EVALUATIVE STUDY OF PLATELET APHERESIS BY HAEMONETICS MCS+ AND COMPARISON WITH RANDOM DONOR PLATELETS

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of Bachelor of Health Sciences (Biomedicine).

March 2005

CERTIFICATE OF APPROVAL

This is to certify that the dissertation entitled,

"EVALUATIVE STUDY OF PLATELET APHERESIS BY HAEMONETICS MCS+ AND COMPARISON WITH RANDOM DONOR PLATELETS"

is the bonafide record of research work done by Haslinda binti Mohd Fauzi during the period from July 2004 to March 2005 under our supervision.

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ACKNOWLEDGEMENT.

In the name of Allah, the most Loving and Merciful, I pray my sincerest thank to Him because of His willing, I am finally able to complete my dissertation.

It is a pleasure to record my gratitude to all the following people who had helped me with the completion of this dissertation, directly or indirectly.

I am very indebted to my supervisor, Dr. Rapiaah Mustaffa and my co-supervisor, Dr. Tariq M. Roshan (both from Haematology Department, School of Medical Sciences, USM) for their willingness in accepting me as their student. Their invaluable and continuous guidance, advice and encouragement throughout the preparation of this dissertation, from the start till the end are highly appreciated.

I want to thank Dr. Noor Izani Noor Jamil, the Coordinator for Final Year Student Research Project, School of Health Sciences (PPSK) for always understanding and tolerant to us, the final year Biomedical students in the completion of our projects.

I would like to thank Assoc. Prof. Pim Chau Dam, the Coordinator of Biomedical Program, School of Health Sciences (PPSK) for his opinion and concern in my project.

I also thank Assoc. Prof. Dr. Syed Hatim Noor and Dr. Norsaadah Bachok from Biostatistics and Research Methodology, PPSP, USM for their helps in solving statistical matters of my project, despite having many other demanding commitments.

My thanks, too, go to all the staff of Transfusion Medicine Unit, HUSM. Your natural warmth made me feel so welcomed and glad to do my project there. To Mr. Lim, the Scientific Officer of Transfusion Medicine Unit, thank you for your guidance in designing method for collection of random donor platelet (RDP) samples and giving the information I need. To all the staff nurses, thanks for being a great help in collecting apheresis platelet concentrates (PCs) samples. To all the laboratory staff, your cooperation in collecting the RDP samples and QC record is very much acknowledged.

My dissertation would also not have been able to be completed without the helps of all the staff of Haematology Laboratory, PPSP. They had given their utmost cooperation and guidance to me despite their daily busy jobs. Thank you very much for teaching and helping me to do hematological laboratory procedures on the apheresis blood samples.

I am also very grateful to all the staff of Unit Kemudahan Makmal (UKM), PPSK for their contribution in culturing of the PCs samples. To Pak Chu, thank you for allowing me to do the culture in PPSK labs. To En. Amri and En. Anizan, thanks for your generous ideas and cooperation with the process of culturing the PCs, even sometimes when it was over the office hours.

To my friends, Haiza, Che Shuraya, Arnida, Shikin and Azim, you all are simply true friends. I doubt I would be able to go through all the turbulences to complete this dissertation without your support, opinion and encouragement.

Last but not least, my special thanks go to my beloved parents. Without their invaluable love, care and never-ending support, I would never have made it.

Thank you, everybody!

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LIST OF ABBREVIATIONS AND SYMBOLS

AABB	–	American Association of Blood Banks
ACD	–	Acid-citrate-dextrose
cm	–	Centimeter.
CMV	–	Cytomegalovirus
Corp.	–	Corporation
DOF	–	Degree of freedom
EDTA	–	ethylene diamine tetraacetic acid
etc	–	et cetera
FBC	–	Full blood count
FNHTR	–	Febrile non-hemolytic transfusion reactions
g	–	Gram
Hb	–	Hemoglobin
Hct	–	Hematocrit
HLA	–	Human Leukocyte Antigen
HUSM	–	Hospital Universiti Sains Malaysia.
IFC	–	Intermittent-flow centrifugation
IgA	–	Immunoglobulin A
IQR	–	Interquartile range
Kg	–	Kilogram
L	–	Liter
MPV	–	Mean platelet volume
mL	–	milliliter
n	–	Number of samples
No	–	Number
PCs	–	Platelet concentrates.
PPSK	–	Pusat Pengajian Sains Kesihatan (School of Health Sciences).
QC	–	Quality control
RBC	–	Red blood cell
RDP	–	Random donor platelets.
SD	–	Standard deviation.
TVP	–	Total volume plasma
UPT	–	Unit Perubatan Transfusi (Transfusion Medicine Unit)
WB	–	Whole blood
WBC	–	White blood cell
WHO	–	World Health Organization
°C	–	Degree Celcius
µL	–	microliter
$>$	–	More than
$<$	–	Less than
$\geq$	–	Same or more than
$\leq$	–	Same or less than
$\pm$	–	Plus minus

