Original article

Essential composition of blood products: what is different in platelet concentrate and pooled leukocyte poor platelet concentrate?

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Abstract:

Background: *Platelet product is used for therapeutic purpose, so the quality control is essential for the blood bank to monitor our products because the amount of platelets in the final product is essential for the clinician to calculate the suitable dosages for treatment. There are several variables in production methods that affected the platelet yield.* ***Objective:*** *To study the quality of platelet concentrate (PC), pooled leukocyte poor platelet concentrate (LPPC) and 4-unit pooled platelet concentrate (PPC) prepared by Siriraj Hospital.* ***Materials and Methods:*** *Two hundred and ten units of PC, 200 LPPC and 200 PPC were tested for platelet count, leukocyte count and red cell count. The volume was measured from the calculated weight. The pH was performed at day 5 of storage.* ***Results:*** *The average number of platelets were 8.8 x 10^{10} ± 2.2, 3.1 x 10^{11} ± 0.5 and 3.5 x 10^{11} ± 0.3 in PC, LPPC and PPC, respectively. The average number of leukocyte were 0.14 x 10^{9} ± 2.2, 0.08 x 10^{9} ± 0.06, and 0.31 x 10^{9} ± 0.7 in PC, LPPC and PPC, respectively. The pH at day 5 were 7.18 ± 0.57, 7.62 ± 0.11, 7.13 ± 0.19 in PC, LPPC, and PPC, respectively.* ***Conclusion:*** *These platelet products tested met all standard requirement of the National Blood Centre, Thai Red Cross Society (NBC TRCS). Since the number of platelets in each product type was higher than the expected value, therefore we use a pool of 4 PC instead of 6 PC to provide platelets for an adult who weights 60 kg. However, an appropriate proportion of platelet cells and plasma volume is essential to maintain appropriate pH which is one of the quality control parameters as guided by European Directorate for the Quality of Medicines & HealthCare (EDQM).*

Keywords : ● *Platelet concentrates* ● *LPPC* ● *Quality control*

J Hematol Transfus Med. 2019;29:91-9.

Received 28 December 2018 Corrected 18 February 2019 Accepted 23 May 2019

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นิพนธ์ต้นฉบับ

องค์ประกอบสำคัญในผลิตภัณฑ์โลหิต: ผลิตภัณฑ์เกล็ดเลือดเข้มข้นและเกล็ดเลือดเข้มข้นชนิดรวมถุงที่มีจำนวนเม็ดเลือดขาวต่ำมีอะไรที่แตกต่างกัน ?

ประพันธ์ กันภัย สายฝน กล้าเด็ด อุษณีย์ ศิริบุญฤทธิ์ วราภรณ์ มีสมรรถ และ ปาริชาติ เพิ่มพิกุล
ภาควิชาเวชศาสตร์การธนาคารเลือด คณะแพทยศาสตร์ศิริราชพยาบาล มหาวิทยาลัยมหิดล

