

**EVALUATION OF A PLATELETPHERESIS TECHNIQUE FOR
THE PREPARATION OF LEUKOCYTE REDUCED PLATELET
CONCENTRATES**



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ABSTRACT

The purpose of this research is to evaluate the performance of a new blood cell separator, Trima Accel (version 5.0) and to compare it with the previous version (the Trima system, version 4.0) in the preparation of leukocyte reduced platelet concentrates.

Donation plateletpheresis was performed using 30 procedures for each machine. Double and triple apheresis platelets were collected. The target platelet yields were 6.0×10^{11} for double-dose and 9.0×10^{11} for triple-dose. From the procedures, plateletpheresis data was compared in terms of platelet yields, residual leukocyte counts, the percentage of platelet collection efficiency, processing times and donor comfort during collections. Platelets were counted with an automated blood cell counter (CELL DYN 1700 version 1.01) and residual leukocytes were counted manually with the Nageotte chamber.

A total of 114 plateletpheresis units were collected from 60 apheresis collections. Each apheresis machines produced the same number (57) of plateletpheresis products, 7 single, 19 double, and 4 triple-dose for Trima Accel and 4 single, 25 double, and 1 triple dose for Trima version 4.0 system. The Trima Accel processed less blood (3526 ± 394 vs. 4206 ± 55 mL, $p = 0.001$) and used less ACD (420 ± 46 vs. 461 ± 55 mL, $p = 0.004$) in a shorter time for a comparable platelet yield than the Trima version 4.0 system (6.98 ± 1.2 vs. $6.63 \pm 0.70 \times 10^{11}$ PLTs, $p = 0.197$). The percentage of collection efficiency was significant higher (83% vs. 65%). The processing time was on average 6 minutes faster (71 vs. 77 min). Regarding leukoreduction, all plateletpheresis units had fewer than 1.0×10^6 WBCs per unit. No severe reactions occurred during and after procedure.

In conclusion, the Trima Accel is a faster and more efficient apheresis procedure, improving the quality of production. All units fulfilled Council of Europe leukoreduction standards. Finally, double-dose and triple-dose leukocyte reduced platelet concentrates can split thereby saving money and reducing the recipient's risk of donor exposure.

KEY WORDS: LEUKOCYTE REDUCED / PLATELETPHERESIS/ BLOOD CELL SEPARATOR/ EFFICIENCY

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การศึกษาเทคนิคการเตรียมเกร็ดเลือดที่ลดจำนวนเม็ดเลือดขาวปนเปื้อน โดยเครื่องแยกส่วนประกอบโลหิตอัตโนมัติ (EVALUATION OF A PLATELETPHERESIS TECHNIQUE FOR THE PREPARATION OF LEUKOCYTE REDUCED PLATELET CONCENTRATES)

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บทคัดย่อ

วัตถุประสงค์ของการวิจัยครั้งนี้เพื่อศึกษาเปรียบเทียบถึง ความสามารถของเครื่องแยกส่วนประกอบโลหิตอัตโนมัติรุ่นใหม่ (Trima Accel, version 5.0) กับเครื่องรุ่นเก่า (The Trima system, version 4.0) ที่ใช้เตรียมเกร็ดเลือดที่มีจำนวนเม็ดเลือดขาวปนเปื้อนน้อย

การศึกษานี้จะทำการแยกเก็บเกร็ดเลือดโดยใช้ เครื่อง Trima Accel และ Trima system, version 4.0 ซึ่งตั้งโปรแกรมการเก็บปริมาณเกร็ดเลือดไว้ที่ 6.0×10^{11} และ 9.0×10^{11} โดยในแต่ละเครื่องจะเก็บเกร็ดเลือดจากผู้บริจาคโลหิตจำนวน 30 ราย และทำการรวบรวมข้อมูลดังนี้ ปริมาณเกร็ดเลือดที่แยกเก็บได้ จำนวนเม็ดเลือดขาวที่ปนเปื้อนในเกร็ดเลือด ประสิทธิภาพของเครื่องแยกส่วนประกอบโลหิตอัตโนมัติ ระยะเวลาในการแยกเก็บเกร็ดเลือด รวมถึงความพึงพอใจของผู้บริจาค นำมาเปรียบเทียบถึงความสามารถในการแยกเก็บเกร็ดเลือดของเครื่องทั้ง 2 รุ่นนี้ โดยปริมาณเกร็ดเลือดที่แยกเก็บได้ จะตรวจนับโดยเครื่อง CELL DYN 1700 version 1.01 ส่วนจำนวนเม็ดเลือดขาวที่ปนเปื้อนในเกร็ดเลือดตรวจนับโดยวิธี Nageotte chamber

ผลการศึกษาพบว่าเครื่องทั้ง 2 รุ่นสามารถแยกเก็บเกร็ดเลือดที่ลดจำนวนเม็ดเลือดขาวปนเปื้อนได้ในจำนวนที่เทียบเท่ากันคือ 57 ถูง โดยปริมาณเลือดที่หมุนเวียนและนำยากันเลือดแข็งตัวที่ใช้ในเครื่อง Trima Accel จะใช้ในปริมาณที่น้อยกว่าอย่างมีนัยสำคัญ (ค่าเฉลี่ย = 3526 ± 394 vs. 4206 ± 55 mL, $p = 0.001$) และ (420 ± 46 vs. 461 ± 55 mL, $p = 0.004$) ในแง่ของปริมาณเกร็ดเลือดที่แยกเก็บได้ เครื่อง Trima Accel ได้ค่าเฉลี่ย = 6.98×10^{11} ต่อเกร็ดเลือดหนึ่งถูง ส่วนเครื่อง Trima system ได้ค่าเฉลี่ย = 6.63×10^{11} ต่อเกร็ดเลือดหนึ่งถูง โดยเครื่อง Trima Accel มีประสิทธิภาพในการแยกเก็บเกร็ดเลือดที่เหนือกว่า (83% vs. 65%) ใช้ระยะเวลาในการแยกเก็บเกร็ดเลือดเร็วกว่าอย่างมีนัยสำคัญ และไม่พบอาการข้างเคียงชนิดรุนแรงเกิดขึ้นในระหว่างและหลังการบริจาค

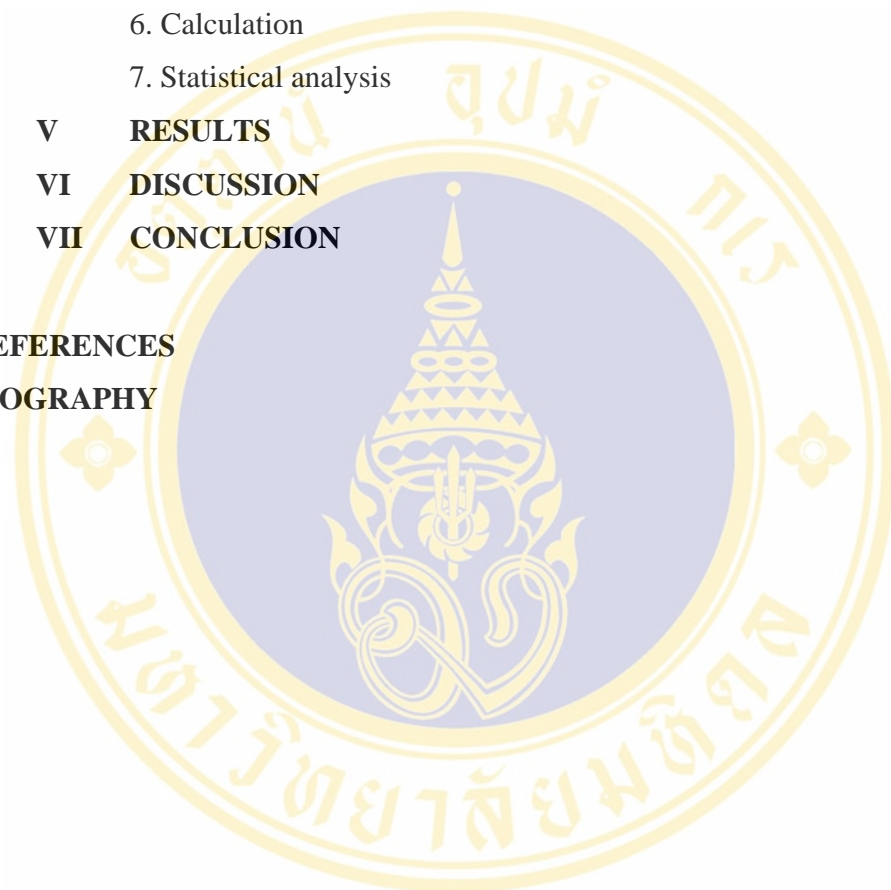
จากผลการวิจัยสรุปได้ว่าเครื่อง Trima Accel ได้รับการปรับปรุงให้มีประสิทธิภาพดีขึ้นในการเตรียมเกร็ดเลือดโดยใช้ระยะเวลาอันสั้นในการเก็บ เกร็ดเลือดที่เก็บได้เป็นชนิด leukocyte reduced platelet concentrates ที่มีคุณภาพตามมาตรฐานที่กำหนด โดยเกร็ดเลือดที่เก็บได้ชนิด double-dose, triple-dose จะถูกทำการแบ่งแยกนำไปใช้รักษาผู้ป่วยเป็นการลดอัตราเสี่ยงการปนเปื้อนจากผู้บริจาคหลายรายต่อผู้รับ อีกทั้งช่วยลดต้นทุนค่าใช้จ่ายในการผลิต

CONTENTS

	Page
ACKNOWLEDGEMENT	iii
ABSTRACT (English)	iv
ABSTRACT (Thai)	v
LIST OF TABLES	viii
LIST OF FIGURES	ix
CHAPTER	
I INTRODUCTION	1
II OBJECTIVES	3
III LITERATURE REVIEW	4
3.1 History	4
3.2 Principles of Operation	4
3.3 Characteristic of Single-Donor and Pooled Platelets	6
3.4 Storage of Apheresis Platelet Concentrates	8
3.5 Indications for Platelet Transfusion	10
3.6 Platelet Dose	10
3.7 Complication due to contaminating leukocytes	11
3.8 Leukocyte Reduction of Apheresis Platelets	14
3.9 Adverse Reactions	15
3.10 Instruments for Plateletpheresis	16
IV MATERIALS AND METHODS	23
1. Study Design, Donors and Apheresis procedures	23
2. Materials and Research Instruments for Plateletpheresis	24
3. Apheresis platelet concentrates by the Trima Sytem (version 4.0)	25
4. Apheresis platelet concentrates by the Trima Accel (version 5.0)	32
5. Nageotte Counting Chamber Method for Counting Leukocytes	39

CONTENTS (Cont.)

CHAPTER		Page
	6. Calculation	40
	7. Statistical analysis	41
V	RESULTS	42
VI	DISCUSSION	50
VII	CONCLUSION	54
REFERENCES		55
BIOGRAPHY		64



LIST OF TABLES

Table		Page
1	Main Chnge: Trima Accel (version 5.0) compared with Trima (version 4.0)	20
2	Donor characteristics before apheresis	44
3	Hematology data for the platelet donors	45
4	Apheresis procedure characteristics	46
5	Plateletpheresis characteristics	47
6	Results of a single-dose, double-dose and triple-dose	48
7	Adverse reactions for different instruments	49

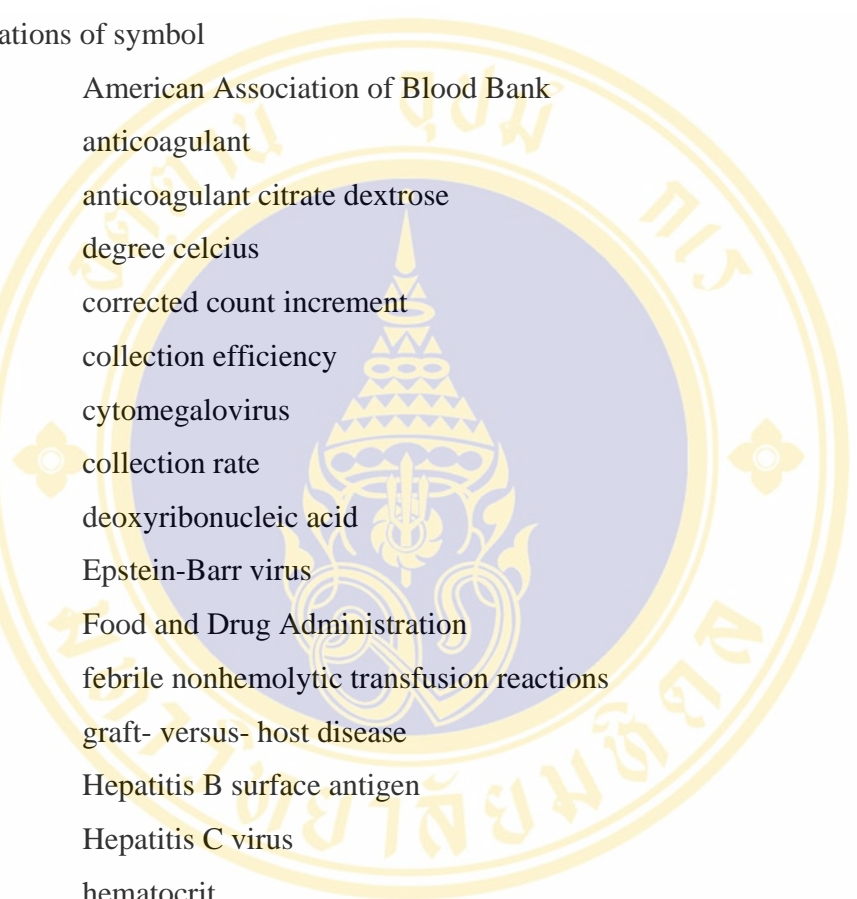
LIST OF FIGURES

Figure		Page
1	Trima Collection System Pumps	21
2	The two different fillers used in the test	22



