A PRELIMINARY STUDY ON PLATELET CONCENTRATES QUALITY COLLECTED FROM TWO DIFFERENT MIXING METHODS: MANUAL MIXING VERSUS AUTOMATED BLOOD COLLECTION MIXER

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UNIVERSITI SAINS MALAYSIA

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BY

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DECLARATION

Here, I declare that this research has been sent to Universiti Sains Malaysia for degree of Master of Science. It is also not be send to any other Universities. With that, this research might be used for consultation and can be photocopied as reference.

LIM WEN SIEW

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LIST OF SYMBOLS

fL	Femtolitre
L	Litre
%	Percent
mL	Millilitre
min	Minute
ºC	Degree Celsius
cm^2	Square centimetre
+	Positive
±	Plus, minus
>	Greater than
≥	Greater than or equal
<	Less than
≤	Less than or equal
rpm	Revolutions per minute
g	Gram
µL	Microliter

LIST OF ABBREVIATION

GPIa	Glycoprotein Ia
GPIb	Glycoprotein Ib
GPIIb/IIIa	Glycoprotein IIb/IIIa
VWF	von Willebrand factor
ADP	Adenosine disphosphate
ATP	Adenosine triphosphate
PDGF	Platelet derived growth factor
HLA	Human leukocyte antigen
HPA	Human platelet antigen
NBC	National Blood Centre
AABB	American Association of Blood Bank
ISBT	International Society of Blood Transfusion
PCs	Platelet concentrates
AP	Apheresis
PRP	Platelet rich plasma
BC	Buffy coat
PRP-PC	Platelet rich plasma derived platelet concentrates
BC-PC	Buffy coat derived platelet concentrates
AP-PC	Apheresis derived platelet concentrates
QC	Quality control

TWBC	Total white blood cell
WB	Whole blood
FFP	Fresh frozen plasma
CPD	Citrate, phosphate, and dextrose
PAS	Platelet additive solution
PVC	Polyvinyl chloride
ESC	Extent shape change
CO_2	Carbon dioxide
PI	Post-transfusion increment
CCI	Corrected count increment
PR	Percentage recovery
TMU	Transfusion Medicine Unit
ADL	Advanced Diagnostic Lab
AMDI	Advanced Medical and Dental Institute
USM	Universiti Sains Malaysia
RBC	Packed red blood cell
EDTA	Ethylenediaminetetraacetic acid
TTI	Transfusion transmission infection
SAGM	Saline, Adenine, Glucose, and Mannitol
JEPeM	Research Ethics Committee (Human)
LDH	Lactate dehydrogenase

ABSTRAK

Terdapat dua kaedah pencampuran darah semasa proses pengumpulan darah, iaitu kaedah pencampuran secara manual dan pengadun pengutipan automatik. Tujuan pencampuran darah yang dikumpulkan dengan antikoagulan dalam beg utama adalah untuk mencegah platelet dan faktor pembekuan awal pengaktifan. Kualiti pati platelet mungkin terjejas akibat daripada proses pencampuran yang tidak mencukupi semasa pengumpulan darah. Oleh sebab itu, kajian ini dijalankan untuk menilai sama ada pencampuran darah secara manual akan menghasilkan kualiti pati platelet yang sama degan pengadun pengutipan automatik. Sebanyak 30 peserta yang layak terlibat diri dalam kajian ini. Sebanyak 15 unit darah dikumpulkan dengan pengadun pengutipan automatik dan 15 unit lagi dikumpulkan melalui kaedah pencampuran manual. Pati platelet dihasilkan menggunakan kaedah “platelet rich plasma”. Pati platelet disimpan pada suhu 20°C hingga 24°C, di rak yang bergoyang selama 5 hari. Parameter kawalan kualiti seperti jumlah platelet sel, jumlah sel darah putih dan pH diuji pada pati platelet pada Hari 1 dan Hari 5. Hasil kajian ini menujukkan semua pati platelet mencapai keperluan kualiti yang dikehendaki daripada garis panduan pusat darah negara, dengan jumlah sel darah putih kurang daripada 0.2×10^9/unit dan nilai pH lebih daripada 6.4.

Walau bagaimanapun, hanya 27% hingga 40% pati platelet memenuhi keperluan standard kebangsaan iaitu bilangan platelet melebihi daripada 60 x 10^9/unit. Parameter jumlah platelet sel menunjukkan perbezaan median yang signifikan ($p< 0.05$) dari Hari 1 hingga Hari 5 bagi kedua-dua jenis kaedah pengumpulan darah. Walau bagaimanapun, untuk jumlah sel darah putih dan pH tidak menunjukkan perbezaan ($p> 0.05$) dari Hari 1 hingga Hari 5 bagi kedua-dua jenis kaedah pengumpulan darah. Terdapat tidak ada perbezaan yang signifikan secara statistik ($p> 0.05$) antara dua jenis kaedah pengumpulan darah pada kedua-dua Hari 1 dan Hari 5 untuk semua parameter yang diuji. Kesimpulannya, kualiti platelet seperti jumlah platelet, jumlah sel darah putih and pH yang dikumpul melalui kaedah pencampuran secara manual adalah serupa dengan pengadun pengutipan automatik.