ABSTRAK

"Platelet concentrates" (PCs) boleh disediakan daripada darah utuh (RDP) atau daripada teknik apheresis. Untuk memastikan kualiti PCs yang baik, beberapa piawai telah ditetapkan oleh Unit Perubatan Transfusi (UPT) dan mengikut piawai ini, PCs apheresis perlu mempunyai kiraan platelet lebih daripada 2 x 10^{11}/ Unit, isipadu melebihi 40 mL setiap 60 x 10^{9} platelet dan kontaminasi sel darah putih (WBC) kurang daripada 0.5 x 10^{9}/ Unit. RDP pula perlu mempunyai kiraan platelet lebih daripada 55 x 10^{9}/ Unit, isipadu antara 40 hingga 60 mL, kontaminasi WBC perlu kurang daripada 0.5 x 10^{9}/ Unit dan kontaminasi sel darah merah (RBC) kurang daripada 1.0 x 10^{9}/ Unit. Kedua-dua RDP dan PCs apheresis perlu bebas daripada mikroorganisma. Objektif utama kajian ini adalah memastikan sama ada piawai-piawai ini dipatuhi atau tidak dan membandingkan kontaminasi bakteria, WBC dan RBC antara PCs apheresis dan RDP. Untuk mencapai objektif ini satu kajian prospektif telah dibentuk untuk dijalankan di UPT dan 47 sampel RDP dan 47 sampel PCs apheresis telah diuji. Kami juga telah mengkaji kesan apheresis ke atas kiraan darah lengkap (FBC) sebelum dan selepas pendermaan, kesan dari aspek hemolisis dan kesan sampingan ke atas 50 penderma yang menggunakan Haemonetics MCS+. Keputusan kajian menunjukkan kriteria untuk RDP dan PCs apheresis dipatuhi kecuali kontaminasi RBC pada kedua-dua teknik dan isipadu RDP. Kontaminasi WBC dan RBC pada PCs apheresis lebih tinggi daripada RDP, tetapi kontaminasi bakteria pada

PCs apheresis lebih rendah. Perbandingan FBC sebelum dan selepas pendermaan menunjukkan penurunan signifikan pada kiraan platelet manakala kiraan WBC, hemoglobin dan hematokrit meningkat dengan signifikan. Secara umumnya tiada hemolisis berlaku pada darah penderma. Toksisiti sitrat adalah kesan sampingan yang tidak serius tetapi paling kerap dialami. Kesimpulannya PCs apheresis dan RDP menepati piawai kecuali untuk kontaminasi RBC, isipadu RDP dan kontaminasi mikroorganisma. Apheresis platelet oleh Haemonetics MCS+ adalah teknik yang selamat dan berkesan.

ABSTRACT

Platelet concentrates (PCs) can be prepared by two methods, including apheresis and random donor platelets (RDP). To ensure good quality of PCs, standards have been set up by Transfusion Medicine Unit and according to these apheresis PCs should have platelet count more than 2.0×10^{11}/ Unit, volume more than 40mL per 60×10^{9} platelets and white blood cell (WBC) contamination less than 0.5×10^{9}/ Unit. Similarly RDP should have platelet count more than 55×10^{9}/ Unit; volume within 40 to 60 mL, WBC contamination should be less than 0.5×10^{9}/ Unit and red blood cell (RBC) contamination should be less than 1.0×10^{9}/ Unit. For both platelet apheresis and RDP there should be no growth of microorganism. The main objective of this study was to determine whether the standards were met or not and to compare the different parameters including contamination with bacteria, WBC and RBC between RDP and apheresis. To meet our objectives we designed a prospective study to be conducted in Transfusion Medicine Unit and 47 apheresis PCs and 47 RDP samples were collected. We also studied the effects of apheresis on pre- and post-donation full blood count (FBC), hemolysis and side effects experienced by 50 donors using Haemonetics MCS+. Results of our study showed that the criteria for apheresis PCs and RDP were met except for RBC contamination in both techniques and volume in RDP, WBC and RBC contaminations in apheresis PCs were unexpectedly higher than in RDP, while bacterial contamination was lower in apheresis PCs. Comparison of pre- and post donation counts showed, significant decrease in platelet count while WBC, hemoglobin and hematocrit levels were significantly increased. In general no hemolysis was observed in apheresis donors by Haemonetics MCS+. Citrate toxicity was mild but it was the most common side effect during apheresis donation. We concluded that quality of apheresis PCs and RDP met the standards except for RBC contamination, volume of RDP and bacterial contamination. Platelet apheresis by Haemonetics MCS+ is a safe, reliable and effective method.

1. INTRODUCTION

1.1 PLATELET CONCENTRATES (PCs).

Platelet concentrates (PCs) are one of the primary products produced from the whole blood (WB). PCs prepared from WB are usually termed as "random donor platelets (RDP)" and the volume of it ranges from 40 to 60 mL to achieve a therapeutic dose 4 to 6 units from different donors are pooled. An alternative of collecting PCs is by platelet apheresis. PCs volume collected from this method range from 200 to 300 mL. Plate 1 shows PCs collected from apheresis while Plate 2 is an example of PCs obtained from whole blood.

Plate 1: Platelet concentrates collected from apheresis technique

Plate 2: Platelet concentrates derived from whole blood/ random donor platelets (RDP)

1.1.1 Indications of PCs.

Platelet concentrates are widely used for a variety of patients. Platelets are transfused to control or prevent bleeding associated with critically decreased circulating platelet numbers or functionally abnormal platelets. Indications of PCs transfusion is shown in Table 1.

Table 1 Indications of PCs transfusion.

Indications	Remarks
Thrombocytopenic patients	Platelet count is less than $50 \times 10^9/L$. Decreased platelet production or decreased platelet function.
Cancer patient	Platelet count is less than $20 \times 10^9/L$. Induced thrombocytopenia during chemotherapy and radiation.
Thrombocytopenic preoperative patient	Platelet count is less than $50 \times 10^9/L$.
Post-operative bleeding	
Post-bone marrow transplant recipient	
Massive transfusion.	Rapid use of platelets for hemostasis and the dilution of the platelets by resuscitation fluids and stored blood can cause thrombocytopenia.

Note: PCs transfusion is not usually effective or contraindicated for patients with increased destruction of circulating platelets caused by autoimmune disorders such as idiopathic thrombocytopenic purpura (ITP).
Source: Modern Blood Banking and Transfusion Practices, D. M. Harmening, 1997.

Each of unit PCs should increase the platelet count $5 \times 10^9/L$ to $10 \times 10^9/L$ in a typical 70-kg individual.

