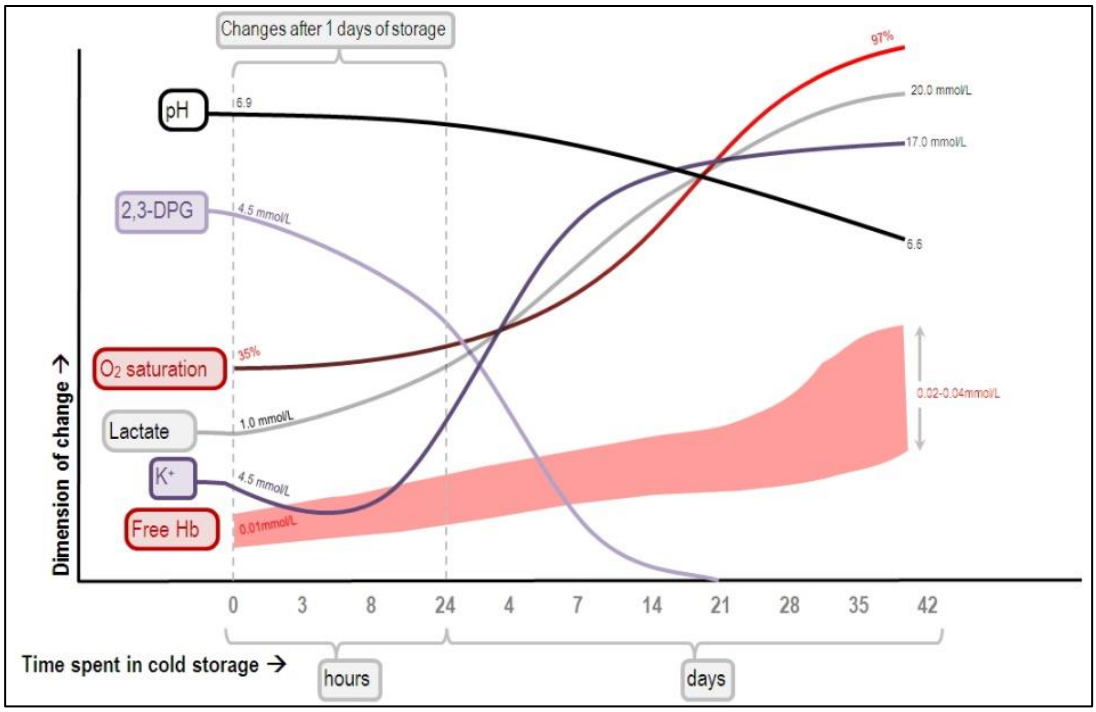


Figure 2.6 The biochemical changes in red blood cells during storage lesion. (Adapted from https://derangedphysiology.com)

2.5.2 Biomechanical effects

During storage, biconcave disc shaped RBC also undergoes slow changes to dense spheroechinocytes shape. The shape of RBC is influenced by the changes cause by ATP depletion and the oxidative damage to their membranes. Cluitmans *et al.*, (2012) suggest that deformability of RBC is a leading determinant of RBC survival, as infer by the association of abnormal RBC shape, anaemia, and splenic sequestration. Meanwhile Chen et al (2016) reported that these storage related RBC shape changes decreased RBC post-transfusion survival due to diminished surface to volume ratio and deformability.

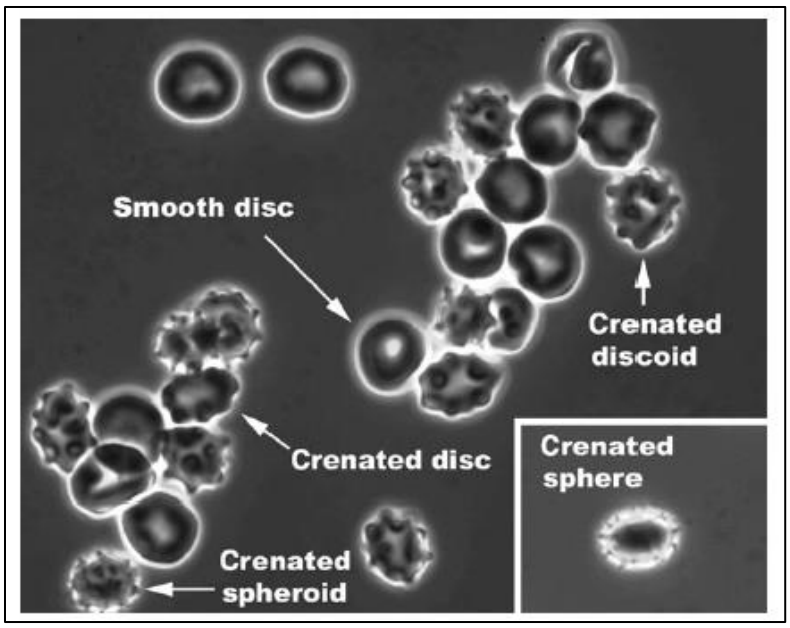


Figure 2.7 RBC morphology changes during storage. RBC become more echinocytic and spherical (Chen *et al.* 2016).