บทคัดย่อ

ความเป็นมา *ผลิตภัณฑ์เกล็ดเลือดเป็นส่วนประกอบของเลือดที่มีปริมาณการใช้อย่างมากในการรักษาผู้ป่วย การตรวจสอบคุณภาพผลิตภัณฑ์จึงเป็นหน้าที่สำคัญประการหนึ่งของธนาคารเลือด เพื่อให้ได้ข้อมูลเรื่ององค์ประกอบในผลิตภัณฑ์เกล็ดเลือด สำหรับให้แพทย์นำไปใช้ประโยชน์ในการสั่งการรักษาได้อย่างเหมาะสม การผลิตผลิตภัณฑ์เกล็ดเลือดมีได้หลายวิธี จึงทำให้ส่วนประกอบต่างๆ ในผลิตภัณฑ์ที่ได้มีความแตกต่างกัน* ***วัตถุประสงค์*** *ศึกษาผลิตภัณฑ์เกล็ดเลือด ที่ผลิตโดยวิธี platelet rich plasma (PRP) และ buffy coat (BC) เพื่อนำข้อมูลไปใช้ในทางคลินิกให้แพทย์สามารถสั่งการรักษาได้อย่างถูกต้องและเหมาะสม* ***วัสดุและวิธีการ*** *ตรวจคุณภาพของเกล็ดเลือดเข้มข้น (platelet concentrates: PC) ซึ่งเจาะเก็บเลือดในระบบถุง triple bags และปั่นแยกด้วยวิธี PRP เกล็ดเลือดเข้มข้นชนิดรวมถุงที่มีจำนวนเม็ดเลือดขาวต่ำ (pooled leukocyte poor platelet concentrates: LPPC) ซึ่งเจาะเก็บเลือดในระบบ quadruple bag:top-bottom system และปั่นแยกโดยวิธีBC และเกล็ดเลือดเข้มข้นชนิดรวม PC 4 ถุง (pooled platelet concentrates :PPC) จำนวน 210, 200 และ 200 ยูนิตตามลำดับ โดยวัดปริมาตร จำนวนเกล็ดเลือด จำนวนเม็ดเลือดขาว จำนวนเม็ดเลือดแดงในวันที่ 1 และวัด pH ในวันที่ 5 และทำการเปรียบเทียบคุณภาพระหว่าง LPPC และ PPC* ***ผลการศึกษา*** *ใน PC, LPPC และ PPC มีจำนวนเกล็ดเลือด 8.8 x 10^{10} ± 2.2, 3.1 x 10^{11} ± 0.5 และ 3.5 x 10^{11} ± 0.3 มีจำนวนเม็ดเลือดขาว 0.14x10^{9} ± 2.2, 0.08 x10^{9} ± 0.06 และ 0.31 x 10 ± 0.7 สำหรับค่า pH เมื่อเก็บไว้ถึงวันที่ 5 มีค่า 7.18 ± 0.57, 7.62 ± 0.11, และ 7.13 ± 0.19 ตามลำดับ* ***สรุป*** *ผลิตภัณฑ์เกล็ดเลือดที่ทำการประเมิน มีคุณภาพตามเกณฑ์มาตรฐานของศูนย์บริการโลหิตแห่งชาติ ซึ่งกำหนดให้ LPPC อย่างน้อยร้อยละ 90 มีจำนวนเกล็ดเลือด > 2.4 x 10^{11} เซลล์ต่อยูนิต จำนวนเม็ดเลือดขาว < 0.2 x 10^{9} เซลล์ต่อยูนิต และ pH ≥ 6.2 เนื่องจากปริมาณเกล็ดเลือดใน PC สูงกว่าเกณฑ์มาตรฐาน ดังนั้นโรงพยาบาลศิริราชจึงนำไปปรับการผลิต PPC จากเดิมใช้การรวม PC 6 ถุง เป็นการรวม PC เพียง 4 ถุง สำหรับผู้ป่วยที่น้ำหนักตัวมากถึง 60 กิโลกรัม อย่างไรก็ตามในการผลิตควรระวังให้มีสัดส่วนจำนวนเกล็ดเลือดเหมาะสมกับปริมาตร เพื่อป้องกันการลดต่ำลงของ pH ด้วย*

คำสำคัญ : ● *เกล็ดเลือดเข้มข้น* ● *เกล็ดเลือดเข้มข้นชนิดรวมถุงที่มีจำนวนเม็ดเลือดขาวต่ำ* ● *การควบคุมคุณภาพ*
วารสารโลหิตวิทยาและเวชศาสตร์บริการโลหิต. 2562;29:91-9.

Introduction

In tertiary care hospital, platelet products are the most frequently requested blood product. They are transfused for the treatment of bleeding in a thrombocytopenic or functionally abnormal platelet as a therapeutic transfusion and to minimize risk of bleeding as a prophylactic transfusion[1,2]. In Siriraj Hospital, there is an increasing number of patients with hematologic malignancies, oncologic patients, stem cell transplant patients. These patients also survive longer because of effective treatment. These factors contribute to the continuous increase in platelet demand. Therefore, blood bank tries to produce platelet product to meet the increasing patient's demand and high quality platelet is expected to provide clinical efficacy determined by post-transfusion platelet count increment, improved hemostasis, and lower risk for adverse reactions in recipients.