1.1.2 Collection, preparation and storage of PCs.

Processing of whole blood in order to prepare PCs should be done within 8 hours of collection. According to World Health Organization (WHO) Technical Report Series, No 840, 1994 Annex 2 – Requirements for the collection, processing, and quality control of blood, blood components and plasma derivatives (Requirements for biological substances, No 27, Revised 1992), blood shall be obtained from the donor by means of a single venepuncture giving an interrupted flow of blood with minimum damage to the tissue. Also it is required that the time and speed of centrifugation used to separate the platelets will produce a suspension without visible aggregation or hemolysis.

Same storage condition applies to both PCs of whole blood and apheresis; PCs must be stored at 20 – 24°C with continuous and gentle agitation by a special agitator to avoid platelet activation. PCs; whether they are from whole blood or apheresis are usually collected or prepared by a closed system and can be stored up to 5 days. If they are prepared or collected from open system, they must be transfused to patients within 6 hours. Pooled platelet concentrates are prepared using open system and this shortened their recommended storage time to 4 – 6 hours.

1.2 QUALITY CONTROL OF PCs

Quality control is operational techniques and activities used to fulfill the requirements for quality. It is also an important element in quality assurance. Performing quality control is an essential practice in blood banks to provide a high level of safe blood donation and transfusion practices to regulatory agencies, accrediting agencies, blood donors, physicians, patients, and patients' families (Daniel M. Harmening, 1997). Quality control of platelet concentrates includes the aspect of its pH, platelet count, volume, sterility and contamination of WBC and RBC. It is recommended by WHO in its Technical Report Series 1994 that PCs units shall be randomly selected at their end of shelf-life and tested at a regular basis. American Association of Blood Banks (AABB) standards, as quoted by Daniel M. Harmening, 1997 stated that 75 percent of plateletpheresis products tested contain a minimum of 3.0 x 10^{11} platelets and 75% of units from RDP tested must contain at least 5.5 x 10^{10} platelets at pH >6.2. The volume of RDP should be 50$\pm$10 mL while plateletpheresis products must be >200mL in volume. (Blaney and Howard, 2000). Blood component quality control used in Transfusion Medicine Unit HUSM, which is used as a reference standard in this study, is adapted from quality control guide of University of Malaya, National Blood Center and AABB. Table 2 shows the summary for quality control of PCs applied by Transfusion Medicine Unit HUSM.

Table 2: PCs quality control applied by Transfusion Medicine Unit HUSM.

Component	Parameters checked	Quality requirements	Frequency
Platelet concentrates or RDP (random units)	Volume	50 $\pm$ 10 mL	1% of annual production with a minimum of 10 units per month
	Platelet count	>55 x 10^9/Unit	
	RBC residuals	<1.0 x 10^9/Unit	
	WBC residuals	<0.5 x 10^9/unit	
	pH	>6.0	
	Sterility	No growth of bacteria	
Plateletpheresis	Volume	>40mL per 60 x 10^9 platelets	1% of annual production with a minimum of 10 units per month
	Platelet count	>200 x 10^9/Unit	
	WBC residuals	<0.5 x 10^9/Unit	

1.3 APHERESIS.

Apheresis is defined as withdrawal of blood from a donor, with a portion (plasma, leukocytes, platelets, etc.) being separated and retained and the remainder retransfused into the donor (Dorland's Medical Dictionary, 26th edition, 2001). Therefore collection of platelet by apheresis is termed as plateletpheresis.

1.3.1 History and development of apheresis in Transfusion Medicine Unit, HUSM.

Increasing needs of PCs, especially PCs that can be collected from a single donor along with the increase in usage of plateletpheresis technique in Malaysia lead to the application of this technique in Transfusion Medicine Unit HUSM. Apheresis technique had begun in this unit since 4th April 2001. Currently there is an average of 5 donors donating platelets and plasma by apheresis technique in every week. Apheresis is mainly used to collect plasma and platelets from donors and occasionally used for therapeutic purposes.

1.3.2 Haemonetics MCS+

Currently two Haemonetics MCS+ apheresis machines are being used in Transfusion Medicine Unit HUSM. This machine employs the system of intermittent-flow centrifugation (IFC); the process of drawing of whole blood, separating into components, retention of desired components and reinfusion of undesired components to the donor needs to be done in one complete cycle before beginning the next cycle. Separation occurs when the anti-coagulated blood enters a disposable centrifugation bowl which is then subjected to various centrifugal forces resulting in the separation of the blood into its various components. In Transfusion Medicine Unit HUSM, Haemonetics MCS+ machines are programmed to collect platelet and plasma products, but they are also able to collect red blood cells, leukocytes and peripheral blood stem cells. Plate 3 shows a photograph of Haemonetics MCS+ apheresis machine. Figure 1 shows a diagram of cross-section of Haemonetics MCS+ centrifuge bowl.

Plate 3: Haemonetics MCS+ apheresis machine

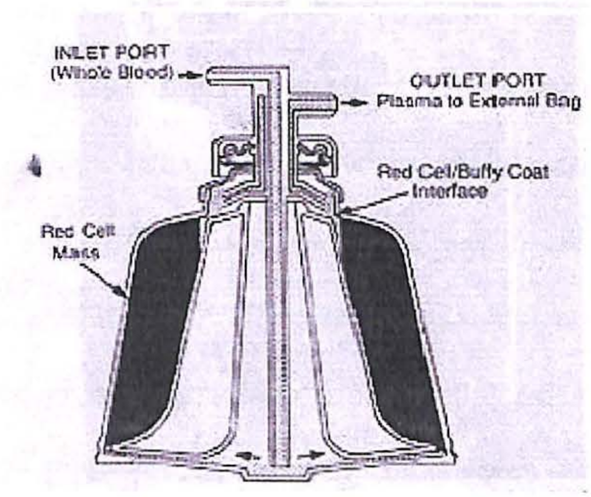

Figure 1: Cross-section of Haemonetics MCS+ centrifuge bowl.