LIST OF ABBREVIATIONS

Abbreviations of symbol

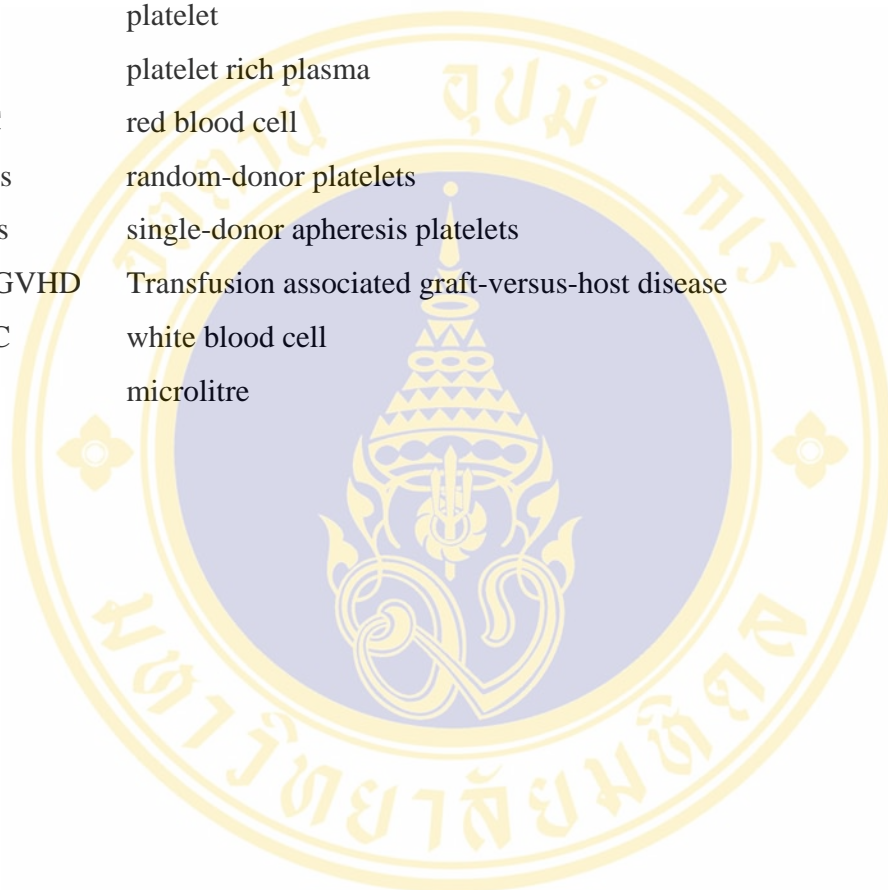


AABB	American Association of Blood Bank
AC	anticoagulant
ACD	anticoagulant citrate dextrose
°C	degree celcius
CCI	corrected count increment
CE	collection efficiency
CMV	cytomegalovirus
CR	collection rate
DNA	deoxyribonucleic acid
EBV	Epstein-Barr virus
FDA	Food and Drug Administration
FNHTR	febrile nonhemolytic transfusion reactions
GVHD	graft- versus- host disease
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
Hct	hematocrit
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
IL	interleukin
LCT	lymphocytotoxic
LRS	Leukocyte Reduction System
mL	microlitre
MNC	mononuclear cell
min	minutes
PCs	platelet concentrates

LIST OF ABBREVIATIONS (Cont.)

Abbreviation of symbol

PLT	platelet
PRP	platelet rich plasma
RBC	red blood cell
RDPs	random-donor platelets
SDPs	single-donor apheresis platelets
TA-GVHD	Transfusion associated graft-versus-host disease
WBC	white blood cell
μL	microlitre



CHAPTER I

INTRODUCTION

The demand for platelet concentrates (PCs) has been steadily increasing. Further, emphasis on reducing the untoward effects of leukocyte has led to an increase in the use of leukodepleted PCs. This is especially due to the lower risk of alloimmunization and transmission of viruses to patients afforded by reduced donor exposure (1). Over the year, plateletpheresis components have been considered superior, for a number of reasons. However, many of purported advantages of plateletpheresis have disappeared, been disproved, or are now recognized as minimal. For example, plateletpheresis components were said to be “fresher” than PCs, which was true when plateletpheresis components were especially collected for special patients. The availability of single-donor apheresis platelets (SDPs) has had a major impact on the treatment of hematology-oncology setting, to prevent HLA alloimmunization and subsequent platelet transfusion refractoriness, providing an efficient means of platelet support while minimizing patient exposure to multiple donors. It remains unclear whether SDPs are more effective than pooled random-donor platelets (RDPs) in minimizing platelet refractoriness (2).

WBC reduction in plateletpheresis can be achieved either by filtration (during or after the procedure) or directly during the procedure on the cell separator (3-6). Filtration results in a platelet loss of about 10%-15% is vulnerable to the risk of filter failure, and if carried out after the apheresis procedure, is cumbersome, increase production costs and calls for extreme rigor on the part of the technicians in charge of the filtration.

The latest generation of blood cell separator such as the Trima (Gambro-BCT) Amicus (Baxter), and ASTEC 204 (Fresenius) allow the collection of leukocyte-reduced platelet concentrates without secondary filtration. So, automated apheresis collection represents a major way of procuring platelets for clinical use (7).

Gambro BCT plateletpheresis technology has been developed to enable blood bank to obtain large number of platelets for transfusion from a single donor. Initially single stage separation was evaluated for platelet collection. It had high collection efficiency but, with $>10^8$ WBC levels, the risk of transfusion reactions was too high. This approach was abandoned in favor of dual stage technology that collected fewer contaminating WBCs. Later, consistent platelet leukoreduction was achieved using a novel fluidized bed LRS chamber, with WBC contamination averaging well below 1×10^6 per unit. Now, single stage platelet separation efficiency is combined with the leukoreduction capacity of the LRS chamber to create a high/efficient, leuko-reduced platelet collection. The benefits of these improvements have been increase platelet collection efficiency and more consistent and enhanced WBC reduction, which may be useful when WBC- reduced plateletpheresis are required.

CHAPTER II

OBJECTIVE

1. To evaluate the performance of a new blood cell separator Trima Accel (version 5.0) with that of the previous version (the Trima system, version 4.0) in plateletpheresis collection.
2. To compare white blood cell (WBC) content, platelet (PLT) yields, the percentage of platelet collection efficiency (CE), processing times and donor comfort between Trima Accel (version 5.0) and the Trima system (version 4.0)

CHAPTER III

LITERATURE REVIEW

3.1 History

Apheresis is a term derived from the Greek that means “take away” by force or “to remove” hemapheresis is the manipulation of circulation blood, leading to remove of normal or abnormal blood constituents. Apheresis may be described as a process by which blood being removed from a subject is a continuously separated into component parts, usually to allow a desired component (or components) to be retained while the remainder is returned to the subject. The apheresis depends on instrumentation, and many advances in the practice of apheresis have been linked to advances in instrumentation. Every general - purpose blood cell separator device released to date has relied on a centrifugation to separate blood into component parts on the basis of differences in density. Apheresis instruments use one of the two versions of a continuous flow centrifugation: either continuously processing or batch processing, the latter having been termed intermittent (or sometimes, discontinuous) flow centrifugation in the apheresis field. Either allows blood to be process in increments small enough that their temporary loss into the instrument is well tolerated by the subject. In this way, it is possible to process total quantities of blood that equal or exceed the subject’s blood volume.

3.2 Principles of Operation

The basis steps in the apheresis are 1) the separation of blood components and 2) the removal of the desired component(s) using an on-line automated system. The ability of various techniques and equipment to carry out these basis steps determines collection efficiency and product purity. Filtration, centrifugation, or a combination of

both can accomplish separation. Filtration takes advantage of differences in particle size to separate blood plasma from the cellular elements. A centrifugation uses differences in specific gravity to separate and isolate blood component.

Centrifugation

In a tube of blood that has reached equilibrium after the application of the centrifugal force, mature red cells (the most dense component) would be located at the bottom while plasma (the least dense component) would have risen to the top. In between, in order of decreasing density, would be neocytes (young red cells), granulocytes, mononuclear cells, and platelets. The granulocyte fraction contains neutrophils, basophils, and eosinophils. The lighter mononuclear fraction contains lymphocytes, monocytes, peripheral blood progenitor cells, in some leukemic patients, blast cells. Unfortunately, a perfectly clean separation is not usually achieved in apheresis instruments. Instead, there is some mixing of the components; for example, small platelets and red cells may be mixed in with the white blood cells (WBCs). In the apheresis field, centrifugal separators are classified as intermittent flow or continuous flow. In intermittent flow devices (also called discontinuous or semi-continuous flow), blood is processed in discrete batches. Separation can occur until the separation container is filled with the dense component; then the container must be emptied before the next batch is processed. It is in contrast to continuous flow devices, in which light, heavy, and intermediate density fractions can all be removed in an ongoing manner so that the separation container need not be emptied until the end of the procedure.

Filtration

Filtration or membrane separators isolate blood component on the basis of differences in particle size. Usually, plasma is separated from the cellular elements. For example, the effective filter pore size may be 0.6 microns whereas the diameter of the platelets, the smallest cellular elements, is 2-3 microns. As whole blood flows by the membrane surface under pressure, plasma passes through the pores and is collected

while the cellular elements are returned to the donor or patient. The main type of membrane separator used in apheresis today is the hollow fiber system. A hollow fiber filter is composed of a bundle of parallel single fibers, each of that resembles a straw with many holes in its wall, confined in a plastic cylinder. Whole blood under pressure enters at one end; as it flows through, plasma is squeezed out the walls and some more concentrated cell suspension exits at the other end. In the most common layout, blood enters the bottom port, cells exit from the top port, and plasma is withdrawn from a side port. An additional side port is usually used to monitor pressure.

Centrifuge-Filter Combination

Filtration and centrifugation can be combined with the use of a rotating fiber. Whole blood enters an upper side port of a stationary container. Rotation of a centrally located fiber causes the blood to rotate and fosters separation of cells and plasma. This pushes cellular components away from the fiber surface (and pores), making the filter less likely to become clogged with cells and therefore more efficient for plasma removal. Plasma passes through the filter membrane and is collected, while concentrated cellular elements are pumped off and returned to the donor. The combination of separation modalities permits the use of a lower g force and a smaller filter surface area than would be required if the modalities were used separately.

3.3 Characteristic of Single-Donor and Pooled Platelets

Both plateletpheresis and whole blood donors must pass the same basis screening criteria. The Food and Drug Administration (8) and the American association of blood bank (9) require that if donors have ingested aspirin or aspirin containing compounds in the previous 36 hours, they not be the sole source of platelets for a given patient. This is especially relevant to apheresis donors because they are likely to be the sole platelet source for a recipient.

Whole-Blood- Derived Platelets

Whole blood derived platelet concentrates are often termed random donor platelet concentrates. This was to distinguish them from apheresis platelet concentrates derived from specific donors for specific refractory patients generally on the basis of HLA matching. In USA, platelet concentrates are separated from whole blood by first preparing platelet rich plasma (PRP) and then concentration the platelet with a second centrifugation. In Europe, the buffy coat technique is most prevalent; the buffy coat isolated from whole blood is resuspended, after which platelets are separated by centrifugation. Mean platelet yields from the two techniques are approximately the same (10). The yield in an individual platelet concentrate depends on the platelet count of the donor, a value that has quite a wide normal distribution. It is also highly dependent on proper technique. Variations tend to even out, however, when the transfusion product is a pool of 4-8 platelet concentrates. In USA, FDA requirements are that platelet content be at least 0.55×10^{11} platelets per unit in 75% of the units tested (11).