To cope with increasing platelet demand, we separated all qualified whole blood units into blood products including platelets besides using apheresis technique to collect platelet from single donor. The majority of our platelet supply came from whole blood-derived platelets prepared by platelet rich plasma (PRP) and buffy coat methods. The different methods have an impact on physical and biological quality of platelet product. The objective of this study is to determine whether the quality of platelet concentrate (PC) and pooled leukocyte poor platelet concentrate (LPPC) prepared meet the parameters specified by the national and international standards and also benchmarking against the applicable standards. The evidence-based data obtained leads to insight for proper management of blood bank and is beneficial to help physicians who are generally unaware of composition content variation for treating patients appropriately and effectively.

Materials and Methods

This study was approved by Siriraj IRB 175/2554. The sample size was calculated to achieve the reliable 95%CI. They were 210 PC, 200 LPPC and 200 pooled platelet concentrate (PPC) which each PPC was prepared from pooling four units of PC.

Qualified whole blood donations at Siriraj hospital were separated into blood components in a closed system within 8 hours of blood collection using validated protocol for platelet-rich plasma or buffy coat production methods which we described elsewhere[3]. All connection was done by the sterile connecting device. Both LPPC and PPC were stored in a 1,000 mL platelet storage bag (Fresenius Kabi AG, Bad Homburg, Germany) and kept in platelet incubator (temperature 20-24°C) with a standard agitator (Helmer, USA).

Platelet production methods: The two methods were used. The platelet rich plasma (PRP) method for PC and PPC. We collected whole blood in triple bag system (Terumo Corp, Tokyo, Japan and Fenwal Inc, Marico, USA). PRP was separated from whole blood by light spin and the platelets were concentrated by heavy spin. PPC was prepared by pooling four units of platelet concentrates.

The buffy coat (BC) method, we collected whole blood in a quadruple bag: top-bottom system (Terumo Corp, Tokyo, Japan and Fenwal Inc, Marico, USA). The whole blood was centrifuged at heavy spin to prepare plasma, buffy coat containing platelets and red cells. Four ABO identical buffy coats were pooled with one plasma unit before light spin centrifugation to separate the LPPC from leukocytes.

The parameters studied were the volume of product, the total number of platelets, leukocytes and red cells at day 1 and pH at day 5. Comparative studies were also carried out between pooled platelet concentrates containing 4 units of PC and LPPC. Data was analyzed using pair-t test for comparative studies. *P-value* less than 0.05 were defined as statistically significance.

All the cell counts were performed by an automated system (Sysmex XS-800i, Kobe, Japan). The volume of blood product was calculated based on weight and specific gravity 1.03 of plasma. The pH of platelet product was also measured using the pH meter (Orion 2 star, Thermo Scientific, Boston, USA).

Results

The composition of PC is shown in Table 1. One unit of platelet concentrate had an average volume of 59 ± 4 mL (range 44-68), platelet number of 8.8 ± 2.2 x 10^{10}(range 4.0-16.3) and leukocytes number of 1.4 ± 1.2 x 10^{8} cells (range 0.0-0.64). The pH at day 5 in 110 PC was 7.18 ± 0.57 (range 5.67-7.83). In Table 1, all parameters were compared with specified standard criteria in the Standard for Blood Bank and Transfusion Service, National Blood Centre, Thai Red Cross Society (NBC TRCS)[4], American Association of Blood Banks (AABB)[5] and Guide to the preparation, use and quality assurance of blood components European Directorate for the Quality of Medicines and HealthCare (EDQM)[6]. Figure 1A shows the distribution of total platelet number and leukocyte number in 210 units of platelet concentrate which showed that majority of PC contained more than 6.0 x 10^{10} platelets.

The composition of LPPC is shown in Table 2. One LPPC had an average volume of 277 ± 34 mL (range 194-368), platelet number of 3.1 ± 0.5 x 10^{11} (range 1.5-4.8) and leukocytes of 0.8 ± 0.6 x 10^{8} cells (range 0.1-6.2). The average pH at day 5 (available in only 10 LPPC) was 7.62 ± 0.11(range 7.40-7.80). Figure 1B shows the distribution of platelet number and leukocyte number in 200 units of LPPC.