1.3.3. Advantages and quality of plateletpheresis

This technique is said to have more advantages compared to whole blood collection technique. A standard platelet concentrates from apheresis contains 3.0 X 10^{11} platelet, while a standard platelet concentrates from whole blood donation has 5.5 X 10^{10} platelet. By collecting a large volume of PCs from a single donor, donor exposure is reduced, thus the risk of disease transmission and exposure to foreign antigens also can be reduced. Besides, a therapeutic dose can be obtained with special characteristics, such as being HLA-matched or IgA-deficient (Ritchard G.Cable and Richard L. Edwards, 2001). Apheresis PCs are usually collected from an HLA-matched donor to transfuse it to a patient who had developed refractoriness or unresponsive to RDP transfusion. This is because the patient had developed alloantibodies to foreign platelet antigen or HLA due to frequent PCs transfusions. As body system is able to replace the loss of the donated platelets within 2 days, platelet apheresis donors can donate up to every 2 weeks compared to whole blood technique which enables donations only once in three months.

A study is carried out by Eugenia Vasconcelos *et al.*, 2003 on quality of platelet concentrates derived by platelet rich plasma, buffy coat and apheresis and they concluded that platelets derived from these three methods differ in terms of in vitro functional activity, aggregation states and storage characteristics, as measured by automated cell counters and pH assessment. This may be related to either differences in the subpopulation of platelets and leucocytes recovered or the processing/storage-induced cellular damage. Leitner GC *et al.* in 2003 in their study reported that the P-selectin expression on multicomponent collection platelets (collected by MCS+ device) was within the range of P-selectin found in platelet concentrates obtained by other apheresis devices. In this study, platelet count and PCs volume were among the parameters that were used to assess the quality of PCs collected by Haemonetics MCS+ and RDP.

1.3.4. Leukodepletion

The prevention of transfusion reactions and transmission of infectious diseases partly relies on the systematic removal of leukocytes. The quality of leukodepletion is dependent on the filtration rate, which should be as slow as possible, as quoted by Daniel Kientz *et al.*, 2001. A study which they conducted in 2001 showed that residual leukocyte level in platelet concentrates collected by Haemonetics MCS+ is approximately 1 x 10^5/ Unit.

Seghatchian J. *et al.*, 2002 in their study on characterization of causes of leukodepletion failures said that based on the large scale United Kingdom's statistical process monitoring data, leukodepletion rate is estimated to be about 20% at 1×10^6 and less than 3% at 5×10^6 by flowcytometry.

Another study of whole blood WBC-reduction filter reported the mean and maximum whole blood-derived PCs WBC counts after filtration were 0.08 x 10^6 and 0.3 x 10^6, respectively per filtered unit, which meets current standard. (Larsson *et al.*, 2001).

R. Moog and N. Müller, 1999 evaluated performance of three blood cell separators (Amicus, AS. TEC 204 and Haemonetics MCS+) on white cell reduction during plateletpheresis and reported that MCS+ gave the lowest WBC count and does not exceed 1 x 10^6/ Unit.

Maximum standard requirement of WBC reduction varies from one country to another. France required that maximal residual leukocytes for both red cell and apheresis platelet concentrates are 1 x 10^6 per filtered unit (G. Andreu *et al.*, 1998). Leukocyte poor PCs with leukocytes <5 x 10^6 may be assumed to fulfill the specification of the Guide to The Preparation, Use and Quality Assurance of The Council of Europe, as reported by H. Olthuis *et al*, 1998.

1.3.5 Full blood count of the donors.

Besides the issue of leukodepletion, sustained decrease of platelet count have been documented to occur in regular plateletpheresis donors as reported by Ellen F Lazarus *et al.*, 2001. Another study done by Cengiz Beyan *et al.* in 2003 on effect of plateletpheresis on donor full blood count (FBC) values using three different cell separator system also revealed that WBC, hemoglobin (Hb), hematocrit (Hct) and platelets were decreased significantly. However, J. Ringwald *et al*, 2003 found no significant decrease in platelet count and no significant increase of WBC, Hct and Hb after donation. Therefore one of the aims this study is to compare the pre and post donation FBC in apheresis donor. From this test we can determine the effect of the apheresis procedure on the above mentioned parameters of apheresis donor. As we collect plasma and platelets but not collect RBC, this should result into increasing count of Hct and Hb after donation.

1.3.6 Hemolysis during apheresis procedure

This study also aims to detect any damage to red blood cells (RBC) that may occur during apheresis procedure. Centrifugation rate is from 3000 to 7000 rpm, and the rate is programmed according to each protocol applied (Haemonetics MCS+ General Operator Manual 1996). Incorrect centrifugation rate can be a factor in causing hemolysis of the blood. Hemolysis risk is also increased in machine malfunction or operational error (Cable RG and Edwards RL, 2001). A study also reported hemolysis as one of the immediate adverse effects in apheresis procedure. (McLeod BC *et al.*, 1998).

1.3.7 Side effects of plateletpheresis

There are also other several side effects occurring to apheresis donors. During the procedure, the most common complaint is that people feel numbness and tingling around their mouths and in their fingertips, caused by citrate – one of the component of the anticoagulant (acid citrate dextrose) used to prevent the blood from clotting inside the apheresis machine. Other side effects are haematoma around the venipuncture site, allergic reactions and dizziness. A growing body of evidence suggests that episodes of fainting can deter volunteer blood donors from returning to donate in the future. In contrast, relatively little is known about the effects of significantly more common mild reactions (e.g., faintness, dizziness, lightheadedness) on donor retention (Christopher R. France *et al.*, 2004).