A PRELIMINARY STUDY ON PLATELET CONCENTRATES QUALITY COLLECTED FROM TWO DIFFERENT MIXING METHODS: MANUAL MIXING VERSUS AUTOMATED BLOOD COLLECTION MIXER

ABSTRACT

There are two different mixing methods used in whole blood collection which are manual mixing and automated blood collection mixer. The purpose of mixing collected blood with anticoagulant in primary bag is to prevent platelet and clotting factors early activation. Platelet concentrates quality might be affected due to inadequate mixing process during collection process. Therefore, this study is to evaluate whether manual mixing method produces similar quality of platelet concentrates with automated blood collection mixer. In this study, total 30 eligible participants were included. 15 units of whole blood were mixed by automated blood collection mixer and another 15 units were mixed by manual mixing method. Platelet concentrates were produced by using platelet rich plasma method. Platelet concentrates were stored at 20°C to 24°C, continuous agitation for 5 days. Quality control parameters such as total platelet count, total white blood cell and pH were tested on these platelet concentrates at Day 1 and

Day 5. This study result showed all platelet concentrates achieved desired quality requirement from National Blood Centre Guideline with total white blood cell less than 0.2 x 10^9/unit and pH value more than 6.4. However, only 27% to 40% of the platelet concentrates met the national standard requirement in which the platelet counts were more than 60x10^9/unit. Total platelet count parameter showed significant median difference (p<0.05) from Day 1 to Day 5 for both types of mixing method. However, there were no statistical significant difference (p>0.05) for total white blood cell and pH from Day 1 and Day 5 for both types of mixing method. There was no statistically significant difference (p>0.05) between two mixing methods at both Day 1 and Day 5 for all the parameters measured. In conclusion, quality platelet concentrates which are total platelet count, total white blood cell and pH collected by manual mixing method has similar outcomes with automated blood collection mixer.

CHAPTER 1

INTRODUCTION

1.1 Background

1.1.1 Platelet morphology and structure

Platelet is small discoid anucleated cell, varying from 1 to 4 microns in diameter, which has mitochondria, produces and stores adenosine triphosphate and is high sensitive to its environment (Thon and Italiano, 2010). The mean volume of platelet cell is 7 to 11 fl, and it is the second most abundant corpuscle in the blood after red blood cell. The normal concentration of platelets in circulating blood is between 150 and 400 x 10^9 /L (Cardigan *et al.*, 2005; Hoffbrand and Moss, 2015). Platelet consists of several important cell structures, organelles, and antigens. The surface of the platelet is coated with glycoproteins molecule, important in platelet activation, adhesion, and aggregation which are the initial events for platelet plug formation during primary haemostasis process. There are several types of glycoproteins present on the surface of platelet which are glycoprotein Ia (GPIa), glycoprotein Ib (GPIb) and glycoprotein IIb/IIIa (GPIIb/IIIa). Each glycoprotein plays different roles in haemostatsis process. GPIa is directly adhesion to collagen when the vascular endothelium exposed. GPIb and GPIIb/IIIa are important in the attachment of platelets to von Willebrand factor (VWF), leads to platelet adhesion to subendothelium, platelet attachment and platelet aggregation subsequently. Besides, GPIIb/IIIa also plays an important role to activate and cross-link with fibrinogen and form a platelet-platelet aggregation. Platelet phospholipid membrane is important in the conversion of coagulation factor X to Xa and prothrombin (factor II) to thrombin (factor IIa) in common pathway of blood coagulation. Platelet consists of three types of storage granules which are dense granule,

alpha granule, and lysosomes. Each granule contains different substance inside it. Dense granules contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin and calcium. Alpha granule is the most frequent granule in platelet, consist of proteins such as clotting factors, VWF, platelet-derived growth factor (PDGF) and others. Lastly, lysosomes consist of hydrolytic enzymes. During platelet activation, the contents of the granules are released into the open canalicular system. The open canalicular system is located at plasma membrane of the platelet which forms an invaginate into the platelet interior structure and provide a large reactive surface to plasma coagulation proteins (Hoffbrand and Moss, 2015).