Apheresis Platelets

One can obtain a therapeutic dose of platelets for one to three adults by apheresis donors over 1-2 hours using a variety of devices (4, 12, 13, 14). There is also wide variation in the number of platelets obtained from plateletpheresis donations. Variability may stem from differences in apheresis instruments used, in donor platelet counts, the volume of blood processed, and the efficiency of the device. The efficiency of the newest devices is such that one should expect to obtain at least 60% of the platelets that pass through them, and most donors begin to be quite restless if the procedure time exceeds more than 90-120 minutes. Within a given collection facility, considerable variation in platelet yield from a given instrument may be attributable to the wide distribution of platelet counts in normal donors. Because a conventional transfusion dose for an adult patient is six-units of pooled platelets, many institutions originally adopted plateletpheresis collection parameters that, on average, yielded the equivalent number of platelets—namely, $4.5-5.0 \times 10^{11}$ platelets. In the past, when there

was no way to directly predict the final platelet yield, procedure endpoints were specified in terms of the collection time, the volume of blood processed, or the number of batches processed; the parameters chosen were those likely to result in the desired yields. More modern apheresis instruments display a predicted yield that is based on the donor's size, platelet count, and hematocrit. Meet 3.0×10^{11} standard this information allows the operator run parameters so that the desired yield is more closely approximated for each donor. FDA requirements (11) stipulate that at least 75% of single-donor platelet concentrates contain at least 3.0×10^{11} platelets. AABB standard for blood banks and transfusion services (9) requires that 90% of concentrates meet the same quantitative standard. This value of 3.0×10^{11} platelets was established many years ago as a process control indicator and was never meant to imply a medical determination that 3.0×10^{11} platelets represented a standard or minimum transfusion dose. The approximate dose of platelets depends on a number of clinical factors, including the patient's underlying condition, the patient size and platelet count, whether the patient is bleeding, and the patient's expected response to a given transfusion dose. With the availability of the predicted yield feature on modern apheresis instruments, the concept arose of dividing the collected dose of platelets into two or more transfusion products. By extending collecting times and /or selecting donors with high platelet counts, collection facilities can achieve yields large enough to divide the product, yet retain enough platelets in each fraction to meet the 3.0×10^{11} platelets standard.

3.4 Storage of Apheresis Platelet Concentrates

Both whole-blood derived and apheresis platelet concentrates may be stored for five days using the same principle: 1) the temperature must be 20-24°C (15) 2) the storage container that allows adequate diffusion of oxygen to meet the cells' metabolic needs 3) continuous agitation during storage. However, the situation is complicated by the great variation in volume and platelet content of the collections depending on the platelet count of the donor, the volume of the donor's blood processed, and the efficiency of the apheresis device. The collected platelet content may range from 2.0×10^{11} to 12.0×10^{11} platelets. Individual collection containers have a 1- liter capacity so that the surface area for gas exchange is enhanced. However, complexity is added

because gas transport varies directly with the volume of the collection in the container. Furthermore, the plastic varies in their gas transport capacity so that there is variability in the maximum platelet content which a container can tolerate without risking hypoxia and pH fall (16). A collection may be small enough to be stored in one container, but large collections may require two or three containers. Using radiolabeling of stored platelets, survival after reinfusion in vivo nearly normal if storage, even for several days, is carried out at 22-24°C. In colder temperature, survival is shortened (15). If oxygen influx is inadequate, the cell will increase their production of lactic acid, leading to depletion of bicarbonate buffer and fall in pH to less than 6.2 (17,18). These acid conditions result in the platelets being rapidly cleared from the circulation after transfusion. A similar fall in platelet occurs if the platelet concentrates are not agitated during storage (18).

In 1986, increasing frequently reports of post-transfusion sepsis, platelet storage duration was reduced to five days from the seven days period according to current FDA regulations, platelets may be stored at room temperature for up to five days if certain approved containers are used (19). Extension of storage to seven days may induce bacterial overgrowth and clinical sepsis in recipient. Bacterial contamination of apheresis platelets resulting in septic transfusion reactions may occur in as many as 1 in 15,100 transfusions (20). Contamination occurs because of inadequate preparation of the skin, venipuncture through skin pores and hair follicles colonized with bacteria, occult donor bacteremia, or contaminating during processing. Many platelet abnormalities have been described after ex vivo platelet storage (21). At present, in vitro characteristics that correlate best with the capacity to circulate in vivo are retention of disc shape and good function in the hypotonic shock response (22). With few exceptions, platelet with normal discoid morphology will circulate normally after transfusion. Platelets that are damaged by cold, acidity, or bacterial contamination generally lose their discoid morphology and become spheres. Normal discoid morphology is reflected by the “swirling” appearance of well-preserved platelet concentrates during gross, visual inspection (23). Blood bank staff and clinical personnel are urged to check platelet concentrates for this phenomenon before transfusion.

3.5 Indications for Platelet Transfusion

Platelet transfusion are indicated for treatment the patients with thrombocytopenia (platelet counts usually below 50,000/ μ L) or patients with functionally abnormal platelets (congenital or acquired) (24, 25). They are also indicated during surgery or before invasive procedures in patients who platelet counts of <50,000/ μ L. Prophylactic platelet transfusions may be indicated for patients who have platelet counts below 5,000 to 10,000/ μ L associated with marrow hypoplasia resulting from chemotherapy, tumor invasion, or primary aplasia (26, 27, 28). This range may be higher for patients with complicating clinical factors (27). There is no evidence that prophylactic platelet transfusion is beneficial in massive transfusion (29) or in cardiac surgery (30).

3.6 Platelet Dose

The Food and Drug Administration (FDA) and American Association of Blood Banks (AABB) require that 75% of RDP contain at least 5.5×10^{10} platelets and 75% of apheresis platelet products contain at least 3.0×10^{11} platelets (31). A six-unit pool of RDP concentrates or equivalent SDP is expected to raise the platelet count in a 70-kg adult by 30,000 to 60,000/ μ L. These platelet increment targets can be used clinically to detect platelet refractoriness, which occurs in 30% to 70% of multiple transfused patients (32). The original principle behind the development of methods for producing platelets by apheresis was that a therapeutic dose for adult could be obtained from one donor at a single setting. Thus, considerations of platelet dose should guide apheresis collection. Assuming that one- third of infused platelets will be pooled reversibly in a spleen of normal size. Therefore, the volume of distribution for transfused platelets is 50% greater than the recipient's blood volume. The average increment after transfusion is approximately half of that expected (33). Most patients do not achieve the optimal, predicted response because of alloimmunization and/or clinical factors such as fever, infection, concomitant use of amphotericin-B, graft-versus- host disease, or other aspects of the clinical setting (34). Observing whether

bleeding stops best assesses the clinical response to platelet transfusion and by measuring the increment in platelet concentration achieves the patient's blood. The post-transfusion platelet count is dependent on the pre- transfusion platelet count, the dose of platelet transfusion, and other factors that affect the recovery, both techniques. Measurement of platelet transfusion recovery in the clinical setting is the post-transfusion corrected count increment (CCI). The CCI is calculated as follows:

$$\text{CCI} = \frac{(\text{Absolute platelet increment}/\mu\text{L}) \times \text{body surface area (m}^2\text{)}}{\text{Number platelet transfused (10}^{11}\text{)}}$$

A CCI of > 7500 to 10,000/ μL from a sample drawn 10 minutes to 1 hour after transfusion, or a CCI of > 4500/ μL from a sample drawn 18 to 24 hours after transfusion is considered acceptable (not indicative of refractoriness) (35,36). Patients who repeatedly have poor clinical or 1- hour CCI responses are more likely to be immune refractory to platelet transfusion. Patients with HLA or platelet alloantibodies usually require HLA-matched or crossmatched platelets (37). They have adequate 1-hour CCI responses, but poor 24- hour CCI recovery, are most likely refractory due to non-immune causes and may require more frequent or larger dose of platelets.

3.7 Complication due to contaminating leukocytes

3.7.1 Alloimmunization

In 1978, Howard JA, et al studied the natural history of HLA alloimmunization in patients receiving platelet transfusions. 60%(n=63) of those not positive for lymphocytotoxic (LCT) antibodies at the beginning of the study were projected to develop LCT antibodies as early as 10 days after primary exposure or 4 days after secondary exposure in patient who had been transfused or pregnant in the past (38). The number of transfusions was not related to likelihood of immunization, an observation that was confirmed by Dutcher JP, et al (39).

In 1986, Slichter SJ, meet 30%-70% of patients receiving chronic transfusion support occurred platelet alloimmunization (40) and may cause substantial difficulty in providing patients with platelet products that are clinically efficacious.

In 1992, Godeau B, et al followed 50 patients who received multiple transfusions for various hematologic and oncologic conditions (41). Four (8%) were immunized to HLA at the start, and a further nine (18%) developed anti-HLA reactivity after the transfusion period was completed.

The major immunization source of HLA in transfused platelets is the donor leukocytes. Studied in animals and humans show that when platelets devoid of lymphocytes are transfused, primary immunization to HLA is very much delayed or does not occur at all (2, 42, 43, 44), whereas unmodified platelet concentrates are associated with a rate of HLA immunization ranging from 25% to 93% (2, 38, 43, 44, 45, 46, 47, 48, 49). These observations implicate the contaminating leukocytes in both platelet and red blood cell transfusions as the source of primary immunization. In addition, animal studies suggest that cell-free plasma supernatants from platelet concentrates stored without prior leukodepletion contain immunizing fragments thought to have been derived from WBCs (50), and these fragments are not significantly removed by leukoreduction filters (51).

3.7.2 Febrile Nonhemolytic Transfusion Reactions (FNHTR)

The development of febrile nonhemolytic transfusion reactions to platelet products has classically been attributed to an alloimmune response to donor leukocytes. Approximately 20% of platelet transfusions were accompanied by FNHTR (52). Some of these reactions were undoubtedly due to antibodies in the patient directed against either leukocyte-specific or HLA antigens on leukocytes contaminating the platelet product. It is now clear that contaminating leukocytes produce inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor alpha during storage at 20-24°C and that these compounds are responsible for many FNHTR since they are not removed by bedside filtration (53, 54). These reactions provide a strong argument for routine, prestorage removal of leukocytes. Nonetheless, FNHTR occur in approximately 2% of platelet transfusions even with prestorage with leukoreduction (55). The cause of these reactions is unknown. They may be related to plasma proteins or products during storage by the platelets themselves.

3.7.3 Graft-versus-Host Disease (GVHD)

Platelet and red blood cell transfusions contain immunocompetent T cells in sufficient numbers to cause GVHD. Factors that determine an individual patient's risk for transfusion associated graft-versus-host disease include whether and to what degree the recipient is immunodeficient, the degree of HLA similarity between donor and recipient, and the number of transfused T lymphocytes capable of multiplication (56). GVHD may occur in an immunologically normal recipient if the donor is homozygous for HLA haplotype for which the recipient is heterozygous, a so-called "one way" HLA match, and if the component contains large numbers of viable T cells. Transfusion associated graft versus host disease (TA-GVHD) is rare in USA transfusion recipients and has been observed almost exclusively in immunocompromised patients. In contrast over 200 cases of TA-GVHD have been described in Japan (57), incidence rates reaching 1:660 patients undergoing cardiovascular surgery (58).

3.7.4 Transfusion-Transmitted Disease

Cytomegalovirus (CMV)

CMV, a member of human herpes virus family, is a ubiquitous DNA virus that causes widespread infection; transmission can occur through infectious body secretions. CMV infections may be transmitted by leukocyte containing blood component transfusions. In asymptomatic carriers, these virus residuals in the nuclei of subsets of leukocytes with little viruses free in plasma. Approximately 50% of blood donors can be expected to be seropositive, it has been estimated that currently less than 1% of seropositive cellular blood components are able to transmit the virus (59).

In 1995, Bowden RA, et al studied that the use of leukoreduced blood components is essentially equivalent to the use of components from CMV-negative donors in a term of risk of CMV transmission (60). CMV infection is a serious complication in immunocompromised patients given an allogeneic bone marrow transplantation. CMV is readily transmitted from donor to susceptible recipient by blood components containing a large number of residual leukocytes content. CMV

appears not to be transmitted to susceptible recipients by either plasma components or by leukoreduced cellular components prepared from healthy CMV+ blood donors.