1.3.8 Bacterial contamination

In the United States, the incidence of bacterially contaminated platelet concentrates is approximately 1 in 1000 platelet concentrates. Skin commensals such as *Staphylococcus epidermidis* and *Bacillus sp.* are the organisms most often implicated in platelet bacterial contamination (Karen-Mae T. Mitchell and Mark E. Brecher, 1999). In Malaysia the incidence is expected to be higher as aseptic technique is sometimes not properly applied during phlebotomy. Another cause of contamination is the transient presence of microorganism in the donor's blood, who is asymptomatic at the time of donation. In this study, bacterial contamination assessment of PCs was included as a part of PCs quality control parameters.

2. OBJECTIVES.

The main objective of this study is to determine the quality of platelet concentrates collected by apheresis machine, Haemonetics MCS+ in terms of platelet count, volume, and WBC, RBC and bacterial contamination and comparing the contaminations with random donor platelets. Secondary objectives of this study are:

- To detect any changes in FBC of apheresis donors by examining the pre and post donation FBC.
- To assess hemolysis caused by apheresis procedure by examining pre- and post-donation of RBC morphology and free hemoglobin in plasma.
- To study side effects of plateletpheresis on donors

3. MATERIALS AND METHODS.

3.1 MATERIALS.

3.1.2 Approvement from Research and Ethics Committee.

This study had obtained ethical approval from Ethical Committee, School of Medical Sciences, Universiti Sains Malaysia. The approval letter is included in Appendix A.

3.1.3 Sample size estimation.

Sample size required for this study was calculated by using the results of hematologic data in a study done by R. Moog and N. Müller, 1999 which is relevant to this study. Sample size was calculated by using Sample Size Calculation Program software. From the calculation, the sample required is 37 samples. By considering drop-out rate of 6% and oversampling of 10 samples for each technique, 50 samples needed to be collected. In this study 50 blood samples before apheresis and 50 blood samples after donation have been taken. 47 PCs samples each; from random donor platelet and apheresis also were tested.

3.1.4 Donors.

The period of study was from September 2004 to January 2005. Apheresis donors who donated platelets by apheresis technique using Haemonetics MCS+ apheresis machine in Transfusion Medicine Unit HUSM were included in this study. Inclusion criteria for the study were similar to apheresis donor qualification criteria and also the willingness of the donor to participate in this study. A detailed criteria of apheresis donor is attached in Appendix B. All donors included in this study have given informed consent. Essential information of the study was given to donors who participated in this study and signed consent form was obtained from them to fulfill the requirement of Research & Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia.

Sample form used to obtain signed consent is included in Appendix C. Donor's particulars relating to apheresis donation were taken after the procedure. A sample of form used to record the particulars is attached in Appendix D. During the procedure, donors were asked if they were experiencing any side effect.

3.1.5 Blood agar preparation.

This was done at Biomedical Laboratory, School of Health Sciences (PPSK). About 10 to 40 blood agar plates were prepared in one batch depending on the number of samples that could be cultured in 2 weeks of time. This was done keeping in view the expiry date for a manually prepared blood agar which is 2 weeks after preparation. About 40 to 45 plates of blood agar can be produced from 1 liter of blood agar. For this study a total of about 30 plates were used to culture RDP and 43 plates for PCs from plateletpheresis; occasionally 2 PCs samples were cultured on a plate.

3.1.5.1 Whole blood.

Expired human whole blood was used and was obtained from Transfusion Medicine Unit HUSM. Several universal bottles were autoclaved before the blood was put into them. After that the bottles were left for at least overnight at 4°C to allow separation of blood into

RBC and plasma. Amount of blood needed to be used was roughly estimated by this way. Usually if the bottles contained 40 – 45 percent of RBC, 3 bottles of whole blood were used to prepare one liter of blood agar.

3.5.1.2 Blood agar base preparation.

Blood agar base powder produced commercially by Oxoid company was used. The amount of powder needed to be used was calculated by using this equation: 36g of powder = 900mL of distilled water. For example, if 500 mL blood agar was desired to be produced, 450 mL distilled water and 18g of powder was used. The distilled water and powder were then put into a 1-liter or 2-liter beaker, mixed and boiled while continuously stirred with spatula until the mixture or blood agar base became clear yellow in color. This was done to ensure proper mixing of the mixture. After that the mixture was poured into a 500 mL Duran bottle and autoclaved. It was then allowed to cool to 55°C and the temperature was maintained by putting it into water bath set at 55°C.

3.5.1.3 Mixing of whole blood and blood agar base.

The universal bottles containing the separated blood were allowed to warm up to room temperature first. After that the separated blood were mixed back to whole blood by gentle rolling of the universal bottle. The next step was pouring the whole blood into the Duran bottle containing blood agar base with proper aseptic technique applied. The Duran bottle was rolled to ensure proper mixing of whole blood and blood agar base. The rolling was gently done to avoid hemolysis.

3.5.1.4 Pouring of blood agar liquid into plates:

This was done in a biosafety cabinet. After mixing, the blood agar liquid was poured into plates as soon as possible before the blood agar liquid turned to be solid blood agar. This step was also done with proper aseptic technique. After that the agar were left to be hardened and cooled at room temperature for 3 to 5 hours before putting the plates into a refrigerator to store them at 4°C.

3.2 METHODS.

3.2.1 Parameter for quality control of PCs.

The parameters used to determine the quality of PCs include its volume, platelet, WBC and RBC count.