Platelet expresses several types of antigens on the surface which are ABO antigen, human leukocyte antigen (HLA) class 1 and human platelet antigen (HPA). These antigens play an important role in platelet-specific autoimmunity and alloimmunity. Each individual consists of two different alleles which are inherited from the parent (Hoffbrand and Moss, 2015). For instances, 28 of HPA biallelic groups have been recovered since 2003 and these are numbered as HPA-1 to HPA-28. Each gene is constructed by 2 different alleles (heterozygosity) or same alleles (homozygosity) termed as "a" or "b" alleles (HPA-1a or HPA-1b) (Nadarajan, 2014). Based on Tan *et al.* study, it showed the most common genotype observed among 17% of population in Malaysia were HPA-1a/1a, HPA-2a/2a, HPA3a/3b, HPA-4a/4a, HPA-5a/5a, HPA6a/6a and HPA-15a/15b. Whereas, 14.33% of Malaysians consist of HPA-1a/1a, HPA-2a/2a, HPA-3a/3a, HPA-4a/4a, HPA-5a/5a, HPA-6a/6a and HPA-15a/15b. The "b" allele generally occurred at low frequency among population. In contrast, from this study result, it was also found that HPA-1b occurred more commonly among Indians compared with Malay and Chinese. HPA-4b homozygous individual was identified in

Malays among other who has no heterozygous HPA-4a/4b individual in Chinese and Indian (Tan *et al.*, 2012).

Platelets originate from bone marrow megakaryocytes by fragmentation of the cytoplasm. Megakaryocytes are the largest cells in the body. Each megakaryocyte can form approximately 1,000 to 5,000 platelets by fragmentation process. The maximum normal shelf life of platelet in bloodstream is 10 days. However, shorten quiescent state of platelets might be due to platelets that are primed to undergo explosive activation after any vessel wall injured and damaged or any pathogenesis disease involved (Cardigan *et al.*, 2005). Besides, the platelet lifespan is also determined by the ratio of apoptotic BAX and anti-apoptotic BCL-2 proteins in the cell (Hoffbrand and Moss, 2015). Due to the uniqueness of cell morphology like the shape and small size, it enables platelet to access the edge of blood vessels and constantly to survey vascular integrity.

1.1.2 Platelet functionality

Once vessel wall integrity is compromised and subendothelial components are exposed, this will activate platelets in response to bleeding. There are three major platelet functions response to bleeding, which are adhesion, aggregation, and release reaction and amplification. Adhesion is the first initial step to create platelet-collagen interaction on the vessel wall for platelet immobilization once shedding has occurred. It is mediated through VWF in plasma which acts as bridge between collagen from vessel wall and glycoprotein GPIb/IX/V complex on the platelet surface. With the initial complex formed, this will slow down the blood flow and enable platelets to enroll and stick on the damaged area of the vessel wall. Subsequently, platelet receptor GPIa/IIa and GPVI will directly bind with collagen to form firm adhesion. Apart from binding to collagen,

platelet also bind to other subendothelial matrix protein such as fibrinogen to initiate adhesion process. Primary activation also induces subsequent platelet activation via various signal transduction pathways by elevating calcium levels in cytoplasmic, cause cytoskeletal change, platelet's shape change into pseudopod formation and platelet spreading. At the same time, granule components for instances serotonin, ADP and VWF are released by platelet intracellular to promote and amplify platelet recruitment, activation, aggregation and plug stabilization. Thromboxane which is the enzyme synthesis by platelet is important in secondary amplification of platelet activation to form a stable platelet aggregate and also promote vasoconstrictive activity (Cardigan *et al.*, 2005; Hoffbrand and Moss, 2015).

Aggregation is characterized by cross link reaction between adjacent platelet receptor GPIIb/IIIa with VWF and fibrinogen bridges. After platelet aggregation and release reaction promoted, phospholipid on the outer membrane of the platelet will change into negative charge. This will enhance the binding sites for several coagulation complexes and promote coagulation cascade to form stable fibrin in secondary haemostasis (Cardigan *et al.*, 2005; Hoffbrand and Moss, 2015). Deficiencies in the number and/or function of platelets may cause abnormalities in coagulation pathway and increase risk of bleeding. Therefore, platelet transfusion is required to treat or prevent haemorrhage in patients with thrombocytopenia or platelet malfunction e.g. Glanzmann´s thrombasthenia, Bernard-Soulier syndrome, Chédiak-Higashi syndrome and Hermancky-Pudlak syndrome (Cardigan *et al.*, 2005). To ensure successful treatment and good haemostatic function achieved by recipient, good quality and functionality of PCs product used for transfusion should be well preserved (Tynngård, 2009).