Table 3 provides data of PPC from 4 units of PC. Figure 1C shows the scatter plot of platelet number and leukocyte number in 200 units of PPC. The average platelet number in one PPC was 3.5 ± 0.3 x 10^{11} (range 2.6-5.0) while containing leukocyte 0.3 ± 0.07 x 10^{9} (range 0.13-0.66) cells. The pH at day 5 measured in 16 available PPC was 7.13 ± 0.19 (range 6.7-7.4). Additionally 186 from 200 PPC contained ± 3.0 x 10^{11} platelets (93%).

The comparison between PPC and LPPC is shown in Table 4 in term of volume, platelet number, leukocyte number and red cell number. All of the parameters were significantly differences between the two types of pooled platelets ($p < 0.0001$). We did not compare between pH because the available data of LPPC was only 10 units, too few for comparison. Figure 2 shows distribution of total platelet number in 200 PPC and LPPC which had normal distribution for number of platelets in both types of platelet pools but PPC had sharp peak distribution between 3.25-3.75 x 10^{11} platelets while LPPC had wider distribution and had peak range from 2.51-3.50 x 10^{11} platelets.

Discussion

In this study, PC and LPPC composition complied with the standard of NBC TRCS, AABB, and EDQM[4-6]. The average platelet number in each unit of PC was 8.8 x 10^{10} with an average residual leucocyte of 0.14 x 10^{9} (0.00-0.64) cells. The platelet yield is higher than all standard requirements of 6.0 x 10^{10} cells but this number is the QC criteria which stated that 90% of units tested must contain as minimal or higher. So the average number will be much higher as we reported in this study. LPPC had an average 3.1 x 10^{11} platelets and only 0.8 x 10^{8} residual leukocyte even though prepared from 4 buffy coats. When compare between pooled 4 PC (PPC) and LPPC produced from 4 BC, PPC product had significantly higher platelet yields ($p < 0.0001$) as the display in Figure 2 but contain 4 times the amount of residual leukocyte. Our finding was similar to the report of Fijnheer R[7], Hirosue[8] and Lozano M[9] but we compared between pooled products while these studies compared between a unit of whole blood-derived platelet.

Refer to the EDQM QC criteria[4], The council of Europe required that the minimum number of platelet per unit must be > 6.0 x 10^{10} for PC and > 2.0 x 10^{11} for PPC and LPPC. From Table 1, with the compliance with these criteria in 54%, we found that 46% of our PC had higher platelet concentration than the allowable limit. The pH at day 5 ranged from 5.67-7.83 and only 90% of tested units passed the criteria, while the leukocyte content is higher than the allowable limit. This negative correlation may be due to a higher number of platelets[10,11] and residual leukocytes which caused increased glucose consumption, lactic acid production and lactate dehydrogenase release during storage which resulted

Table 1 Platelet concentrate (PC) composition (n = 210)

Parameter	Mean ± SD	Standard	Criteria	Meet the required value (%)	Pass/Fail
Volume (mL)	59 ± 4	AABB	NA	NA	NA
(range)	(44-68)	EDQM	90%, > 40 mL/6.0 x 10^{10} platelets**	54.0	fail
		NBC TRCS	90%, > 40-70 mL	100	pass
Platelet count (x10^{10})	8.8 ± 2.2	AABB	90%, platelet > 5.5 x 10^{10}	96.7	pass
(range)	(4.0-16.3)	EDQM	90%, platelet > 6.0 x 10^{10}	91.9	pass
		NBC TRCS	90%, platelet > 6.0 x 10^{10}	91.9	pass
Residual leukocyte (x10^{9})	0.14 ± 0.12	AABB	NA	NA	NA
(range)	(0.002-0.635)	EDQM	90%, leukocyte < 0.2x10^{9}	77.1	fail
		NBC TRCS	NA	NA	NA
pH at day 5*	7.18 ± 0.57	AABB	90%, pH > 6.2	91.8	pass
(range)	(5.67-7.83)	EDQM	90%, pH > 6.4	90.0	fail
		NBC TRCS	90%, pH > 6.4	91.8	pass
Red cells (x10^{12})	0.006 ± 0.005	NA	NA	NA	NA
(range)	(0.000-0.032)				

*N = 110 PC for pH measurement at day 5; **define volume 40 mL per number of platelet 6 x 10^{10}