2.5.3 Oxidative effects

RBC function and viability fall apart as the oxidative damage persists throughout storage (Chen *et al.* 2016). RBCs are continually exposed to a pro-oxidative environment under aerobic storage conditions. RBCs are normally armed with superoxide dismutase and methaemoglobin reductase for oxidative damage repairing (Zimrin & Hess, 2009). However, RBC's antioxidant defence mechanisms become overwhelm as hydroxyl radical increases via fenton reaction with declining glutathione stores during storage. Equally important, RBC also go through continuous oxidative insult to protein and lipid during storage. For example, progressive oxidative stress cause the spectrin damaged correlates well to macrovesicles formation during storage (Chen *et al.*, 2016). From their study, Zimrin & Hess (2009) proposed that oxidative damage can generate lysophospholipid formation, which has correlation to transfusion-related acute lung injury (TRALI) incident.

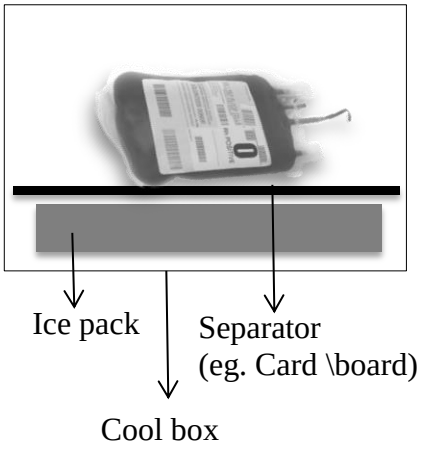
2.6 PRBC Transportation

PRBC should be supply to the ward only when patient is ready for transfusion. Ward or clinics personnel will pick up the PRBC unit from the blood bank and transport it to the ward using cool box and ice pack. The box/container used to transport the PRBC shall be the cool box which has insulated lid and body, robust, and tamper proof (Amin *et al*., 2016). The size commonly used is between 5 Qt (4.7 L) to 10 Qt (9.5 L) (Yusof *et al*., 2011). Currently in Malaysia, there are no strict rules for a standard type of cool box to be used but is acceptable as long as the box used is well insulated and able to keep the allowable cold temperature. The allowable PRBC transportation temperature is between 2-10ºC and the ratios of ice pack (coolant) to blood shall be validated (Amin *et al*. 2016). In practice, the cool box is seldomly validated for their capacity of ice retention. A study done by Yusof et al (2011) in Hospital Universiti Kebangsaan Malaysia conclude that the temperature chain of blood bags were not well maintained mainly because of the usage of non-standardised blood box and the non-compliance to the standard operating procedure (SOP) and guidelines.

During transportation, PRBC must be accompanied by a decent frozen ice pack in order to maintain the appropriate transportation temperature in the cool box (WHO, 2002). To prevent haemolysis, the ice packs must not directly touch the PRBC unit. A separator like cardboard or strawboard must be placed in between (Figure 2.8). The number of blood to ice pack ratio also play important role in keeping the temperature low. However, the quantity of blood taken for patients at the ward is normally between one to four units only at a time. Thus, minimum ice pack is required (1 or 2 depending size). It is important to note that currently the

temperature of cool box for blood transportation to the ward is not monitored by any thermometer or temperature tracking device.

a) Cool box & Ice packs
(Adapted from www.google.com)



b) Ice packs and PRBC placement in the cool box

Figure 2.8 PRBC transportation to the ward or clinic using (a) cool box and ice packs and (b) their placement in the cool box.

2.7 30 minutes and 4 Hours Rules

Based on Council of Europe and US guidelines, transportation of PRBC must maintain the temperature of the components below 10°C, where in the United States it does not have time limit and in Europe this is allowed for maximum of 24 hours (Thomas *et al.*, 2013). While in Malaysia, the allowable PRBC transportation temperature is between 2-10ºC also with no time limit (Amin *et al.* 2016). In blood transfusion service across the world, there are two rules that have been implemented to limit the PRBC exposure to outside storage temperature conditions: the 30 minutes rules and 4 hours rules. Among these two rules, 30 minutes rules is widely used and

studied (Brunskill *et al.*, 2012; Dumani *et al.*, 2013; Sandra Ramirez-Arcos *et al.*, 2013; Thomas *et al.*, 2013).

2.7.1 30 minutes rule

For the 30 minutes rule, any PRBC that left out from blood refrigerator and being exposed to uncontrolled temperature for more than 30 minutes must not be place back into storage for reissue but it shall be discarded (Carson et al. 2011; Brunskill *et al.*, 2012). In Malaysia, according to Amin *et al* (2016) PRBC and whole blood should be transfused within 30 minutes of removal from the blood refrigerator. The rationale to this rule is that the risk of bacterial proliferation will increase in time when PRBC warm up prior to the exposure of uncontrolled temperature. Besides that, the effects of temperature on the PRBC quality also need to be considered (Brunskill *et al.*, 2012). This rule is believed originated from a studies by Pick and Fabijanic conducted in the early 1970s where they found when blood temperature approached 10°C, the viability of RBCs was significantly diminished and blood unit left out at room temperature would reach core temperature of 10ºC in 45 to 60 minutes (Sandra Ramirez-Arcos *et al.*, 2013).