Transfusion medicine is not a laboratory-based discipline but more likely clinical oriented specialty which requires to ensure safe transfusion process from donor's vein

to patient's vein. This process involves blood collection, blood component processing, storage, testing, transfusion, and monitoring on patient transfusion outcome. There are several guidelines being referred for processing blood components, such as National Blood Centre (NBC) Guideline, American Association of Blood Banks (AABB) Guideline, International Society of Blood Transfusion (ISBT) Guideline, and others. According to each guideline, platelet concentrates (PCs) can be obtained through 3 different methods which are automated process of apheresis (AP), from WB donation via centrifugation using the platelet-rich plasma (PRP) method and from WB via buffy coat (BC) method. For the WB collection, 2 difference mixing methods can be employed which include manual mixing with scale and by using automated blood collection mixer. Uneven mixing process between citrate and blood immediately in primary bag will cause inadequate anticoagulation to react with cells component, resulting in decrease of labile coagulation factors, loss of quality for platelets and form of clumps that consist of erythrocytes, platelets, and some leucocytes (Amin *et al.*, 2016; Armstrong, 2008; Carson *et al.*, 2011). Therefore, mixing process during blood collection is important to ensure anticoagulant in blood bag will mix evenly and adequately with the collected blood. However, there was only scanty published data on this topic. Hence, a study to look at the quality of PCs collected using two types of mixing method either through manually or by using automated blood collection mixer is needed to ensure that the platelet product transfused meets the standard quality control and is effective in treating patient requiring platelet transfusion (Amin *et al.*, 2016; Armstrong, 2008; Carson *et al.*, 2011).

Quality control (QC) of PCs is the tool available to assess component quality result and play as troubleshooting references to adjust production techniques when necessary. There are several methods that can be performed to assess the quality of PCs which are

tests of platelet function and metabolism *in vitro*, animal models of thrombocytopenia, recovery and survival of labeled autologous platelets in normal volunteers and platelet increment studies in thrombocytopenic patients (Cardigan and Williamson, 2003). However, platelet function tests are not easy to perform but also time-consuming, difficult to control test quality, lack sensitivity and usually require specialist laboratory support (Cardigan *et al.*, 2005). In this study, only 3 parameters of platelet QC which are total platelet count, total white blood cells (TWBC) and pH will be assessed as per recommendation from NBC Guideline (Amin *et al.*, 2016).

1.2 Research Justification

This study shall compare any significant difference on quality of PCs collected from two different mixing methods of blood collection which are manual mixing and automated blood collection mixer.

The rationales of this study are:

(1) To conduct first validation study on automated blood collection mixer, Bag Mixer BIO33, Italy based on quality of PCs produce.
(2) To evaluate whether manual mixing method produces similar quality of PCs with automated blood collection mixer.
(3) To conduct first study in Malaysia regarding comparison of PCs quality collected from two different mixing methods which are manual mixing and automated blood collection mixer.

1.3 Objectives

General Objective

To study the quality of PCs collected from two different mixing methods which are manual mixing and automated blood collection mixer.

Specific Objectives

1. To determine the PCs quality which are total platelet count, TWBC and pH collected from two different mixing methods: manual mixing and automated blood collection mixer.
2. To determine the changes of PCs quality which are total platelet count, TWBC and pH over 5 days of storage collected from two different mixing methods: manual mixing and automated blood collection mixer.
3. To compare the quality of PCs which are total platelet count, TWBC and pH collected from two different mixing methods: manual mixing and automated blood collection mixer.

1.4 Hypothesis

Study Hypothesis

There is a difference between quality of PCs which are total platelet count, TWBC and pH collected from two different mixing methods: manual mixing and automated blood collection mixer.

CHAPTER 2

LITERATURE REVIEW

2.1 Platelet concentrates collection methods

2.1.1 Apheresis (AP)

As per ISBT guideline, for AP procedure, the blood from the donor is processed with a cell separator with an in-line centrifuge for platelet collection. The platelets are transferred to a collection bag, whereas the other blood cells and most of the plasma are returned to the donor. Various cell separators are available for platelet collection and have different collection principles which are intermittent-flow and continuous flow. Whole AP blood donation usually requires about 2 hours (J.Hardwick, 2008). Based on NBC Guidelines, AP donor shall have to fulfill the standard criteria as per whole blood (WB) donor selection criteria and usually they are converted from regular WB donor (more than 5 times WB donation) with good vein. AP donor is allowed a maximum donation of total volume of 15 liters platelet and/or plasma in 24 times, in a year time. AP donor shall be subjected to have minimum interval of 2 weeks rest time between successive donation. The minimum pre-donation platelet count for a prospective platelet AP donor must be more than 150 x 10^9/L. The PRP and BC methods require pooling of platelets from several donors (usually 4-6) to obtain a platelet dose equivalent to that which can be obtained from a single donor by AP collection. The yield of the platelets count in a bag is more than 200 x 10^9/unit, standard adult therapeutic dose (Amin *et al.*, 2016). Since only one donor is required to produce a transfusion unit by AP technique, the risk for HLA antigens alloimmunization is reduced as the recipient in this case is exposed to less antigen stimulation. Besides, this method also limits the risks of transfusion transmission infections expose to recipient. AP platelet is the first-choice

platelet product use for transfusion, however, the limitations are high producing cost involved and high technology cell separator required (J.Hardwick, 2008). Therefore, not every institute can afford to have AP technology to collect PCs.