3.2.1.1 Apheresis PCs.

Volume reading of PCs harvested was taken from Haemonetics MCS+ machine parameter readings which the procedure was done with. 3 to 5 mL of PCs was taken by Transfusion Medicine Unit HUSM staff nurse from sample pouch attached to the platelet concentration bag into EDTA tube. Platelet, WBC and RBC count of the PCs were performed using hematological analyzer, Sysmex KX – 21N (Sysmex Co.). These were part of routine apheresis donation procedure.

3.2.1.2 RDP.

The following data of RDP platelet concentrates (PCs) as listed below are taken from book record of Quality Control of Blood Components, Transfusion Medicine Unit HUSM dated from January 2004 to January 2005.

- Beg no.
- Date of collection.
- Date of quality control procedure
- PCs volume.
- Platelet, WBC, RBC count.

3.2.1.3 Calculating volume of PCs, and platelets, WBC and RBC count.

Calculation was done according to quality control manual by Transfusion Medicine Unit HUSM.

Platelets: Platelet count performed by hematological analyzer was per microliter (/µL) in unit. To convert the units from per µL to per unit PCs (/per unit), this formula was used:

Platelets/ unit = total volume plasma /TVP (ml) x platelet count x 10^3

For example: Reading obtained from analyzer was 1312 x 10^3/µL platelets and TVP was 285 mL, which means 10^{-3} mL (1 µL) plasma/PCs contains 1312 x 10^3 platelets. If the total volume of unit is 285mL then

1 mL of plasma/ PCs contains 1312 x 10^3 platelets.

Therefore, 285 mL of plasma/ PCs would contain

285 mL x 1312 x 10^3 x 10^3 platelets = 3.74 x 10^{11} platelets/ Unit.

RBC: RBC count performed by hematological analyzer was in microliter (/μL) unit. To convert the units from per μL (/μL) to per unit PCs (/per unit), this formula was used:

RBC/ Unit = TVP (mL) x RBC count x 10^3

For example, RBC count obtained from analyzer was 0.02 x 10^6/μL and the volume was 219 mL, which means1μL (10^{-3}mL) plasma/ PCs contains 0.02 x 10^6 RBC.

Therefore, 219 mL of plasma/ PCs would contain

219 x 0.02 x 10^6 x 10^3 RBC = 4.38 x 10^9 RBC/ Unit.

WBC: WBC count performed by hematological analyzer was in number of WBC per microliter (/μL) in unit. To convert the units from per μL (/μL) to per unit PCs (/per unit), this formula was used:

WBC/ Unit = TVP (mL) x WBC count x 10^3.

For example, WBC count obtained from hematological analyzer was 0.1 x 10^3/ μL and volume was 270 mL, which means 1μL (10^{-3}mL) plasma/ PCs contains 0.1 x 10^3 WBC.

Therefore, 270 mL of plasma/ PCs would contain

270 x 0.1 x 10^3 x 10^3 WBC = 27 x 10^6 WBC/ Unit.

Volume of apheresis PCs: PCs volume reading by Haemonetics MCS+ is in mL per unit bag. Standard requirement of Transfusion Medicine Unit is >40 mL per 60 x 10^9 platelets. To convert the units from mL per Unit to mL per 60 x 10^9 platelets, this formula was used:

$$\text{Volume per } 60 \times 10^9 \text{ platelets} = \frac{\text{Volume per bag/ Unit}}{\text{Platelet count } (\times 10^{11})} \times \frac{60}{10^2}$$

For example, PCs volume per Unit was 313mL, and platelet count was 3.0×10^{11} per Unit, which means the volume was 313mL per 3.0×10^{11} platelets. Therefore, volume per 60×10^9 platelets was:

$$\text{Volume per } 60 \times 10^9 \text{ platelets} = \frac{313}{3.0} \times \frac{60}{10^2} = 62.6 \text{ mL.}$$

3.2.2 Apheresis pre- and post-donation FBC:

FBC of apheresis pre- and post-donation samples was also a part of routine apheresis procedure. 3 - 4 mL pre-donation blood sample was obtained at the blood sampling site of the tubing just before the apheresis procedure began by staff nurse who handled the procedure. The sample was then filled into an EDTA tube. After the procedure finished a post-donation blood sample (3 - 4 mL) was taken from the same blood sampling site by the same staff. Platelet, WBC, RBC and hematocrit (HCT) count was performed on both the pre-and post-donation samples using hematological analyzer, Sysmex KX – 21N. This was done in Hematology Laboratory, School of Medical Sciences. USM.

3.2.3 Hemolysis assessment of apheresis.

3.2.3.1 Peripheral blood film.

After FBC was done, a smear is made on each of the remaining blood samples of apheresis pre- and post-donation on clean glass slides. As it ideally should be, the smears were made within 3 hours of collection, but some of the samples had to be smeared more than 3 hours after collection (but no more than 6 hours) due to unavoidable problems faced. The slides were then dried by using a desiccator for 15 to 20 minutes. After that the slides were kept in a slide holder. Staining was done on the smears at least after 24 hours the smears were made. For this purpose Wright staining was used. Staining procedure was done according to Hematology Laboratory HUSM standard procedure: The slides of the smears were flooded with Wright stain for 4 minutes, after that an equal volume of phosphate buffer was added, mixed well with the Wright stain by gentle blowing and were left for 12 minutes. The slides are then washed with running water briefly but adequately before drying them using a desiccator. The slides were kept for at least 24 hours after staining before being mounted with DPX and being put a cover slip over them. Microscopic evaluation of the pre- and post-donation blood films was performed by a hematologist.

3.2.3.2 Free hemoglobin/ plasma hemoglobin.

After smears were made, the remaining apheresis pre- and post donation blood samples in EDTA tubes were centrifuged by using refrigerated centrifuge ALC PK B012 to separate the blood into red cell and plasma components. The temperature of centrifugation was set to 4°C and centrifugation rate was set at 3000rpm for 15 minutes. After that the hemoglobin count of the plasma was performed by using Sysmex KX - 21N.