In regards of implementing this rule, the interpretation and compliance is broadly varies between countries as they defined "controlled environment" quite differently (Sandra Ramirez-Arcos *et al.*, 2013). As from European guidelines, they defined uncontrolled environment as been out of the blood refrigerator, while for Canadian Standard Association Standard, uncontrolled environment is when there is in the absence of temperature-monitoring system. According to National Blood

Centre, uncontrolled temperature is when the blood is removed from the blood refrigerator (Amin *et al.*, 2016).

2.7.2 4 hours rule

The four hour rule states that transfusion of PRBC units should be completed within four hours of their removal from controlled temperature storage (Brunskill *et al.*, 2012; Sandra Ramirez-Arcos *et al.*, 2013). This means that for a unit of PRBC, the time of transfusion into the patient must not exceed four hours. The blood is hang at the patient's bed side at room temperature during transfusion. Thus, the blood is actually being exposed to higher temperature than allowable storage and transport temperature.

The bacterial proliferation risk in blood unit is increased at higher temperature. D'Alessandro *et al.*, (2010) reported in their study that the high temperature changes over time may compromise the safety and efficacy of stored red blood cells, reducing their capacity to carry and release oxygen, increase haemolysis rate, decrease pH value and accumulating of free potassium which can harm the patient. Consequently, storage of RBCs at temperatures outside specified temperature ranges only will be accepted for a very limited period of time and that RBCs are often destroyed as a consequence. The question is raised whether some of those units as a matter of fact might have been suitable for transfusion (Gulliksson & Nordahl-Källman, 2014).

The evidence base to support current guidelines of 30 minutes and 4 hours is weak where very little current published report is available (Brunskill *et al.*, 2012).

Not only it is important to ensure the quality and safety of PRBC unit, it is also important to minimise the unnecessary wastage. Each unit of PRBC is precious and it must be manage wisely. It was reported by Thomas *et al.*, (2012) that in 2010 and 2011, more than 10,000 units of red blood cells in United Kingdom were discarded because of being out of controlled temperature for more than 30 minutes where the estimated wastage cost is over £1.2 million. In United Kingdom, it has been reported that 8005 (0.56%) pint of blood was discarded due to out of temperature control outside laboratory in 2017/2018 which equates to £1.0 million (Demand, 2017). While in United State, Whitney *et al.*, 2015 reported that 749 (4.0%) blood units were discarded upon return to blood bank after issue due to unsuitable for transfusion.

2.8 Returned Untransfused PRBC

Once the PRBC is taken out from blood bank, it is now exposed to uncontrolled temperature where it is been out of the blood refrigerator with no temperature-monitoring system. Therefore it is crucial to start the transfusion as soon as possible after reaching the ward (Amin *et al.* 2016) and blood should not be taken out from the blood box until it is ready to be transfused. The blood should not been left out on the nurse counter or at patients bed side. The high environment temperature may affect the PRBC. Therefore, in the event when the transfusion is postponed or cancelled, the ward or clinic shall return all untransfused PRBC immediately to the hospital blood bank. It is recommended in our national guideline that any untransfused blood must be return to the blood bank **immediately (**Amin *et al.* 2016).

Throughout 35 days of shelf life, PRBC is repetitively being in and out of blood bank refrigerator for processing, testing, transportation to wards/ blood facilities and as well as issuing to the patients. The returned untransfused blood which accepted back into the storage will then again use for testing and reissued to another patient and of course there is chances of this cycle being repeated for several time. To date, most of the hospital blood bank practice that any returned untransfused PRBC which exceeds temperature of 10°C and/ or being out from blood refrigerator for more than four hours should be discarded. To minimise risk of harm and for safety reason, the PRBC shall not be placed back into storage pool for reissue because it might affects the recipients of the blood. This practise is set based on the maximum allowable transportation temperature for PRBC which is below 10°C whereas the four hours' time limit is based on the rule that the transfusion of each unit shall not exceed four hours.

2.9 PRBC Quality Assessment

In common blood banking practice, the parameters used to study PRBC quality includes haemoglobin level, haematocrit, percentage of haemolysis and the sterility (Amin *et al.* 2016; Europe 2015). These are the minimum quality parameters requirement to assess PRBC quality which have been produced and supplied to patients. There are few international guidelines that can be referred to determine quality requirement and specification of PRBC (Europe 2015; Carson *et al*. 2011; Devine & Chen, 2014). While in Malaysia, minimum quality requirements for PRBC are haemoglobin level more than 45g/ unit, haematocrit between 65-75%, volume range between 191 mL to 265 mL, haemolysis less than 0.8% of red cell mass, and sterility test detect no microorganism growth (Amin *et al.*, 2016).