2.1.2 Whole blood collection by manual mixing and automated mixing

Platelets derived from WB is another product commonly used for transfusion. WB is the source material for blood component preparation such as packed red blood cell (RBC), fresh frozen plasma (FFP) and PCs. Among three components obtained from a unit of WB collected, platelets are the most extremely labile, very easily activated and it's quality easily affected during sample collection and processing (Greening *et al.*, 2011). During WB collection, it is important to ensure the blood is adequately mixed with anticoagulant from the time it starts entering the primary bag. Mixing is important to prevent platelet consumption and clotting factor activation (Van Der Meer and Pietersz, 2006). Low platelet count in PCs and low levels of coagulation factors including factor VIII in FFP can be the major negative consequences if inadequate mixing of WB with anticoagulant which subsequently cause the initiation of coagulation process in WB collected (Armstrong, 2008).

During blood donation, approximately 450mL of WB from each donor is collected directly into bag containing CPD (citrate, phosphate and dextrose) anticoagulant, to prevent the blood from clotting. Citrate binds to calcium, and therefore it prevents the activation of clotting factors and thus clot formation. Citrate is the most commonly used anticoagulant in blood component collection (Green *et al.*, 2015). According to ISBT guideline and NBC guideline, there are two methods use to mix the anticoagulant with the WB in primary bag which are manual mixing, immediately and continuously

upside-down of the bag on the scale every 30 seconds during donation and by using automated blood collection mixer (Armstrong, 2008; Amin *et al.*, 2016).

Blood collection mixer had been developed to automate several steps in the blood-collection process. The bags of the blood collection system are placed in the mixer, and once the vein is punctured, the device mixes the blood with the anticoagulant. Automatic mixer measures and checks the volume of blood collected according to type of blood bag used along the donation process. Once the desired volume is collected, the mixer automatically clamps the donation tubing to prevent overfilling of the bag. The ratio between volume of blood collected and volume of anticoagulant in the bag should be correct. Overweight blood bag indicates insufficient anticoagulant reacts with blood collected and this may lead to initiation of clotting process. Underweight blood bag means there is an excessive anticoagulant within blood collected which could denatures the blood properties during storage. Underweight and overweight blood collection are not suitable for transfusion and usually will be discarded. Besides, mixer measures the flow speed, and can be programmed to sound an alarm when the blood flow falls below a predetermined level. The duration of the donation is very important to prevent initiated coagulation especially during slow flow. PCs cannot be processed if the duration of donation was longer than 12 minutes. Furthermore, FFP and cryoprecipitate cannot be produced if donation took longer than 15 minutes. Hence, it was important to record the donation duration (Van Der Meer and Pietersz, 2006; Armstrong, 2008).

Besides, mixing the collected blood with anticoagulant, blood collection mixer also measured the volume donation accurately and monitored the blood flow rate and time taken for the donation. However, there are some limitations by using blood collection mixer which are high cost of the equipment, approximately 5 times higher than scale

and practical difficulties in terms of difficult for transportation, battery loading and the bulky size of the mixer (Follea *et al.*, 1997).

In the absence of an automated blood collection mixer, the bag should be hung upside down on the scale to allow the inflowing blood mix with the anticoagulant immediately upon entering the bag. The content of the collection bag should be manually mixed every 30 second throughout the collection period. There is risk for inadequate mixing process with anticoagulant in primary bag if WB is collected manually using scale (Armstrong, 2008; Amin *et al.*, 2016).

In late 19 centuries, G.Follea *et al*. conducted a similar study regarding comparative manual and automated methods for mixing and volume control in WB collection. Three different brands of automated blood collection mixers which were Compomixer (NPBI, Germany), Optimix (Baxter, Belgium) and Hemo-matic (Hemopharm, France) were used to compare with manual scale in the study. The study showed that manual or the automated mixing procedures had equal accuracy and reproducibility of the blood volumes obtained, collection times and flow rates. The quality of all these products including red blood cell concentrates, PCs and plasma units collected from automated procedures were found to be comparable to that manual procedure. The study concluded that there was no significant advantage from the use of automated blood collection monitors as compared to simple scale with manual mixing method at that upon of time (Follea *et al.*, 1997).