Epstein -Barr Virus (EBV)

Transfusion-transmitted EBV is usually asymptomatic, but has been a rare cause of the postperfusion syndrome that follows massive transfusion of freshly drawn blood during cardiac surgery and is a rare cause of post transfusion hepatitis (61). EBV infection targets B-lymphocytes, which undergo polyclonal proliferation and then induce a T- lymphocyte response, observed as “atypical lymphocytes”. EBV contributes to the development of lymphoproliferative disorders in immunosuppressed recipients of hematopoietic and organ transplants.

3.8 Leukocyte Reduction of Apheresis Platelets

WBC-reduced blood components have become a mainstay in supporting multiply transfused patients, mainly in hemato-oncologic settings, to prevent HLA alloimmunization and subsequent platelet transfusion refractoriness and the transmission of infectious agents such as CMV (62, 63, 64). Moreover, they might be responsible for the generation and release of the cytokines in PCs, and previous studies suggested an importance role of cytokines in febrile and non-febrile hemolytic transfusion reactions (65, 66, 67, 68, 69). PCs can be filtered during infusion at the bedside, but it may be preferable to perform leukocyte reduction at the time of preparation of the platelet concentrates. WBC reduction can be performed by preparative techniques or by filtration.

Guidelines developed by the FDA (70) and AABB (9) has established a standard of $< 5 \times 10^6$ WBCs per leukocyte- reduced unit. The council of Europe has defined acceptable leukocyte reduction at a more stringent level of $< 1 \times 10^6$ WBCs (71). Many newer apheresis devices incorporate leukocyte reduction hardware or software, allowing the production of product. Consistently containing $< 5 \times 10^6$ WBCs without secondary filtration. Several studied have shown significant apheresis platelet loss (16-24%) and unacceptably high filtration failure (7%) when prestorage or poststorage conventional fiber leukocyte reduction filters are employed (72, 73). Additionally, bradykinin production during beside platelet filtration with negative charged fiber-based filters is purporting to cause rare hypotensive responses (74).

3.9 Adverse Reactions

Common adverse reactions associated with plateletpheresis include chills, citrate toxicity, and vasovagal reactions. Plateletpheresis requires extracorporeal circulation. Thus, apheresis donors can experience acute adverse reactions, in addition to the risks of whole blood donation. Cooling of the blood in the extracorporeal circuit and infusion of anticoagulants frequently causes the donor to feel cold and/or to have chills. This reaction is usually self-limited and may be easily treated with hot packs and blankets.

Citrate Toxicity

ACD (Anticoagulant citrate dextrose) has been safely and successfully used as the anticoagulant of choice for apheresis procedures. Toxicity of ACD is related to binding of ionized calcium by citrate. Returning anticoagulant plasma may cause clinical signs ranging from mild systems such as circumoral paresthesia (e.g. numbness or tingling around the mouth and face), (progressive paresthesia, nausea, vomiting, abdominal cramping, etc.) to more severe effects (e.g. muscle cramps, loss of consciousness, hypotension, cardiac arrhythmia, neuromuscular irritability, etc). The severity of the clinical sign and symptoms during apheresis is primarily dependent on the rate of citrate infusion. This is determined by several parameters including hematocrit, blood return rate, and anticoagulant: whole blood ratio. Citrate toxicity is common, but it usually is easily managed in apheresis procedures. Oral calcium supplementation, either before or during plateletpheresis procedure, may be useful in preventing mild reactions. Slowing of intravenous calcium should be administered for severe citrate reactions.

Vasovagal Reactions

Vasovagal reactions are also occurring during or after apheresis. Apheresis donors generally manifest as pallor and diaphoresis, with associated hypotension and bradycardia. More severe vasovagal reactions may progress to nausea, vomiting, and syncope, with involuntary defecation and/or convulsions. These clinical sign and symptoms in donors that treated by maintaining the donor in a supine position with the

head below the legs (Trendelenberg position). The procedure stopped. Vital signs should be monitored and recorded until the reaction is reverse to normal. Vasovagal reactions are more commonly seen in first time donors.

Hyperventilation

Hyperventilation (rapid breathing) may cause the anxious or excited donor to lose excessive amounts of carbon dioxide. To divert the donor's attention by engaging in conversation, to interrupt the hyperventilation pattern and/or stimulate the donor rebreathe air from the paper bag.

Hemolysis

Hemolysis is usually caused by a mechanical problem with the equipment, such as a kink in the plastic tubing, machine malfunction or operator error. Observing the return line is important to avoid this problem.

Air Embolism

Donor safety has been a major concern throughout apheresis procedure. Air embolism is very rare complication. Modern apheresis instruments are rapidly advancing due to improvements in technology. They have safety features to detect air in the system and prevent such an occurrence.

3.10 Instruments for Plateletpheresis

Modern cell separators can be programmed for collecting single, double and triple units of SDPs with a high platelet yield and a low WBC contamination (75, 76, 77, 78, 79, 80, 81). These products may be split thereby reducing costs and donor exposure; if double dose SDPs are consecutively transfused to the same patients. If the criterion for leukodepleted blood components is fulfilled (82) there is no further need

for WBC filtration so that costs for the beside WBC reduction filter are saved. Furthermore, the inevitable platelet loss due to filtration process is avoided (83, 84, 85, 86, 87), if leukocyte-reduced components are produced during an apheresis. Very recently, a new device (Leukocyte Reduction System, LRS) for the preparation of leukocyte-depleted platelet concentrates was investigated. This device utilized the “fluidized particle bed technology”, a non-mechanical principle, which effectively reduces the WBC contamination.

Trima Collection System

The Trima system uses a continuous flow centrifuge to separate whole blood into its major components. Whole blood is drawn from the donor and mixed with an anticoagulant (AC). The blood and AC are pumped into a channel--a plastic passageway that sits a specially designed groove in the centrifuge filler and spun at high speed in the centrifuge, separating the blood into its component parts. Combinations of platelets, plasma, and red blood cells (RBC) are collected in bags and the remaining components are returned to the donor. The Trima system uses five pumps to draw and return blood from the donors, as **Figure 1**.

During the collection procedure, AC and whole blood are mixed at a manifold near the needle site. The inlet pump draws the blood and ACD mixture into the system, where the mixture enters the channel. As the whole blood is separated within the channel, pumps remove the platelets and plasma. The pressure created from the blood being continuously pumped into the centrifuge pushes RBCs out of the channel. Depending on the selected procedure, platelets are collected in the product bags or, if not collected, they are routed to the return reservoir to be returned to the donor. When the return reservoir contains a sufficient volume, the return pump is activated. The contents of the return reservoir are returned to the donor until the level of blood reaches the lower level sensor. During platelet and plasma collection, the inlet pump remains at a constant speed, and a small amount of return blood is drawn back through the inlet line and into the channel. This recirculated blood allows the Trima system to maintain a continuous flow of blood through the channel.

Dual Stage Separation

“Dual Stage” refers to the method of separation in the centrifuge channel. The current dual stage design is an excellent separation technology in many years. Anticoagulated-whole blood enters the channel and is separated in two-stage. The first stage separates the RBCs and most of the WBCs from the platelet rich plasma. In the second stage, the platelets are separated from the plasma and further leukoreduced in the LRS chamber.

Single Stage Separation

The new “single stage” design has only one stage of separation. Whole blood enters the inlet port and travels around the channel to the collection chamber. The majority of platelets are removed from the plasma and “lay down” on the interface above the mononuclear cell (MNC) layer on the buffy coat interface. The platelets remain on the buffy coat/RBC interface and have very little contact with the plastic of the channel. However, the MNC layer also separates quite thoroughly. To ensure that a platelet product contains consistently less than 1×10^6 WBCs, the Trima® Accel™ system uses the larger LRS chamber originally designed for use with the Spectra Apheresis system.

The Trima system (Version 4.0)

The Trima system is an apheresis system designed for the collection of Red Blood Cells (RBC), Platelets (PLT) and plasma components from a single donor, uses “dual stage” technology to separate whole blood into the desired blood components. The Trima system is an automatic centrifugal system that is run and controlled by a computer, which on the basis of donor data such as gender, blood type, height, body weight, and platelet pre-count. The system is based on a continuous flow cell separator principle, but with a discontinuous donor flow through a single needle peripheral vein access. The continuous flow cell separation maximizes the platelet collection efficiency due to the dual stage separation chamber that, together with the integrated

LRS, a Gambro BCT proprietary and patented technology, allow one to obtain leukocyte-reduced platelet units in at least 99% of procedures without subsequent filtration. The Trima system has a very low priming volume (approximately 180 ml after the reinfusion phase) and a very limited volume variation during the draw/reinfusion cycles ($\approx 53\text{ml}$). This allows a very high level of comfort for the donor and a reduce incidence of hypovolemic events.

The Trima Accel (Version 5.0)

In 2002, Gambro BCT has redeveloped its single-stage separation technology in the format of the Trima Accel System to increase platelet collection efficiency. Both channel and software were changed, as shown in **Figure 2**. With the single-stage channel, PLTs are continuously collected without the need for any dam separating the channel into separate sections. Alterations of the tubing set and the recirculation of plasma resulted in a lower extracorporeal volume with a new device. The new channel is a single-stage channel similar to, but the LRS chamber is larger in size in the Trima Accel than in the Trima (version 4.0)

There are several articles with which to study. McAteer and coworkers (88) compared the Trima system (version 4.0) to the Trima Accel for the collection of platelets in the same donor. The Trima Accel processed less blood in a shorter time (2400 ± 223 vs. $2802 \pm 454\text{mL}$) (37 ± 6 vs. 48 ± 7 min), whereas preapheresis PLT counts and PLT yields were similar (268 ± 37 vs. $263 \pm 45 \times 10^3/\mu\text{L}$) (3.8 ± 0.5 vs. $3.7 \pm 0.5 \times 10^{11}/\text{unit}$)

Jacobson and associates (89) reported processing time using Trima Accel was shorter than using the Trima system for single products (19-22 min) and double products (14-30 min). The split rate was higher in the Trima Accel, (48.1% vs. 32.2%). Additionally, there was significantly shorter in procedure times for the Trima Accel (single product 48 ± 8.7 min and double product 91 ± 12.7 min) (90) and other studies have reported that procedure time of 62 ± 15 for Trima Accel vs. 83 ± 16 min for the Trima system. (91). Collection rate of $0.068 \pm 0.025 \times 10^{11}\text{PLTs}/\text{min}$ have been reported for Trima Accel (92).

Recently, Ringwald and coworkers (93) compared the productivity and donor citrate toxicity between the Trima system (version 4.0) and the Trima Accel. The procedure time was shorter for Trima Accel (38.9 ± 5.68 vs. 46.1 ± 5.25 min, 15.6% on average). The mean collection efficiency (CE) was higher in the Trima Accel (70.8 ± 7.84 vs. $63.2 \pm 10\%$), the processed volume and ACD-A volume were less in the Trima Accel (2126 ± 340.8 vs. 2350 ± 382.9 mL) (261.6 ± 38.18 vs. 326.3 ± 41.12 mL) and the collection rate were higher (8.8 ± 1.46 vs. $7.3 \pm 1.37 \times 10^9$ PLTs/min). None of the donors had severe citrate effects. Donor comfort was better in the Trima Accel.

Other studies (94) have reported donor satisfaction of platelets collected with both devices. 80% of donors found the experience both better than before and 18% claimed it was the same. 81% of those surveyed thought the procedure was shorter.