3.2.4 Culturing of PCs samples.

Culturing of samples was done in Biomedical Laboratory PPSK. Before doing so, the blood agars were left for 1 hour at room temperature to make sure the agars were dried.

3.2.4.1 Apheresis.

After sampling of the PCs was done on sample pouch, the remaining PCs in the sample pouch was kept in same storage condition as in an actual PCs; agitated and under temperature between 20 -24°C. it was kept for 5 to 7 days. The sample pouch was then brought to Biomedical Laboratory PPSK in a special polystyrene container which is suitable to maintain the temperature from 20 to 24°C if transported within short distances. Before culturing was done, the sample pouch was checked to ensure no leaking occurred. Streaking of the samples was done in a biosafety cabinet with full aseptic technique applied – the biosafety cabinet was disinfected with 70% alcohol before culturing was done, wire loop used to streak the samples onto blood agar was sterilized by burning the end of it with flame before streaking and the part of sample pouch that will be cut to obtain a drop of the PCs was properly disinfected by 70% alcohol before cutting it with a scissor which was also properly disinfected by 70% alcohol. After streaking, the plates were labeled with the samples' bag number, sealed with parafilm and incubated in incubator Binder at 37°C overnight. The cultures were examined after that, and if no growth was detected, the cultures were incubated for another three days. Contaminated growth (e.g. fungal colonies) was not considered as a positive growth. In total 47 samples were cultured using the above mentioned technique.

3.2.4.2 RDP.

47 samples were randomly selected and cultured. Majority of the sampling was done by cutting a portion from remaining tube segment of RDP bag that had not been transfused yet to the patient. The PCs in the remaining tube segment was pushed into the bag and mixed well with the PCs in the bag. After that a little amount of the PCs were pushed back into the tube. The tube segment was properly sealed two times using a tube sealer before cutting it to ensure no leakage occurred. The cut portion of the segments was

1.5 to 2.5 cm in length. After that the cut segment of tube was labeled with the same number used to label the RDP bag and kept agitated in an agitator at 20 - 24°C for as long as platelet expiry time. For instance if the sampling was done on day 2 after the RDP was prepared, the sample was kept for another 3 days (overall storage period of PCs are 5 days). Transportation of the samples to the Biomedical Laboratory PPSK and culturing of the samples were done in the same manner as apheresis PCs samples described above.

3.2.4.3 Identification tests for positive growth:

If positive growth was detected, Gram stain was done on the colonies. Catalase test and/ or coagulase test was/ were done to Gram positive bacteria. However, identification tests for Gram negative bacteria were not performed.

3.2.5 Statistical analysis.

Statistical analyses are done by using SPSS software version 11.5. Normality test is done on all results except for side effects during plateletpheresis and hemolysis assessment. A p value less than 0.05 was considered to be statistically significant. Independent t-test is used to test the equality of RBC contamination between apheresis PCs and RDP, while non-parametric test (Mann-Whitney test) is used to compare WBC contamination between apheresis PCs and RDP since the two populations are not normally distributed. Paired t-test was done to compare apheresis pre- and post-donation FBC.

Figure 2 shows the flow chart of the methods used in this study as previously explained.