Another study by De Korte D and Veldman HA in 2001 showed that certain blood mixing devices failed to mix effectively, especially at low bleeding rates. Manual mixing of the collection containers showed sufficient mixing of WB with anticoagulant compared to blood collection mixer (de Korte and Veldman, 2001).

Subsequently, another evaluation study on automated blood collection mixers with latest improvement features were done by P. F. van der Merr and & R.N. Pietersz, in 2006. Two different brands of automated blood collection mixers which were CompoGuard (Fresenius Hemocare, Netherlands) and HemoLight (Fresenius Kabi AG, Germany) were evaluated in the study. The result showed that CompoGuard blood collection mixer gave sufficient mixing at low flow rate (30 mL/min), normal flow rate (60mL/min) and high flow rate (90 mL/min). HemoLight only able to mix well at normal and high flow rate. However, the overall mixing results obtained from this study showed great improvement compared with the similar study done before by De Korte D and Veldman HA in 2001. This study suggested that the quality of blood collection mixers had improved over the year (Van Der Meer and Pietersz, 2006).

2.2 Platelet concentrates derived from whole blood

2.2.1 Platelet rich plasma (PRP) method

Platelets derived from WB are produced by the PRP method in the USA, whereas the BC method is used in Europe and Canada (Vassallo and Murphy, 2006). Both PRP and BC methods are used to separate blood components from WB based on principle of centrifugation; each cellular has different size and density properties. This will make each cellular sediment at different rates when centrifugal force is applied. Red cells have greater mass and highest density, it will settle at the bottom of the blood bag. Whereas, white cells will sediment above the red cells, platelet form a layer above the white cells and leave the plasma and anticoagulant at the top of suspending fluid (J.Hardwick, 2008).

PRP method involved soft spin centrifugation to obtained PRP followed by a high-speed centrifugation to obtain a platelet poor plasma and platelet pallet (J.Hardwick,

2008). Most of the plasma is removed, and the platelets are stored in a reduced volume of remaining plasma. Alternatively, the platelets are re-suspended in a synthetic medium such as platelet additive solution (PAS) (Ringwald *et al.*, 2012). The critical step in the production of PCs by using PRP method is the validated first soft centrifugation at a speed and time needed to maximize the number of platelets, and minimize the number of red cells and leucocytes in plasma. The most challenge step using PRP method to produce PCs is removal of buffy coat from red cell concentrates after soft-spin, as the interface between these 2 components are not sharply defined (J.Hardwick, 2008). Furthermore, if the second centrifugation is performed too hard, the platelet button will form the stubborn aggregated platelets in the bottom of the bag. This will make irreversible platelet aggregation in PCs and significantly compromised the PCs quality produced (Ringwald *et al.*, 2014).

2.2.2 Buffy coat (BC) method

There are two methods to prepare BC which are conventional quad bag method and top and bottom bag method. With the BC processing method, WB is centrifuged at high-speed to prepare a BC. The BCs are pooled and centrifuged to a platelet-rich supernatant that is transferred to the storage container. The critical step to produce a high number of platelets with low white and red cells count in PCs by BC method is the validated second centrifugation setting. It is important to sediment the residue red cells and white cells whereas maximized platelet remain suspended in plasma as supernatant. Besides, due to the volume of BC is small, the bag fails to insert and sit vertically in centrifuge bucket and this will cause pockets cells trapped in the fold. This will interface between residue red cells and PRP after centrifugation. Proper supports like special plate or dummy bag shall be inserted to withstand the BC in the centrifuge bucket (J.Hardwick, 2008).

2.2.3 Comparison between platelet concentrates produced by three different processing methods

The selection of PCs preparation methods should be based on each institution availability of resource and efficacy. There is substantial variability of QC of the products due to the different preparation methods. In the study of RP Singh *et al*. (2009), there was no significant different in terms of platelet count per unit, swirling score and pH between platelet rich plasma derived platelet concentrates (PRP-PC) and buffy coat poor derived platelet concentrates (BC-PC) units. However, BC-PC had been found less white blood cell contamination compared with PRP-PC unit. AP-PC units showed better swirling score and platelet count than PRP-PC and BC-PC units (Singh *et al.*, 2009). Besides, apheresis derived platelet concentrates (AP-PC) units contained less WBC contamination compared with PRP-PC and BC-PC units. The study suggested that the quality of AP-PC was superior than PRP-PC and BC-PC. AP-PC may be recommended and indicated only in selected patients or HLA-matched platelet transfusion (Singh *et al.*, 2009). Another study was done by RS Mallhi *et al*., in year 2015, also found that quality of AP-PC was better than BC-PC. However, one AP-PC unit cost approximately five to six times higher than 6 BC-PC units. AP-PC should be reserved for more deserving multi-transfused immunocompromised patients and HLA-matched/HPA-matched/crossmatched platelet transfusion are indicated (Mallhi *et al.*, 2015).