Table 1. Main change: Trima Accel (version 5.0) compared with Trima (version 4.0)

Trima Accel (version 5.0)	Trima (version 4.0)
Single-stage filler/channel	Dual-stage filler/channel
One-piece filler	Two-piece filler
Recirculation of plasma	No recirculation of plasma
Extracorporeal volume ≈ 75 mL	Extracorporeal volume < 100 mL
Draw flow ramping	Not present
TBV-based draw/return flow management	Not present
Needle stick evaluation	Not present
Automatic air removal from product bags	Not present
Added process control messages	Not present
Option to limit donor volume body weight	Not present
Red cell calibration factor	Not present

TBV, total blood volume. Ringwald et al., 2003

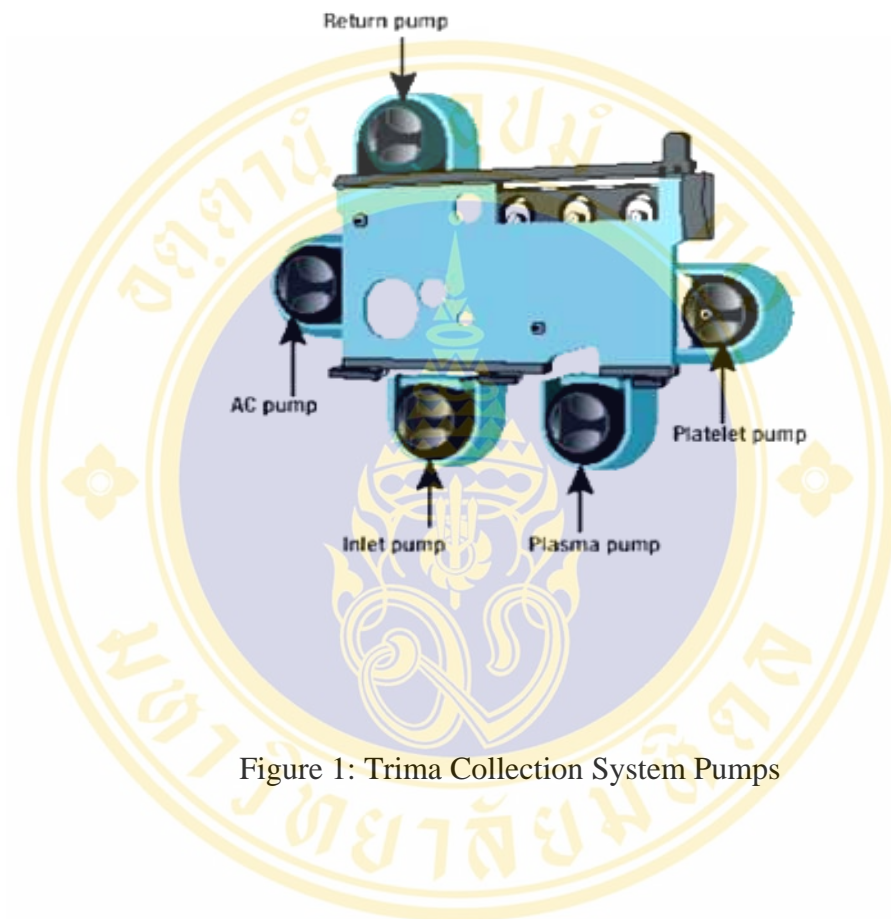


Figure 1: Trima Collection System Pumps

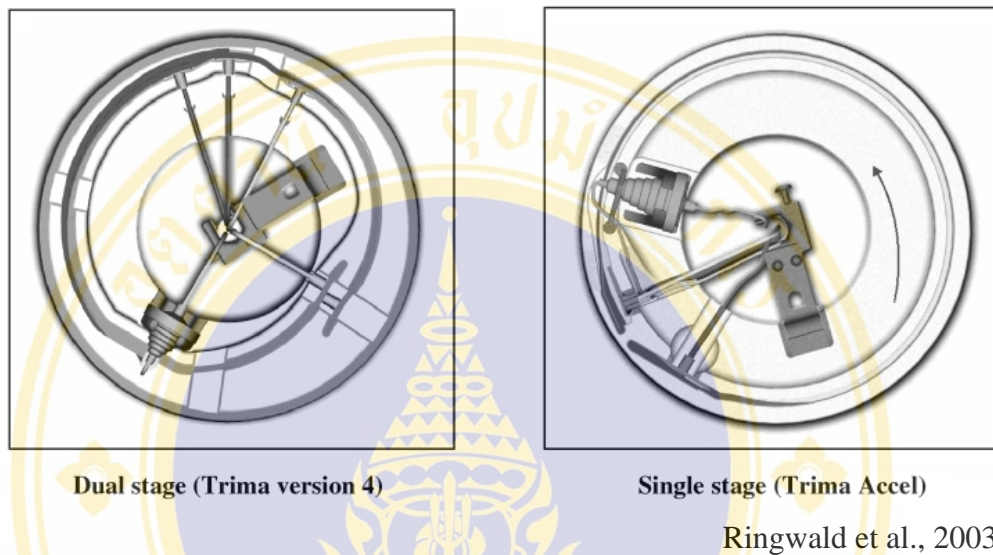


Figure 2. The two different fillers used in the test. The Trima dual-stage filler is shown on the left [Trima version 4]. Cells separate over one-third of the channel circumference (bidirectional flow). The Trima single-stage filler is shown on the right (Trima Accel). Cells separate at maximum radius and over full channel circumference (unidirectional flow).

CHAPTER IV

MATERIALS AND METHODS

Study Design

Cross sectional study design

Timing of the study

Plateletpheresis procedures were collected since November 2004 to March 2005.

Donors and Apheresis procedures

Donor Selection

All volunteer and repeat apheresis donors were recruited from the healthy donor population with no known mental or physical disability or abnormality and receiving no drug therapy. All donors met the requirements of AABB and the FDA blood donors. Donors signed an informed consent document that was approved by the Ethics Committee. Donors' pre-donation platelet count was $\geq 200 \times 10^3/\mu\text{l}$ and venous accesses were available. All procedures were performed by using single needle system without concomitant plasma collection.

Platelet Collection by Apheresis

A total of 60 successful plateletpheresis procedures were performed at the Department of Transfusion Medicine, Siriraj Hospital. 30 procedures were performed by the Trima system (version 4.0) and other 30 procedures were performed by Trima Accel (version 5.0). Blood samples were obtained from each donor the procedure and

tested by using the standard blood-borne pathogen test panel (HIV antigen, HIV antibody, HBsAg, HCV antibody, etc). Complete blood counts were performed in donors' pre-donation apheresis and post-donation apheresis samples with an automated counter (The Cell-DYN 1700 Blood Cell Counter). Post-donation samples were taken immediately. To prevent sample dilution, 7-10 mL of blood was discarded from the tubing line before taking the blood sample. Then, platelet samples were stored at 22°C-24°C for at least 2 hours after the procedure, and then overnight under continuous agitation in a PLT incubator. The following day, all plateletpheresis units were analyzed for their PLT content using the same instrument and WBC counting was performed by using Nageotte chamber.

Plateletpheresis system

The Trima system collects blood components for transfusion. It uses single-needle access and an ACD-A solution as anticoagulant, and does not require the infusion of 0.9% sodium chloride to the donor. The Trima (version 4.0) and Trima Accel (version 5.0) were programmed to obtain PLT yield 3.0×10^{11} for single-dose, 6.0×10^{11} for double dose, and 9.0×10^{11} for triple dose. The ACD-A: whole blood ratio of 1:10.

Materials and Research Instruments for Plateletpheresis

Equipment

1. Trima® Automated Blood Collection System (version 4.0)
2. Trima® Accel™ Automated Blood Collection System (version 5.0)
3. Trima Accel Automated Blood Collection System Tubing Set
4. The Cell-DYN 1700 Blood Cell Counter
5. Autodilution or Autopipe
6. Donation Chair
7. Pressure cuff or tourniquet
8. Heat sealer or Tube sealer

9. Scissors
10. Nageotte Counting Chamber
11. Trima Accel Automated Blood Collection System Operator's Manual, Version 5.0

Supplies

1. Set COBE® Trima™ LRS® Platelet, Plasma, RBC set
2. Set Trima® Accel™ LRS® Platelet, Plasma, RBC Set
3. Anticoagulant Solution: ACD solution USP formula A, 500 ml
4. Test tube 13x125
5. 70% alcohol
6. 2.5% Tincture Iodine
7. Sterile gauze and cotton
8. Transpore and adhesive bandages
9. Leucoplate

METHODS

Procedure of each apheresis instrument: follow the list below

1. Apheresis platelet concentrates by the Trima System (version 4.0)
2. Apheresis platelet concentrates by the Trima Accel (vesion5.0)

Apheresis platelet concentrates by the Trima System (version 4.0)(95)

Principle of the Procedure

The Trima was introduced at the end of 1997, uses “dual stage” technology to separate whole blood into the desired blood components and designed for donor procedures only. The Trima system is an automatic centrifugal system that is run and controlled by a computer which on the basis of donor data such as gender, blood type, height, body weight, and platelet pre-count. The system is based on a continuous flow cell separator principle, but with a discontinuous donor flow through a single needle peripheral vein access. The continuous flow cell separation maximizes the platelet

collection efficiency due to the dual stage separation chamber. First, the PLT-rich plasma is separated from the red blood cells (RBCs) and most of the white blood cells (WBCs). The resulting blood components flow in opposite directions in the first stage, whole blood and PLT-rich plasma counterclockwise, RBCs and most WBCs clockwise. In the second stage, the PLTs are concentrated and drawn off through a small collection line, where they pass through a leukoreduction chamber (LRS). The LRS chamber is a conical-shaped chamber that uses saturated, fluidized, particle-bed filtration technology to remove WBCs from the PLTs before they enter the storage-bag.

A. Installation the Disposable Set

1. Turn the power switch on the upper right side of the machine.
2. The system runs self-diagnostic tests, and the main screen appears.
3. Touch load system.
4. Have the graphics that represent the steps in loading the tubing set, as follow
 - 4.1 hang the bags on the IV pole.
 - 4.2 close the blue slide clamp on the line to one of the platelet bags.
5. Remove the donor line from the package and remove the white paper tapes from the tubing.
6. Rest the coil in the groove in the top left side of the machine or hang the donor line on the left side of the IV pole.
7. Press both upper corners of the cassette to snap the cassette into place.
8. Open the centrifuge door by squeezing the handle to release the door lock.
9. Turn the centrifuge so that the loading port opens to the front.
10. To raise the filler latch, release the filler latch pin by pushing the pin toward the center of the centrifuge.
11. Tuck the LRS chamber inside the flattened channel. Gently feed the channel into the loading port and push it up through the opening at the top of the centrifuge.
12. Lower the filler latch and press it down to lock it into place.
13. Press the channel into its slot in the filler.

14. Press the channel where its ends overlap into the filler.
 - 14.1 Press the rest of the channel into place.
 - 14.2 The LRS chamber will fall into place in its holder.
15. Make sure that the channel is pressed completely into its slot so that the top edge is flush with the locator slots in the top edge of the filler.
16. Position the centrifuge collar over the centrifuge collar holder.
 - 16.1 Aligning the yellow plasma line with the round indent.
 - 16.2 Ensure the plasma line is not blocked by any other line.
17. Pushing from the top, push the centrifuge collar into the holder until the silver release pin extends.
18. To load the lower bearing, push the narrow part of the bearing down into the lower hook until it clicks.
19. To load the upper bearing.
 - 19.1 Push the narrow part of the bearing sideways into the upper hook.
 - 19.2 Push the bearing down into the hook firmly.
 - 19.3 Tug very gently to test that the bearings do not slide.
 - 19.4 Check that there is no kink tubing.
20. Load the upper collar by inserting the tubes into the holder with the collar below the holder.
 - 20.1 Pull the tubing upward until the collar is fully seated in the holder.
21. Rotate the centrifuge to check that the tubing and bearing are secure and not twisted.
 - 21.1 Close the centrifuge chamber door firmly.
22. Ensure that the lines to the product bags are not caught underneath the pump headers.
23. Touch continues. The cassette and pump headers are loaded into place.
24. After the pumps have stopped turning, check that all pump headers have been properly loaded.
25. Close the white pinch clamps on the donor line and the sample bag line as indicated on the screen.
26. Touch continues. The system performed tubing set test.

B. Priming the System

1. After the test is complete, prepare the AC line.
 - 1.1 Get an AC bag (ACD-A) and connect to the set using the orange spike.
 - 1.2 Hang the ACD-A bag next to the product bags on the IV pole.
2. Prime the drip chamber.
 - 2.1 Pinch the drip chamber until the AC reaches the level line, then release.
3. Insert the AC tubing into the AC sensor, located above and to the left of the cassette.
 - 3.1 Use a flossing (back-and-forth) motion.
 - 3.2 Do not twist the tubing.
4. Touch “continue”. The status line shows “Priming AC”.
 - 4.1 When AC priming is complete, begin donor preparation.

C. Connecting the donor

1. Touch “begin donor prep”.
2. Perform the venipuncture.

D. Taking a Blood Sample

1. Touch “begin donor prep”.
2. Perform the venipuncture on the donor.
3. Open the white pinch clamps on the donor line and on the tubing leading to the blood sample bag.
4. Maintain a tourniquet or a cuff pressure of 10 to 20 mmHg on the donor’s arm to improve draw flow.
5. Allow the blood sample bag to fill to the desired volume.
 - 5.1 Close the white pinch clamp on the sample bag tubing.
 - 5.2 Leave the pinch clamp on the donor line open.
6. Touch “start draw”.
7. Seal the blood sample bag line.