NA = not applicable

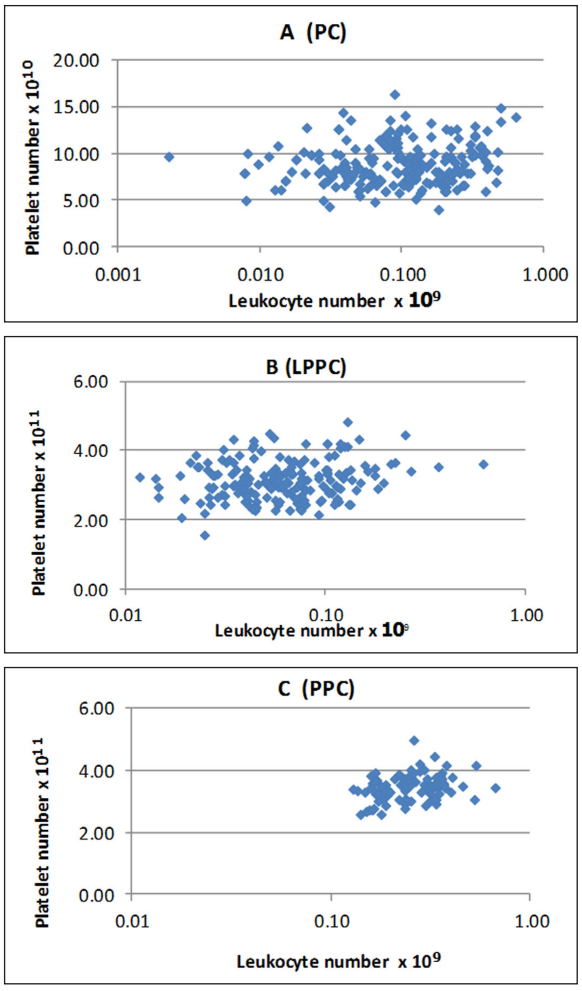

Figure 1 Platelet number and leukocyte number in platelet products: PC (A), LPPC (B), PPC (C)

Table 2 Pooled leukocyte poor platelet concentrate (LPPC) composition (n = 200)

Parameter	Mean ± SD	Standard	Criteria	Meet the required value (%)	Pass/Fail
Volume (mL)	277 ± 34	EDQM	90%, > 40 mL/6.0 x 10^{10} platelets**	91.0	pass
(range)	(194-368)	NBC TRCS	NA	NA	NA
Platelet count (x10^{11})	3.1 ± 0.5	EDQM	90%, platelet > 2.0 x 10^{11}	99.5	pass
(range)	(1.5-4.8)	NBC TRCS	90%, platelet > 2.4 x 10^{11}	93.0	pass
Residual leukocyte (x10^{9})	0.08 ± 0.06	EDQM	90%, leukocyte < 1 x 10^{9}	100	pass
(range)	(0.01-0.62)	NBC TRCS	90%, < 0.2 x 10^{9}	97.0	pass
pH at day 5 *	7.62 ± 0.1	EDQM	100%, pH > 6.4	100	pass
(range)	(7.40-7.80)	NBC TRCS	90%, pH > 6.2	100	pass
Red cells (x10^{12})	0.015 ± 0.005	NA	NA	NA	NA
(range)	(0.005-0.030)				

*N = 10 LPPC for pH measurement at day 5; ** define volume 40 mL per number of platelet 6 x 10^{10}; NA = not applicable
Note : AABB do not have QC criteria for LPPC

Table 3 Pooled platelet concentrates (PPC) composition (n = 200)

Parameter	Mean ± SD	Standard	Criteria	Meet the require value (%)	Pass/Fail
Volume (mL)	214 ± 5.8	EDQM	90%, > 40 mL/6.0 x 10^{10} platelets**	14	fail
(range)	(196-243)				
Platelet count (x10^{11})	3.5 ± 0.3	EDQM	90%, platelet > 2.0 x 10^{11}	100	pass
(range)	(2.6-5.0)				
Residual leukocyte (x10^{9})	0.31 ± 0.07	EDQM	90%, leukocyte < 1 x 10^{9}	100	pass
(range)	(0.13-0.66)				
pH at day 5*	7.13 ± 0.19	EDQM	90%, pH > 6.4	100	pass
(range)	(6.7-7.4)				
Red cells (x10^{12})	0.018 ± 0.005	NA	NA	NA	NA
(range)	(0.005-0.040)				