2.3 Storage Requirement

The shelf life of PCs varies between 3 and 7 days, accordingly to local regulatory authorities enforcement. Malaysia is practicing maximum 5 days shelf life of the PCs used for transfusion (Amin *et al.*, 2016). PCs are stored at room temperature (20°C to 24°C) with constantly agitating. It is necessary to maintain platelet viability during storage.

2.3.1 Storage lesion

Storage at 20°C to 24°C is susceptible in promoting bacteria growth and increase the risk of bacterial contamination in PCs. Therefore, short shelf life of the PCs was implied (Tynngård, 2009). There are many factors influence the quality of platelets during storage which include PCs preparation method, type of plastic material used for storage bag, permeability of the bag surface to allow gas exchange, storage temperature, type of anticoagulant used, total volume of the PCs in the bag and the motion of agitation (Murphy and Gardner, 1975; Slichter and Harker, 1976). Theoretically, every manipulation of platelets may lead to the activation or apoptosis of the cells. Platelets exposure to plastics, centrifugation, filters, gases, solution and temperature changes may create a stress, leading to alterations (Garraud *et al.*, 2016). This is commonly defined as storage lesion where changes that alter the physiological properties in collected blood over time. Platelets lose their functionality and viability during *in vitro* storage and are not suitable to use for treatment of patient who requires platelet therapy (J.Hardwick, 2008).

2.3.1.1 Storage temperature

Decrease storage temperature will increase platelet stickiness and lead to aggregation. In 1967, Pert *et al*., showed more clumping in PCs from chilled blood compared with the blood kept at room temperature (Pert *et al.*, 1967). In 1971, another study done by Kattlove *et al.,* reported higher aggregation tendency from PCs prepared from blood stores at low temperatures (0°C to 6°C) than blood kept at 23°C and 37°C. From the study, they postulated that low temperature may cause conformational changes in platelet membrane protein and induced aggregation (Kattlove and Alexander, 1971). In a study conducted by Welch *et al*., in 1985, result showed number of platelet clumps reduced when the temperature of centrifuge and storage environment increased from 20°C to 24°C (Welch and Champion, 1985). As per discussed above, platelet aggregation may increase due to lower temperature exposure. Therefore, PCs shall always process and keep in optimal storage temperature 20°C to 24°C.

2.3.1.2 Storage container

PCs shall be kept in good bag container, to ensure proper gas exchange throughout 5 days lifespan. The most common problem with platelet container is lack efficiency of gas permeability and exchangeability from the surface of the container. If platelets are in hypoxia condition, this will lead to increase platelet glycolysis and increase lactate acid production. Subsequently, this will induce low pH value and ATP level, and cause insufficient energy supply (Gulliksson *et al.*, 1992). Hence, changes in pH in PCs indicate significant defect on platelet viability. First-generation storage bag made of polyvinyl chloride (PVC) plasticized with di(2-ethylhexyl)phthalate had poor oxygen permeability and therefore PCs only compromised for 3 days storage. With improvement, second-generation storage bags produced by different material which is

polyolefin (Murphy *et al.*, 1982) and PVC plasticized with tri(2-ethylhexyl) trimellitate (Simon *et al.*, 1983) which had greatly increased on gas permeability and allowed the storage period to be extended to 5 days. Furthermore, PVC plasticized from butyryl-tri-n-hexyl-citrate have shown good gaseous permeability and good *in vivo* viability of platelets upon transfusion (Turner *et al.*, 1995).

2.3.1.3 Overloading platelet

Overloading platelet contents in storage container will cause increased capacity of oxygen transportation from storage container which leads to immediate negative effects on platelet metabolism and energy supply. This will cause delayed effects on platelet function and some platelet disintegration (Gulliksson *et al.*, 2012). Another factor that could potentially cause loss of viability by storage of PCs in volume less than 35mL. Lesser platelets volume will increase container surface-to-PC volume ratio and lead to more frequent platelet container wall interaction and cause platelet activation (Singh *et al.*, 2009). A study done by Bode *et al.* (1989) showed that it was important to reduce container surface area-to volume ratio from $7cm^2$ to $4cm^2$, to prevent less platelet activation during storage (Bode and Miller, 1989). By reducing this factor, less lactate produced, platelet morphological and function integrity were greatly improved during storage (Bode and Miller, 1989).