8. Draw blood samples as soon as possible from the sample bag for infectious markers lab.

E. Entering Donor Information into the system

1. Touch “donor info”.
2. The system displays donor’s gender, height, and weight.
3. Touch the picture of the male or female.
4. Touch donor height.
 - 4.1 Use the keypad to enter the donor’s height.
5. Confirm the information entered is correct.
6. Touch donor weight.
 - 6.1 Enter the donor’s weight.
 - 6.2 Confirm the information entered is correct.
 - 6.3 Touch enters.
7. System uses the donor’s gender, height, and weight to calculate total blood volume (TBV), and displays this number in the middle of the screen.
8. Touch “confirms info”.
9. Enter donor blood type, hematocrit, and pre-donation platelet count.
10. Touch blood type.
 - 10.1 Enter the donor’s blood type on the letter keypad that replaces the blood type button.
11. Touch hematocrit.
 - 11.1 Enter the donor’s hematocrit value.
 - 11.2 Touch enters.
12. Touch platelet precount.
 - 12.1 Look at the donor’s history and enter the platelet pre-count.
 - 12.2 Touch enters.
 - 12.3 Touch confirms info.
13. To select a procedure.
 - 13.1 Touch the less Preferred or More Preferred.
 - 13.2 Touch confirms procedure.
14. After touch confirms procedure, the system returns to the main screen.

F. The collection run

Touch star draw. Whole blood is drawn from the donor and mixed with AC at the donor manifold. The separated components are removed from the channel by pumps and are either collected in the product bags or routed to the return reservoir.

When the collection run is complete, the system beeps once and automatically continues into rinse back.

1. Touch end run, and then confirm rinse back.
 - 1.1 Rinse back takes about 5 minutes, empties the channel and returns the remaining blood to the donor.
 - 1.2 Rinse back is complete.
2. Touch continues.
 - 2.1 Seal the product bag. Remove them and lay them aside.
 - 2.2 Seal and remove the AC bag.
3. Close the white pinch clamp on the donor line.
 - 3.1 Touch continues.
4. Disconnect the donor.
 - 4.1 Touch confirms disconnect.
 - 4.2 The system raises the cassette for removal of the disposable tubing set.
5. Remove the channel from the filler.
 - 5.1 Begin by gently pulling the upper collar down from the holder until it is free.
 - 5.2 Remove the upper and lower bearings from the hooks.
6. Press inward on the platelet port to release it while gently lifting the tubes and channel from the filler.
7. Push in the release pin on the centrifuge collar holder and remove the centrifuge collar.
 - 7.1 Raise the filler latch and pull the channel down through the loading port.
8. Pull the AC line out of the AC sensor.

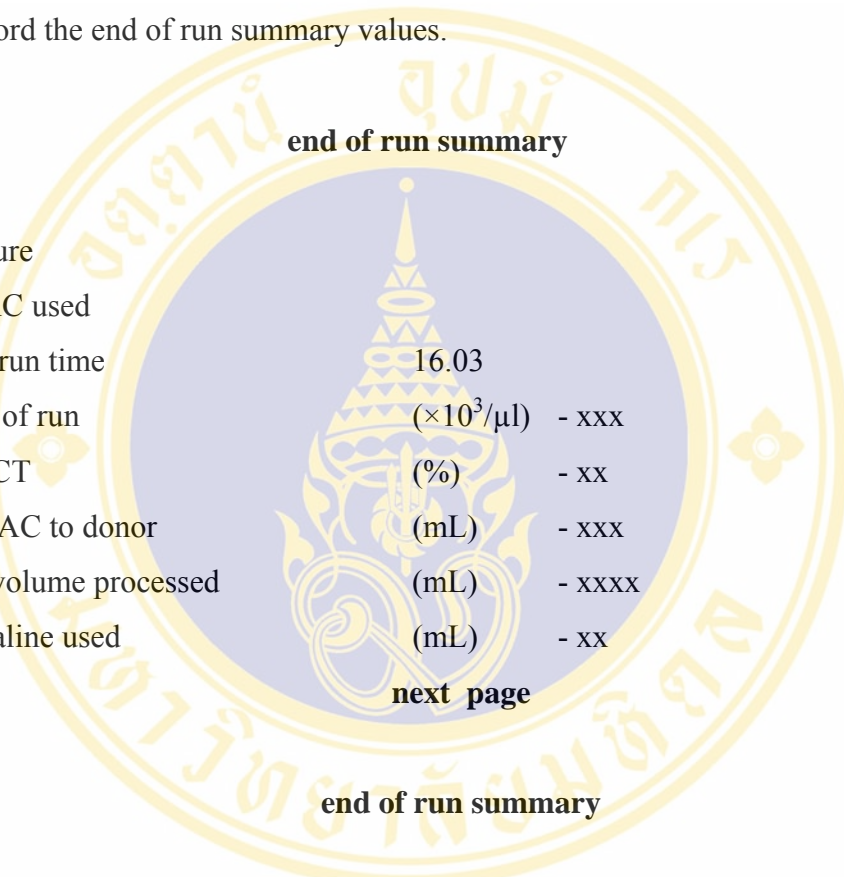
9. Remove any remaining bag.

9.1 Remove the cassette from its holder by pressing the latch in the upper right corner of the cassette.

9.2 Remove the top edge of the cassette, and lift the cassette off the rails.

10. Touch continues.

11. Record the end of run summary values.



end of run summary

Procedure		
Total AC used		
End of run time	16.03	
Length of run	($\times 10^3/\mu\text{l}$)	- xxx
Post HCT	(%)	- xx
Actual AC to donor	(mL)	- xxx
Blood volume processed	(mL)	- xxxx
Total saline used	(mL)	- xx

next page

end of run summary

Platelet Product

Platelet volume	(mL)	- xxx
Yield of platelets	($\times 10^{11}$)	- xx
Volume of AC in platelets	(mL)	- xxx

Plasma Product

Plasma volume	(mL)	- xxx
Volume of AC in plasma	(mL)	- xx

RBC Product

RBC volume	(mL)	- xx
Volume of AC in RBC	(mL)	- xx

Apheresis platelet concentrates by the Trima Accel (Version 5.0)(96)

Principle of the Procedure

The Trima Accel uses a single-stage channel with the same radius throughout. The whole blood enters the channel and travels counterclockwise. The RBCs, WBCs, PLTs, and plasma separate into layers according to specific gravity owing to centrifugal force with all of the blood components flowing in one direction. A shelf in the buffy coat area helps separate the PLTs from the WBCs. As the PLTs are continuously collected without the need for any dam separating the channel into separate sections, they pass through a larger capacity LRS chamber than that used in the Trima (version 4.0).

A. Installation the Disposable Set

Unpacking the Tubing Set and Loading the Cassette

1. Turn the power on the upper right side of the machine.
2. The system runs self-diagnostic tests, and the main screen appears.
3. Touch “load system”
4. Choose the platelet disposable tubing set
5. Open the tubing set package.
6. Inspect the tubing set. Do not use if:
 - 6.1 the needle cap is off,
 - 6.2 the AC spike cap is not in place,
 - 6.3 the tubing shows severe kinks or other signs of damage, or
 - 6.4 the tubing set is incorrectly assembled.
7. Remove the vent, RBC, plasma and platelet bags, and hang the bags on the IV pole
 - 7.1 For platelet collection procedure, close the slide clamp on one platelet bag and collect the product in one bag.
8. Remove the donor line from the package and remove the white paper tapes from the tubing.
9. Place the donor line in the indent on the left side of the machine.

10. Remove the cassette, and position the bottom of the cassette on the bottom rail of the cassette holder, positioning the pump headers over the pumps.
11. Press the upper corners of the cassette to snap it into the cassette clamp.

Loading the Channel into the centrifuge filler

1. Remove the channel from the package.
2. Open the centrifuge door by squeezing the handle to release the door lock and gently lower the door.
3. Turn the metal arm of the centrifuge to the left, so that the loading port is open to the front.
4. Raise the filler latch by pushing the pin toward the center of the centrifuge.
5. Fold the channel in half and tuck the LRS chamber to the folder channel, aligning the chamber's length with the channel.
6. Without stretching any of the tubes.
 - 6.1 carefully feed the channel into the loading port, and
 - 6.2 gently pull the channel up through the opening at the top of the filler.
7. Lower the filler latch and lock it into place.
8. Starting with the collection chamber on the left side of the channel.
 - 8.1 Press the channel into the filler, loading the inlet port last.
9. Load the LRS Chamber into the bracket.
10. Ensuring that the channel is properly seated and flush with the top edge of the filler and the LRS chamber is properly loaded into bracket.
11. Position the centrifuge collar above the holder and push the collar into the holder until hear the "click" of the silver locking pin popping out.

Loading the Lower and Upper Bearings

1. Firmly push the narrow part of the lower bearing into the lower holder.
2. Push the narrow part of the upper bearing into the upper holder.
 - 2.1 Check the tubing in the centrifuge collar line and the white braided portion of the loop to ensure that neither is twisted.

Load the Upper Collar

1. Insert the tubes into the holder with the collar below the top of the holder.
2. Pull the tubing upward until the collar is fully seated in the holder. (The upper collar will be locked into place when the centrifuge door closes.
3. Rotate the centrifuge one full revolution.
 - 3.1 Check the tubing and bearing are securely loaded and that the tubing and white braided portions of the loop are not twisted.
4. Close the centrifuge chamber door.

Loading the Pumps and Lowering the Cassette

1. Check that the lines to the product bags are not caught underneath the pump headers.
2. Check that the clamp on the needle line and sample bag line is not clamped.
3. Touch continues. The Trima system automatically lowers the cassette and loads all pumps, valves, and sensors.
 - 3.1 The system starts the pumps to remove air from the product bags.

B. Preparation the donor tubing and anticoagulant (ACD) bag for the collection procedure.

1. Close the white pinch clamps on the donor line and the sample bag.
2. Touch continues.
3. The Trima Accel system performs a tubing-set test.
 - 3.1 When the tubing set test is complete, connect the AC bag to the AC line.
4. Prime the drip chamber to its level line by pinching the drip chamber and releasing.
5. Using an up-and-down “flossing” motion.
 - 5.1 Seat the AC tubing in the AC sensor.
 - 5.2 Make sure the AC filter is below the AC sensor.
6. Touch continues to perform AC prime.
7. When priming is complete, the Trima main Donor Info/Load System screen displays.

C. Connecting the Donor

1. Touch begins donor prep.
2. Perform the venipuncture.

D. Take a donor samples and prim the system

1. Open the white pinch clamps on the donor line and on the tubing leading to the blood sample bag.
2. Allow the blood sample bag to fill to the desired volume
3. Close the white pinch clamp on the sample bag tubing
 - 3.1 Leave the clamp on the donor line open. To maintain a closes system tubing set and avoid clotting at the needle, clamp the blood sample bag line.
4. Touch star draw.
 - 4.1 The Trima system primes the tubing set with the donor blood, separates it in the centrifuge.
 - 4.2 Begin collecting the blood component.
5. Seal the blood sample bag line as close as possible to where it joints the donor line.
6. Draw samples as soon as possible from the sample bag, using evacuated blood collection tubes.
7. Take care and maintain a cuff pressure 10-20 mmHg on the donor's arm to improve access flow.

E. Entering Donor Information

1. Touch “donor info” on the main screen.
2. The system display donor's gender, height, and weight.
3. Touch the picture of the male or female.
4. Touch “donor height” and enter the height using the number keypad.
5. Touch “enter”.
6. Touch “donor weight” and enter the weight using the number keypad.
7. Touch “enter”.
8. Touch “confirm info”.

9. Enter the donor's blood type
10. Touch "blood type". Enter the type on the letter keypad.
11. Touch "enter".
12. Touch "hematocrit". Enter the donor's hematocrit using the number keypad.
13. Touch "enter".
14. Touch "platelet precount" and enter the donor's platelet pre-count using the number keypad.
15. Touch "enter".
16. Touch "confirm info".
17. To select a procedure.
 - 17.1 Touch the less Preferred or More Preferred.
 - 17.2 Touch "confirm procedure".
18. After touch confirms procedure, the system returns to the main screen.

F. The Collection Procedure

The Trima system automatically collects the products selected. The Trima system first completes a Blood Prime, during which the status line message "blood prime in progress" displays. Anticoagulant blood is pumped up the draw line until the blood reaches the centrifuge. The return pump then reverses and draws anticoagulant blood up the return line until the low sensor in the return reservoir detects fluid. At this point blood prime is complete. The Trima system generates an audible tone to alert the operator to the start of the first return cycle. Collection of the products begins after the first 2-3 draw and return cycles.

G. Performing the Post- Collection Procedures

Seal the product bags and disconnect the donor

1. When rinse back is complete, the screen displays "run complete"
 - 1.1 Touch continues.

2. Seal the collect lines just above the cassette and remove the product bag and the AC bag. Do not seal the vent bag.
3. Assure the product bags are labeled with the donor identification number.
4. Close the white pinch clamp on the donor line.
5. Disconnect the donor.
6. Touch continues.
7. Touch confirms disconnect.