*N = 16 PPC for pH measurement at day 5; **define volume 40 ml per number of platelet 6 x 10^{10}; NA = not applicable
Note: NBC TRCS and AABB do not have QC criteria for PPC

Table 4 Comparison of PPC (n = 200) and LPPC (n = 200)

Parameter	PPC	LPPC	p value
Volume	214 ± 5.8	277 ± 34	< 0.0001
Platelet count	3.5 ± 0.29	3.1 ± 0.5	< 0.0001
Leukocyte count (10^{9})	0.31 ± 0.27	0.08 ± 0.06	< 0.0001
Red cells (x10^{9})	0.023 ± 0.076	0.025 ± 0.134	< 0.0001
pH at day 5*	7.13 ± 0.19	7.62 ± 0.11	

*Only 16 PPC and 10 LPPC were available for measure pH on day 5

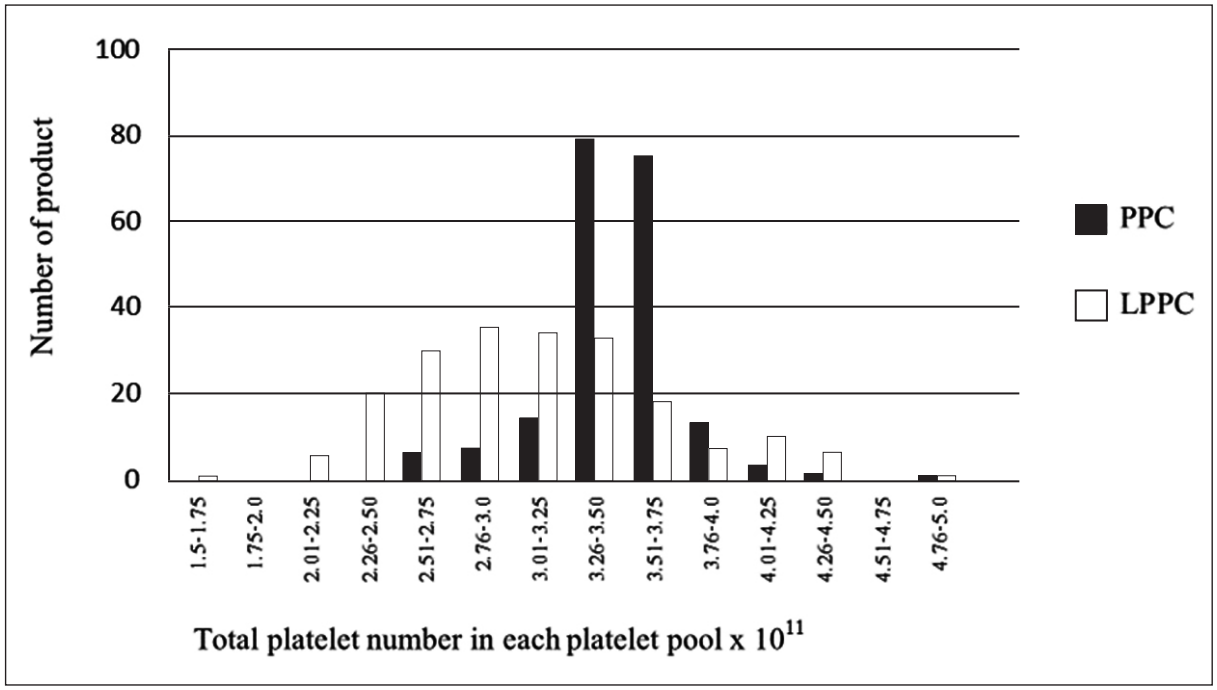

Figure 2 Distribution of total platelet number in PPC and LPPC

in lower pH [12] in the products. The possible solution is to increase plasma volume of product in order to support platelet metabolism and lower residual leukocytes contaminated. The increased in the plasma volume of PC had been tried and the data showed better trend of pH. All LPPC which produced from semi-automated separation system complied with EDQM and NBC TRCS QC criteria and from available 10 units tested for pH, all pH was higher than 6.4 at day 5 of storage.