2.3.1.4 Duration of storage

It is known that the metabolic activity of platelets continues throughout storage and even with all the procedures have been taken to preserve platelet haemostatic viability. A study done by Maria *et al.* (2011) showed throughout storage of PCs for 5 days, platelet aggregation decreased with storage whereas platelet count and pH increased with storage (Maria José Dantas *et al.*, 2011).

Similarly, another study showed that Day 6 samples platelet yield/unit and pH value were higher than Day 1 samples. Day 1 samples indicated low CD62P expression compared with Day 5 samples. This showed most of the platelets were undergo degranulation and activation at Day 5 (Mastronardi *et al.*, 2013). The study result also implied slightly reduction of morphology score and percentage of extent shape change (ESC) for Day 1 sample compared with Day 5 sample (Mastronardi *et al.*, 2013). Another study done showed increase in percentage of platelet expressed CD62P, 20-30% at Day 4/5 to 30-50% at Day 7/8 (Dumont *et al.*, 2002). There was a 30% decreased in the ESC between Day 1 and Day 7 of PCs stored in plasma (Holme *et al.*, 1990). Another study also showed 20%-45% decreased in platelet morphology score between Day 1 and Day 7 storage (Dumont *et al.*, 2002). By decreased morphology score and ESC, platelets showed loss of discoid shape and poor recovery and survival rate *in vivo* (Holme *et al.*, 1978).

2.4 References guidelines for platelet concentrates quality requirement

QC parameters that were used for assessment of PCs included swirling, volume, platelet count and white blood cell count per bag and pH change. There were few international guidelines that can be referred to determine quality requirement and specification of platelet components produced (Amin *et al.*, 2016; Carson *et al.*, 2011; WHO, 2002; Europe, 2015).

In Malaysia, minimum quality requirements of random PCs are platelet count more than 60 x 10^9/ unit, volume remain 50mL to 70mL, TWBC count less than 0.2 x 10^9/ unit prepared from PRP method, TWBC count less than 0.05 x 10^9/ unit prepared from BC method, pH more than 6.4 and sterility test detect no microorganism growth (Amin *et al.*, 2016).

Summary of parameters for assessment of PCs quality from different guidelines are shown in table 2.1 (Amin *et al.*, 2016; Carson *et al.*, 2011; WHO, 2002; Europe, 2015).

Table 2.1: Summary of platelet concentrates quality requirement from different guidelines

Parameters / Guidelines	**Platelet Count**	**WBC Count**	**pH**	**Sterility**	**Volume**	**Acceptance Range of % Tested Products to Meet Criteria**
NBC PRP method: BC method:	60 x 10^9/unit	 <0.2 x 10^9/unit <0.05 x 10^9/unit	> 6.4	No growth	50-70mL	75% for other testing parameters except for total white blood cell count 90% for total white blood cell count
AABB Pool platelets leucocytes reduced: single filtrated PC:	55 x 10^9/unit	 <5 x 10^6/unit <8.3 x 10^5/unit	> 6.2	No growth	40-70mL	90% for total platelet count and pH 95% for total white blood cell
WHO	55 x 10^9/unit	<0.12 x 10^9/unit	Not specify	Not specify	50-60mL	Not Specify
Council of Europe	60 x 10^9/unit		> 6.4	Not specify	50-70mL	75% for total platelet count

Parameters / Guidelines	Platelet Count	WBC Count	pH	Sterility	Volume	Acceptance Range of % Tested Products to Meet Criteria
PRP method: BC method:		$<0.2 \times 10^9$/unit $<0.05 \times 10^9$/unit				90% for total white blood cell count

2.5 Quality control of platelet concentrates

2.5.1 Total platelet count

Platelet count is the total number of platelets exist in the final storage bag. Platelet count is not only interfered by platelet metabolism activity throughout storage but also with all procedures that allow platelet haemostatic viability (Seghatchian and Krailadsiri, 2000; J.Hardwick, 2008). Platelet count is an important assessment required to perform and to check sufficient cells content in PCs. Low platelet yield in PCs will cause ineffective platelet increment post transfusion (Maria José Dantas *et al.*, 2011).

2.5.2 Total white blood cell

Leucocytes contamination in PCs compete for available oxygen and this will result in giving negative effects on platelet metabolism, increase glucose consumption, decrease energy supply, increase lactic acid production, significant decrease in pH value, but also delayed effects on platelet function and some platelet disintegration (Gulliksson *et al.*, 2012; Cardigan and Williamson, 2003). High concentration of leucocytes in 5 days storage of PCs will cause high excretion of β-TG, loss of platelet nucleotides, decreased ability to incorporate H-adenosine and poor platelet morphology (Saran, 2003; Pietersz