F. Recording End of Run Summary Values

end of run summary

Procedural

Total AC used	(mL)	- xxx
End of run time	14.30	
Length of run	(min)	- xxx
Post platelet count	($\times 10^3 / \mu\text{l}$)	- xxx
Post HCT	(%)	- xx
Actual AC to donor	(mL)	- xxx
Blood volume processed	(mL)	- xxxxx
Total saline used	(mL)	- xxx

next page

end of run summary

Platelet product

Platelet volume	(mL)	- xxx
Yield of platelets	($\times 10^{11}$)	- xx
Volume of AC in platelets	(mL)	- xxx

Plasma Product

Plasma volume	(mL)	- xxx
Volume of AC in plasma	(mL)	- xxx

RBC Product

RBC collect volume (mL) - xxx

Volume AC in RBC (mL) - xxx

next procedure**G. Removing the Tubing Set**

1. Open the centrifuge door
2. Gently remove the upper collar from the collar holder.
3. Gently remove the upper and lower bearings from the bearing holders.
4. Remove the LRS chamber.
5. Remove the channel from the filler.
 - 5.1 Put the finger through the finger access hole on the underside of the filler.
 - 5.2 Push the Collection chamber up, loosening the channel in the filler groove.
6. Push in the locking pin on the centrifuge collar.
 - 6.1 Remove the centrifuge collar from its holder by grasping the tubing above the centrifuge collar and pulling the tubing upwards.
7. Push the filler latch pin toward the center of the centrifuge and raise the filler latch.
8. Pull the channel, LRS chamber, and centrifuge collar down through the loading port.
9. Remove the AC line from the AC sensor.
10. Remove any remaining bags.
11. Remove the cassette from the cassette holder by pressing the latch located in the upper right hand corner of the cassette.
 - 11.1 Pull the top edge of the cassette toward and lift the cassette off the rails.
12. Place the tubing set in a biohazard disposable container.
13. Touch “continue”
14. The screen display end-of run summary
15. Record procedure results.

Nageotte Counting Chamber Method for Counting Leukocytes in Apheresis Platelet Concentrates

1. Label donor number on a test-tube for identify it with the platelet product
2. Apheresis platelet concentrate samples were diluted 1:5 by adding 100 µl of each sample to 400 µl leucoplate.
3. Mix diluted sample well by gentle vertexing and allow to mix for 10 minutes.
4. Carefully place the cover slip on a clean, dry Nageotte chamber. The cover slip must be entered exactly on the chamber. Mix diluted sample again and withdraw sample into a pipettor. Carefully load both slides of Nageotte counting chamber, without disturbing the cover slip. Do not disturb the cover slip once the chamber is loaded.
5. Place the Nageotte chamber to rest in a moistened petri dish for 15 minutes to allow leukocytes to settle. Count the sample within 30 minutes.
6. Use a microscope with a 20x objective, count leukocytes by scanning back and forth across the gridded area.
7. To calculate leucocytes/µl use the following formula:

$$\text{Leukocyte}/\mu\text{l} = \frac{\text{Cells counted} \times \text{Dilution}}{\text{Volume Counted } /\mu\text{l}}$$

8. The volume of the gridded areas counted is 100µl.
9. When no leukocytes were found, calculations were performed as if 1 leukocyte was seen in the volume actually counted.

Donor's complete blood counts analysis by the Cell-DYN 1700 Blood Cell Counter

Complete blood counts were performed in donors' pre-donation apheresis and post-donation apheresis samples with an automated counter (The Cell-DYN 1700 Blood Cell Counter) and in samples from apheresis platelet concentrates. The latter were diluted 1:10 before being counted.

Calculation

Platelet Yield

The PLT yield given by the machines at the end of the procedure were compared with laboratory measurements of the products. And the PLT yield was determined from the formula

$$\text{PLT yield} = \frac{\text{Product Volume (mL)} \times \text{Product Count (platelets/}\mu\text{L)}}{1000\mu\text{L}} \times \text{Conversion Factor (1000}\mu\text{L)}$$

The Percentage of Platelet Collection Efficiency (CE)

CE was determined from the formula

$$\text{CE} = \frac{\text{Platelet Yield}}{\text{Total Platelets Processed}} \times 100$$

$$\text{Total Platelets Processed} = \frac{\text{Pre+Post Counts (platelets/}\mu\text{L)}}{2} \times \text{Total Blood Volume Proc. (mL)} \times \text{Conversion Factor (1000}\mu\text{L/mL)}$$

$$\text{Total Blood Volume Proc.} = \text{Blood Volume Proc (mL)} - \text{Anticoagulant (mL)}$$

WBC Contamination

WBC counting was determined from the formula

$$\text{WBC/}\mu\text{L} = \frac{\text{cells counted} \times \text{dilution}}$$

$$\text{Total WBC/unit} = \text{WBC/}\mu\text{L} \times 1000 \times \text{PLT volume (mL)}$$

When no WBCs were seen, calculations were performed as if 1 WBC was seen in the volume actually counted.

Statistical analysis

All data were compared by using statistical software (SPSS for Windows, version 11.5; SPSS, Chicago, IL). The results are reported as mean±SD. Results were tested for normal distribution that tested using the Kolmogorov-Smirnov test. For statistical analysis, it was performed with unpaired *t* test. Differences are analyzed by using a two-tailed *t* test. A $p < 0.05$ was considered significant.



CHAPTER V

RESULTS

The characteristics of an apheresis donor are shown in Table 2. PLTs counts before apheresis (325 ± 42.92 vs. $326 \pm 42.72 \times 10^3/\mu\text{L}$) and donor blood volume (4681 ± 383 vs. $4720 \pm 406 \times 10^3/\mu\text{L}$) were similar in the Trima system and Trima Accel, but WBCs, and HCT in the Trima system were significantly higher. Donor PLT counts after apheresis were lower in Trima Accel than in the Trima system (Table 3)

Procedure characteristic are shown in Table 4. The mean amount of blood volume processed and ACD used were significantly higher for the Trima system than for Trima Accel (4206 ± 555 vs. 3526 ± 394 mL, $p=0.001$)(461 ± 55 vs. 420 ± 46 mL, $p=0.004$). The Trima Accel had a significantly shorter processing time (71 vs. 77 min, $p=0.044$), being 7.7% shorter than the Trima system.

Product characteristic are shown in Table 5. The number of collected PLTs was $6.63 \pm 0.7 \times 10^{11}$ for the Trima (version 4.0) and $6.98 \pm 1.2 \times 10^{11}$ for Trima Accel (version 5.0). There was not significant difference between both devices ($p=0.197$). PLTs per liter of processed volume were significantly better in the Trima Accel ($1.99 \pm 0.32 \times 10^{11}/\text{L}$ vs. $1.61 \pm 0.33 \times 10^{11}/\text{L}$, $p=0.001$). The CR was higher in the Trima Accel ($0.098 \pm 0.016 \times 10^{11}$ PLTs/min vs. $0.087 \pm 0.018 \times 10^{11}$ PLTs/min, $p = 0.020$). The Trima Accel had a significantly higher CE, being 21% higher than the Trima system. The WBC contamination per unit was similar in the Trima system ($0.042 \pm 0.56 \times 10^6$) and Trima Accel ($0.041 \pm 0.48 \times 10^6$ WBCs/unit, $p=0.315$), which complied with European and AABB guidelines for leukoreduction (93).

All of units from both devices met the minimum yield of 3.0×10^{11} PLTs (100%) required by AABB standard. (9) Double products ($\geq 6.0 \times 10^{11}$ PLTs) were obtained in 83% (25/30) of the collections for the Trima (version 4.0) and 63% (19/30) for the Trima Accel, whereas triple products ($\geq 9.0 \times 10^{11}$ PLTs) were obtained in 3.33% (1/30) and 13.33% (4/30) with the Trima (version 4.0) and the Trima Accel (Table 5).

Adverse reactions in apheresis donors are shown in Table.7. The adverse reactions during or after the procedures were minimal. We only observed mild citrate reactions (including circumoral numbness, paresthesia, and a sensation of vibration), which associated only with return cycles. All reactions responded rapidly to decrease the ACD-A infusion rate and /or oral calcium supplementation. No severe reaction to citrate was seen with either device.

Apheresis of a single-dose, double-dose, and triple-dose SDPs

Compared to the Trima system (version 4.0) as shown Table 6, the Trima Accel processed less blood volumes and ACD-A volume (3397 ± 466 vs. 4280 ± 295 mL) (403 ± 52 vs. 470 ± 28 mL). The Trima Accel collected higher PLT yields (5.76 ± 0.15 vs. $5.64 \pm 0.29 \times 10^{11}$ PLTs). The mean processing time was shorter in the Trima Accel procedures, being 12% (68 ± 14.34 vs. 78 ± 6.58 min). Thus, the collection rate (CR) was higher in Trima Accel (0.087 ± 0.016 vs. $0.072 \pm 0.009 \times 10^{11}$ PLTs/min). A single-dose SDPs (3.0×10^{11}) was obtained in 100% of the collections for both devices.

For double-dose platelet concentrates, both devices were programmed for a target of 6.0×10^{11} PLTs. The Trima Accel collected higher PLT yields compared to the Trima system. The mean PLT yield of both devices exceeds the programmed target by 83% (The Trima system) and 63% (Trima Accel). The mean processing time was shorter in the Trima Accel, being 9% (70 ± 8.38 vs. 77 ± 13.94 min). Thus, the collection rate was higher in the Trima Accel device compared to the Trima system (0.099 ± 0.012 vs. $0.089 \pm 0.019 \times 10^{11}$ PLTs/min).

For triple-dose platelet concentrates were programmed for a target of 9.0×10^{11} PLTs. The mean PLT yield of both devices exceeded the programmed target by 13.33% (the Trima Accel) and 3.33% (the Trima system). The mean processing time was shorter in the Trima Accel, being 8.9% (81 ± 9.81 vs. 89 min). The collection rate was higher in the Trima Accel device compared to the Trima system (0.118 vs. 0.101×10^{11} PLTs /min).

The collection efficiency (CE) was higher in the Trima Accel (a single-dose; $77.2\% \pm 1.90$ vs. $56.1\% \pm 3.29$, triple-dose; $88.8\% \pm 4.75$ vs. 85.8%) except double-dose; $66.6\% \pm 5.29$ vs. $84.6\% \pm 8.7$

Table 2. Donor characteristics before apheresis

	Trima system (version 4.0)	Trima Accel (version 5.0)	p value
Number of procedures	30	30	
Donor blood volume (mL)	4681.10±383.60	4720.63±406.05	0.700
Preapheresis PLT count ($\times 10^3/\mu\text{L}$)	325.90±42.92	326.33±42.72	0.969
Preapheresis WBC count ($\times 10^3/\mu\text{L}$)	7.06±1.17	6.25±1.12	0.008
Preapheresis HCT (%)	45.20±2.82	42.57±3.24	0.001

Table 3. Hematology data for the platelet donors

	Trima system (version 4.0)	Trima Accel (version 5.0)	N	P-value
Donor PLT concentration before collection ($10^3/\mu\text{l}$)	325.90 ± 42.92	326.30 ± 42.72	30	0.969 ^a
Donor PLT concentration after collection ($10^3/\mu\text{l}$)	222.73 ± 44.23	215.66 ± 34.29	30	0.492 ^a
Donor RBC concentration before collection ($10^6/\mu\text{l}$)	5.09 ± 0.45	4.89 ± 0.39	30	0.073 ^a
Donor RBC concentration after collection ($10^6/\mu\text{l}$)	4.91 ± 0.42	4.75 ± 0.40	30	0.153 ^a
Donor Hct before collection	45.20 ± 2.82	42.57 ± 3.24	30	0.001
Donor Hct after collection	43.70 ± 2.74	41.42 ± 3.14	30	0.004
Donor WBC concentration before collection ($10^3/\mu\text{l}$)	7.06 ± 1.17	6.25 ± 1.12	30	0.008
Donor WBC concentration after collection ($10^3/\mu\text{l}$)	8.44 ± 2.04	7.19 ± 1.93	30	0.018

^a Not significant

Table 4. Apheresis procedure characteristics

	Trima system (version 4.0)	Trima Accel (version 5.0)	P value
Number of procedures	30	30	
Blood volume processed (mL)	4206±555	3526±394	0.001
ACD-A used (mL)	461±55	420±46	0.004
Processing time (min)	77±13	71±10	0.044