Before this study was done, we provided one PC for body weight 10 kg of patient as recommended by blood transfusion therapy: A physician's handbook[13], and a LPPC for one adult therapeutic dose (ATD) to cover the need of patient with 40-60 kg body weight. After finding out the amount of platelets in each PC, we switched to pool 4 PC units instead of 6 units; the target of 3 x 10^{11} cells was achieved in 93%. Therefore, there was more PPC available for transfusion and could also reduce the number of donor exposure to each patient. By doing this, it provided 20% more ATD from a same number of blood donation.

Considering the three standards namely NBC TRCS, AABB, and EDQM, each standard had focused on the same important issues but determined the little different acceptable limit. One main different issue was the volume of platelet product, NBC TRCS, and AABB guide specified the acceptable volume while EDQM specified the maximum concentration of platelet which was useful for the preservation of platelets and better support metabolism of a viable platelet. The results showed that the concentration of platelet was important for the blood bank to preserve the platelet well, as described earlier. The number of platelet in a unit of LPPC was different with 2.0 x 10^{11} platelets in EDQM and 2.4 x 10^{11} platelets in NBC TRCS. In our opinion, the different number in the final product such as 2.4 x 10^{11} platelets in LPPC and 3.0 x10^{11} platelets in apheresis (4,5,6) unit make clinician bias and order apheresis platelet without other proper reason but to get higher platelet number for transfusion. This is why we manipulate our production to provide the standardized dose of platelet from a different type of product, we aim for 3 x 10^{11} platelets for every PPC, LPPC, and apheresis and we can achieve this protocol without difficulties.

The acceptable pH issue was also different in each standard, The NBC TRCS and AABB required pH 6.2 while EDQM required pH 6.4. From our data, all LPPC product and PPC had pH more than 6.4 at day 5. Our

interpretation is, if we have enough volume, proper storage container and keep in platelet incubator with an agitator to support platelet metabolism, the pH will be in an acceptable range of maximum pH 6.4 at day 5 of storage.

In general practice, attending physician who is taking care of a patient must consult the blood bank physician before transfusion of a platelet product. Evidence-based data form this study lead to insight for the decision about the proper dose of platelet combination with relevant patient information. Blood product should be considered as the drug which defines the amount of active gradient to meet patient demand as well as impurity that may affect patient such as residual red cells. The amount of average residual red cell in our platelet products were 0.006 x 10^{12} cells (0.5 mL) in PC, 0.015 x 10^{12} cells (1.35 mL) in LPPC and 0.018 x 10^{12} cells (1.62 mL) in PPC. They all met the standards that required < 0.5 mL of PC, < 2 mL for PPC and apheresis unit. However, no standard exist for residual red cell in LPPC. The volume of red cell was calculated follow Misso S, et al[14]. This data provided evidence-based for prescribe proper dose of Rh immunoglobulin to Rh-negative patient who received Rh-positive platelets.

For blood bank, this study can help us to modify our process for a better outcome and we would like to look for the highest standard of blood products. However, this study was emphasized on the composition of platelet product as guided by standard, the majority of criteria emphasize the number of platelets while the platelet function and clinical outcome are also importance to be assessed[15,16] and the further study should be done. We would like to convince blood bank to study and know their product so they can have evidence-based and confidence data to provide for a clinician. They can also benchmark their product with others and find a way to improve the production method to achieve their goal.

Conclusion

From this study we have evidence-based data of platelet product composition in term of number of platelets, residual leukocytes and red cells in each product type. We realized the importance of platelet concentration in final product which must be enough to support metabolism. We could modify our production to have more available platelet dose for transfusion without compromising number of transfused platelets, this lead to better utilization and lower donor exposure. We encourage blood bank to use the QC results as the monitoring tool for quality improvement of blood products which will finally benefit to our patients.

Acknowledgments

This study was supported by Siriraj Research Fund. We would like to thank our blood component preparation staff and blood component QC for their professional performance in daily routine work. We also sincerely thank Assoc. Prof. Sasitorn Bejrachandra who established the blood component QC lab and provided valuable suggestion.

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