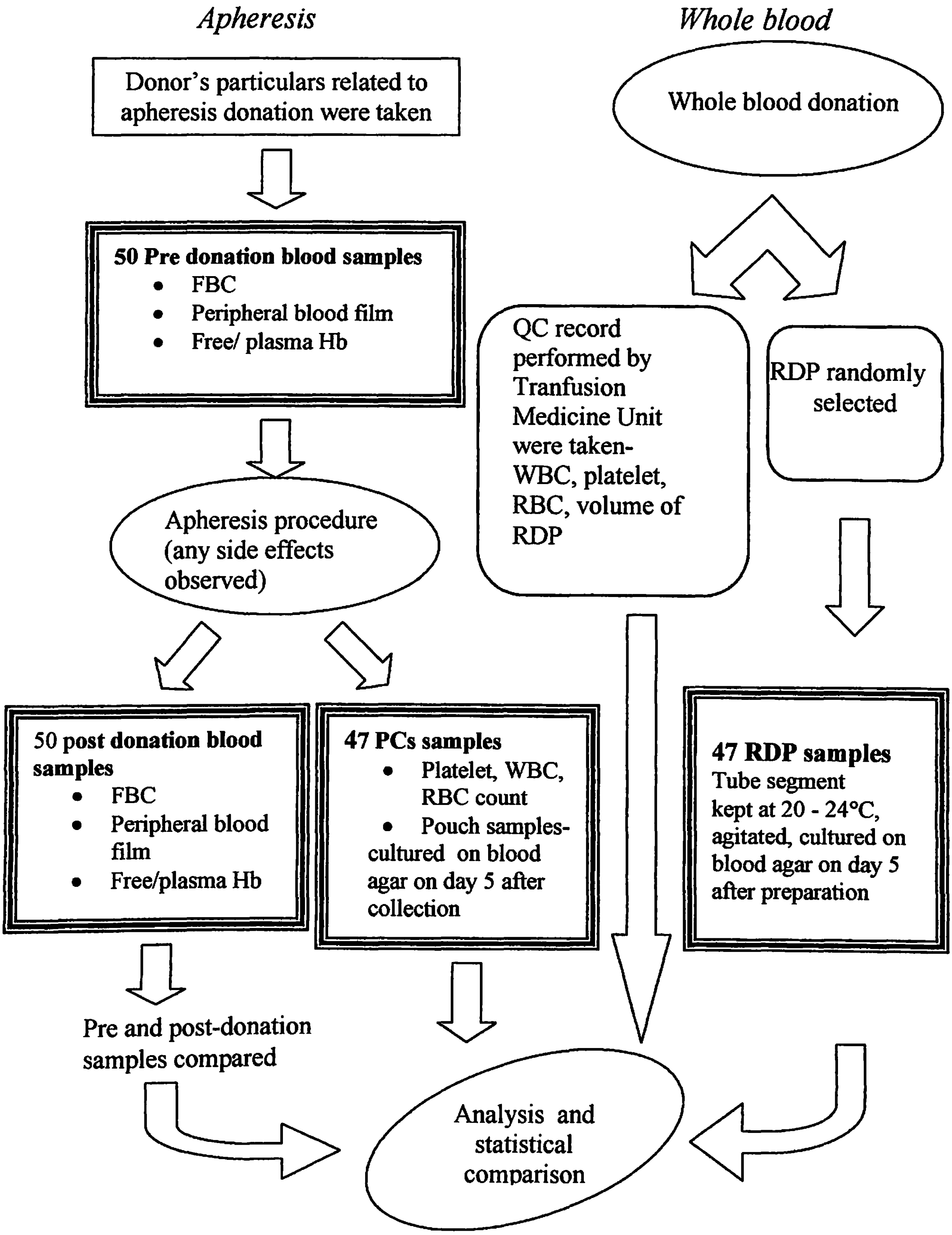

Figure 2: Flow chart of overall methods used in this study.

4. RESULTS.

A prospective study was performed at the Transfusion Medicine Unit, HUSM from September 2004 to January 2005. Sample size was 50 healthy apheresis donors; from them 50 pre- and 50 post-donation blood samples were analyzed and 47 PCs samples were analyzed. 47 RDP were randomly sampled to test for sterility and 47 RDP QC record performed by Transfusion Medicine Unit were also analyzed. From the 50 apheresis donors, 3 of them were female. Mean age of the donors was 38.8 years, ranging from 29 – 52 years. 44 (88%) of them were Malays, while the rest were Chinese. Frequency of donations is shown in Figure 3, while mean apheresis procedure time was 74 minutes.

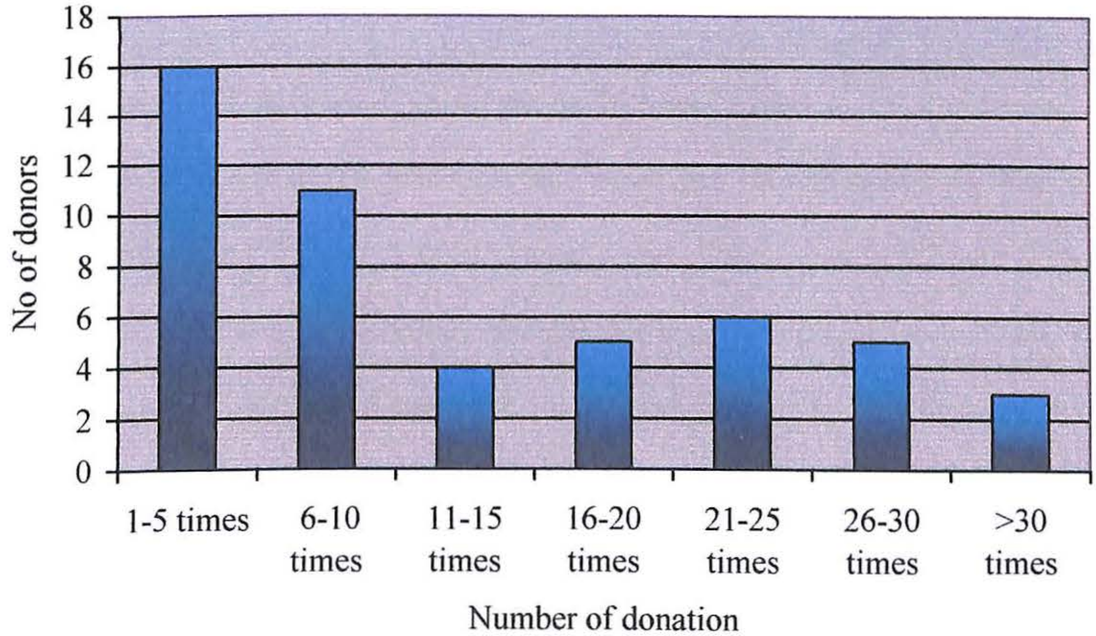

Figure 3: Frequency of donations among apheresis donors.

4.1 PCs QUALITY DETERMINATION AND COMPARISON.

4.1.1 WBC, RBC and bacterial contamination.

Since distribution of WBC contamination of both apheresis PCs and RDP are not normal, non-parametric test (Mann-Whitney test) were used to compare the equality between them. Because of the non-normal distribution too, median and interquartile range (IQR) were considered instead of mean ± standard deviation (SD). As shown in Table 3, in apheresis PCs, WBC contamination per microliter (μL) is significantly lower than RDP. However, WBC contamination per Unit is significantly higher (p<0.001) in apheresis PCs compared to RDP. Standard requirement of Transfusion Medicine Unit HUSM for WBC contamination in both apheresis PCs and RDP is <0.5 x 10^9/ Unit. All units tested complied with this standard (Table 6).

Contamination of RBC per μL in apheresis PCs was found to be significantly higher compared to RDP (p<0.001), while contamination per Unit in apheresis PCs is significantly far higher than RDP (p<0.001). Table 4 shows the mean ± SD and 95% Confidence Interval for RBC contamination between the two populations while Table 5 shows the statistical values of independent t-test used to compare the equality between the two means. RBC contamination requirement in RDP is <1.0 x 10^9/ Unit. No standard was established for RBC contamination in apheresis PCs. However, if RBC contamination requirement in RDP is applied to apheresis PCs, none of the units met the requirement, compared to RDP which 38% of them met the requirement (Table 6).