Table 5. Plateletpheresis characteristics

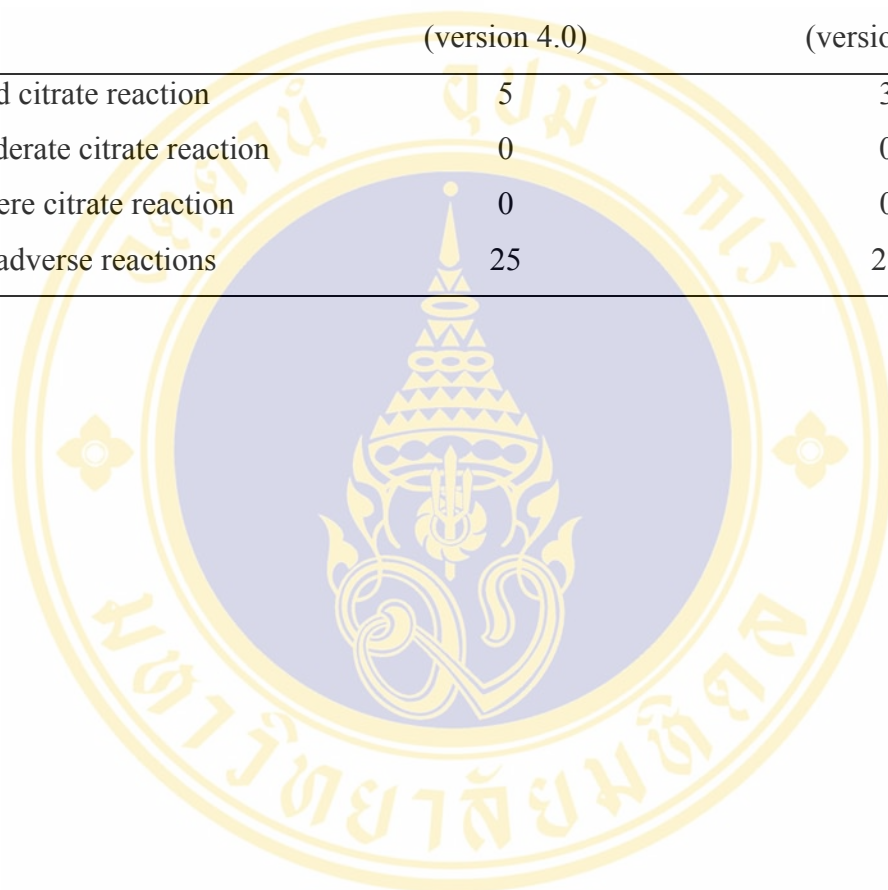
	Trima system (version 4.0)	Trima Accel (version 5.0)	p value
PLT volume (mL)	423±5.6	399±46	0.008
PLT yield ($\times 10^{11}$)	6.63±0.70	6.98±1.2	0.197
PLT yield (PLT $\times 10^{11}$ /L)	1.61±0.33	1.99±0.32	0.001
CE (%)	65±7.2	83±8.0	0.001
CR (PLTs $\times 10^{11}$ /min)	0.087±0.018	0.098±0.016	0.020
WBC contamination ($\times 10^6$ /unit)	0.042±0.56	0.041±0.48	0.315
Log WBC per unit	4.62±0.01	4.61±0.05	0.180
Frequency			
$\geq 3.0 - 5.99$ ($\times 10^{11}$ PLTs)	5.64 ± 0.29	5.76 ± 0.15	
(%)	(13.33%)	(23.33%)	
$\geq 6.0 - 8.99$ ($\times 10^{11}$ PLTs)	6.69 ± 0.42	6.94 ± 0.66	
(%)	(83.33%)	(63.33%)	
≥ 9.0 ($\times 10^{11}$ PLTs)	9.05	9.62 ± 0.35	
(%)	(3.33%)	(13.33%)	
< 1.0 ($\times 10^6$ WBCs) (%)	100	100	

Table 6. Results of a single-dose, double- and triple-dose SDPs

	Single-dose SDPs		Double-dose SDPs		Triple-dose SDPs	
	T4 (N=4)	T5 (N=7)	T4 (N=25)	T5 (N=19)	T4 (N=1)	T5 (N=4)
Preapheresis						
PLT count ($\times 10^3/\mu\text{L}$)	313 \pm 18.87	309 \pm 32.99	328 \pm 46.28	317 \pm 27.76	314	398 \pm 50.38
Blood volume processed (mL)	4280 \pm 295	3397 \pm 466	4186 \pm 598	3522 \pm 352	4396	3802 \pm 380
ACD-A used (mL)	470 \pm 28	403 \pm 52	459 \pm 60	418 \pm 42	478	465 \pm 29
PLT volume (mL)	421 \pm 2.36	381 \pm 3.28	423 \pm 6.06	392 \pm 47.66	423	469 \pm 22.14
Processing time (min)	78 \pm 6.58	68 \pm 14.34	77 \pm 13.94	70 \pm 8.38	89	81 \pm 9.81
PLT yield ($\times 10^{11}$)	5.64 \pm 0.29	5.76 \pm 0.15	6.69 \pm 0.42	6.94 \pm 0.66	9.05	9.62 \pm 0.35
TBV	4830 \pm 526	4639 \pm 395	4672 \pm 365	4774 \pm 455	4309	4641 \pm 93

Table 7. Adverse reactions for different instruments

Type of adverse reactions	Number of donors	
	Trima system (version 4.0)	Trima Accel (version 5.0)
Mild citrate reaction	5	3
Moderate citrate reaction	0	0
Severe citrate reaction	0	0
No adverse reactions	25	27



CHAPTER VI

DISCUSSION

The main objective of this study was to evaluate and compare whether the performance of the Trima Accel (version 5.0, dual stage) and the Trima system (version 4.0, single stage) can collect large of leukocyte reduced platelet concentrates (double-dose, and triple-dose). Focusing on the processing time of the procedure, the yield of platelet, which yield and time considered together as collection rate, and WBC contamination.

Comparison of donor characteristics before apheresis in our study showed that apheresis donor underwent plateletpheresis procedures with both devices, there were no significantly differences in preapheresis PLT counts except preapheresis WBCs and HCT in the Trima system were higher significantly. After apheresis, although PLT counts were lower in Trima Accel donors (34%) than Trima system (31.6%) but within normal range. These differences are considered not to be clinically relevant for apheresis donors.

Collection efficiency (CE) is a practical way used to compare apheresis system. CE compares the amount of PLT collected versus the calculated amount of PLTs that pass through the machine, which volume of blood processed plays a major role in determining CE. Both devices obtained similar PLT yields, but the Trima system had to process significantly more blood to obtain them, which resulted in a significantly lower CE. In our study, the CE for the Trima Accel was higher (83%). Others have reported collection efficiencies of 70%-75% for Trima Accel (93, 97). Increased collection efficiencies may translate into several benefits, including increased double and triple dose SDPs, and decrease donor processing time.

Comparison of plateletpheresis characteristics, the Trima Accel processed less blood volumes with high CE, and high CR in a shorter time (Table 2, 3).

And if we compared platelet yield per liter of blood processed, there was a significant difference between the Trima system and the Trima Accel ($p < 0.001$). For that reason, is due to the improvement of platelet collection efficiency and a new design single-stage channel with the Trima Accel, which use less blood processed, but high collection efficiency.

Focusing on the PLT products show a significant difference in the PLT volume, but the PLT yields were not significantly different. PLT yield in our study included a single, double, and triple-dose platelet concentrates (before splitting). A finding of more practical meaning than mean PLT yield is the frequency at which yields of at least 3.0×10^{11} , 6.0×10^{11} , and 9.0×10^{11} PLTs were obtained. As can be seen in Table 3, the frequencies were different in both devices. All of the platelet collections met the minimum yield in the AABB standard (98). The split (double) rate of 83% for the Trima system was higher than the Trima Accel (65%), whereas the split (triple) rate of 13.33% for the Trima Accel was higher than the Trima system (3.33%). However, apheresis instruments from both devices were able to produce platelet products equally that was 57 units in total 7 single-dose (100%), 19 double-dose (65%) and 4 triple-dose (13.33%) for Trima Accel, whereas 4 single-dose (100%), 25 double-dose (83%), and a triple-dose (3.33%) for Trima system. Finally, double-dose and triple-dose leukocyte reduced platelet concentrates can be split. The splitting of SDPs reduces costs (for disposable kits, laboratory testing, filter) and donor exposure of the recipients when double, and triple-dose of SDPs are transfused to the same patient. Furthermore, because a number of blood donors are limited, providing an increasing demand for SDPs, we must optimize the productivity of plateletpheresis with respect to the effectiveness, economy, and consistency in platelet collecting. Additionally, we ought to emphasize donor safety and donor satisfaction (e.g. donor adverse reaction and the duration of procedure).

The duration of procedure has been identified as another important element in apheresis PLT donor retention (99), and this variable should be considered as one of the most important factors for both donors and blood banks when evaluating and choosing an apheresis system.

In our study, comparison of apheresis procedure characteristics shows that the processing time for the Trima Accel was significantly shorter.

Collection rate (CR) is probably a practical way to compare plateletpheresis system because it addresses PLT yield and processing time simultaneously. The CR was higher for the Trima Accel (0.098 ± 0.016 vs. $0.087 \pm 0.018 \times 10^{11}$ PLTs/min). Others have reported collection rates of 0.115 and 0.068×10^{11} PLTs/min (92, 93). Therefore, our results for Trima Accel fit well within this range.

In addition to PLT yields, CE, CR, and processing times, consistent leukoreduction is a key element in plateletpheresis. At present WBC reduction in blood components represents an important improvement in transfusion (100). In our study, all of apheresis platelet concentrates from both devices were capable of consistently leukoreduced platelets less than 5×10^6 WBCs as same as published results (93). This low number of the contaminating WBCs reduces the risk of alloimmunization, generation of interleukins during storage, and transmission of CMV (4). Moreover, the SDPs fulfill the requirements for WBC-reduced blood components as stated by AABB (9) and European regulations (71).

Regarding donor adverse reactions, we only observed mild adverse reaction and no calcium gluconate was given. The mean processing time of not greater than 77 minutes that our donors' experience can tolerance to apheresis instrument very well. Lower or equivalent reaction rates have been reported with Trima Version 4.0 versus Trima Accel (90, 91, 93, 94).

From these results, we also suggest that if apheresis donor have preapheresis PLT count $\geq 315 \times 10^3/\mu\text{L}$, we can collect double-dose or triple-dose of leukocyte-reduced platelet concentrates with these machines that spent more 10-12 minutes in triple-dose. We should also asked donors whether they have enough time to donate in completely procedure, which depending on a satisfaction of donors to make a decision. Therefore, donor selection for providing large PLT yield and donor satisfaction are the high priorities in modern apheresis collection, which should not exceed 90 minutes. Because most donors begin to be quite restless if the processing time exceeds more than 90-120 minutes.

For these apheresis instruments, we would like to discuss some of weak points that we observed and donor complain in regarding to the use of pressure sleeve that facilitates extraction cycles (draw or return cycle). Both devices had no pressure

sleeve compare to another machine (e.g. Amicus, MCS plus). Donors are confused because they can't extract cycle.

Secondly, the disposable kit from these machines, there are no connecting joint for collecting post-apheresis sample in the disposable kit. This will be a problem when we have to change to the new needle.



CHAPTER VII

CONCLUSION

This study aimed to evaluate and compare the performance of a newly cell separator Trima Accel (version 5.0) with that previous the Trima system (version 4.0), which used in platelet collection. A total of 60 volunteer and/or repeat apheresis donors were recruited to donate platepheresis at the Department of Transfusion Medicine, Faculty of Medicine, Siriraj Hospital since November 2004 to March 2005. As for data analysis, descriptive statistics, unpaired *t* test was employed.

In this study, the Trima Accel shows further improvements apheresis platelet production in a shorter time. Regarding, WBC- reduction the newly Trima Accel already complied with the existing guidelines when using the previous Trima system. None of apheresis platelets from the Trima Accel contained more than 1×10^6 WBCs, which resulted in WBC contamination that was not significantly different from that obtained in collections using the Trima system.

Furthermore, the Trima Accel can collect large of platelet concentrates (double-dose, triple-dose) that used less blood and reduced load of citrate on the donor. We may conclude that the Trima Accel has the ability to produce platelet concentrates without increasing incidence of citrate reactions.

In conclusion, the Trima Accel is directed at collecting faster apheresis procedure, improvements in the production of high-quality products, and few adverse reactions for donors. The advantage of this technique reduces the recipient's risk of donor exposure, the production cost, loss of platelets, and carries the risk of filter failure.

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