QUALITY OF SINGLE DONOR PLATELETS IN ADDITIVE SOLUTION

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Finally, I would like to dedicate the usefulness of this thesis to my beloved family, Dad and Mom, I would like you to know how proud I am to be your child and I'll try my best to make you proud of me. Thank for your endless love.

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ABSTRACT

The requirement of single donor platelets (SDP) for thrombocytopenic patients has been increasing. To extend platelet shelf-life for more than 5 days, special type of a plastic bag and platelet additive solution (PAS) have been developed.

The purpose of this study is to evaluate the quality of single donor platelet in PAS. Two hundred and forty units of SDP from 120 repeated donors were prepared using fully automated blood cell separator. SDP in plasma and PAS were collected from the same donor and were tested in parallel in vitro for platelet counts, residual white blood cell count, pH, swirling phenomenon, bacterial contamination and CD62p expression on day 0, 3, 5 and 7 days. The effectiveness of platelet transfusion of both SDP in plasma and PAS in patients were evaluated by platelet count increment (CCI).

The results showed comparable results for platelet yields, residual WBC and swirling phenomenon. CD62p level were higher in SDP in PAS but 1 hour and 24 hour CCI were not significantly different in both SDP in plasma and PAS. In conclusion, SDP in PAS have a comparable effectiveness for transfusion as SDPin plasma.

KEY WORDS: SINGLE DONOR PLATELET (SDP)
PLATELET ADDITIVE SOLUTION (PAS)

61 pp.

การศึกษาคุณภาพของ SINGLE DONOR PLATELETS ใน ADDITIVE SOLUTION

(QUALITY OF SINGLE DONOR PLATELETS IN ADDITIVE SOLUTION)

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บทคัดย่อ

เนื่องจากความต้องการเกร็ดโลหิตชนิด single donor platelets (SDP) ที่เพิ่มมากขึ้นของผู้ป่วยที่มีภาวะเกร็ดโลหิตต่ำ ทำให้เกร็ดโลหิตเป็นส่วนประกอบของโลหิตที่มีความขาดแคลน จึงมีความพยายามที่จะเก็บรักษาเกร็ดโลหิตให้นานขึ้น อันนำไปสู่การพัฒนาเทคโนโลยีในการเก็บรักษาเกร็ดโลหิต โดยพัฒนาคุณภาพของ พลาสติก ที่นำมาผลิตเป็นถุงสำหรับเก็บเกร็ดโลหิต นอกจากนี้ยังมีการสังเคราะห์น้ำยาสำเร็จรูปขึ้นมาเพื่อนำมาใช้ในการเก็บรักษาเกร็ดโลหิต ทำให้สามารถเก็บเกร็ดโลหิตได้นานกว่า 5 วัน

ในการศึกษาครั้งนี้ต้องการศึกษาคุณภาพของเกร็ดโลหิต ที่เตรียมโดยใช้เครื่องแยกส่วนประกอบโลหิตอัตโนมัติ เพื่อให้ได้ส่วนประกอบของโลหิตชนิด single donor platelets (SDP) จำนวน 120 ถุง ที่เก็บรักษาใน plasma เปรียบเทียบกับการเก็บรักษาใน platelet additive solution (PAS) จากผู้บริจาคโลหิต จำนวน 120 ราย โดยศึกษาเปรียบเทียบ ปริมาณเกร็ดโลหิต จำนวน residual white blood cell , pH, swirling phenomenon, การปนเปื้อนของแบคทีเรีย และ CD62p expression ในวันที่ 0, 3, 5 และ 7 พร้อมทั้ง ศึกษาถึงคุณภาพของ SDP ที่เก็บรักษาใน plasma และ PAS เมื่อให้แก่ผู้ป่วย โดยดูจากค่าของ platelet count increment (CCI).

ผลการศึกษาพบว่า ปริมาณเกร็ดโลหิต จำนวน residual white blood cell, pH, swirling phenomenon, CCI ของ SDP ที่เก็บรักษาใน plasma และ PAS ไม่มีความแตกต่างอย่างมีนัยสำคัญ ไม่พบการปนเปื้อนของแบคทีเรีย CD62p expression ของ SDP ที่เก็บรักษาใน PAS มีค่ามากกว่า SDP ที่เก็บรักษาใน plasma จากผลการศึกษาสรุปได้ว่า SDP ที่เก็บรักษาใน PAS มีคุณภาพดี และสามารถที่จะนำมาใช้ทดแทน SDP ที่เก็บรักษาใน plasma

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

µL	=	microliter
µM	=	micrometer
ACD	=	acid-citrate-dextrose
CCI	=	corrected count increment
CD	=	cluster of differentiation
DIC	=	disseminated intravascular coagulation
EDTA	=	ethylene diaminetetra-acetatic acid
FITC	=	fluorescein isothiocyanate
h	=	hour
ITP	=	idiopathic thrombocytopenic purpura
m^2	=	square meter
min	=	minute
mL	=	milliliter
mm	=	millimeter
mm^3	=	cubic meter
mMol	=	millimolar
NAIT	=	neonatal alloimmune thrombocytopenia
^oC	=	degree Celsius
PAS	=	platelet additive solutions
PBS	=	phosphate buffer saline
PC	=	platelet concentrates
PE	=	phycoerythrin
PF4	=	platelet factor 4
PPP	=	platelet poor plasma
PRP	=	platelet rich plasma
rpm	=	revolutions/round per minute

LIST OF ABBREVIATIONS (continued)

RT	=	room temperature
S.G	=	specific gravity
SDP	=	single donor platelets
SPSS/PC+	=	statistic package for the social science personal computer plus
TTP	=	thrombotic thrombocytopenic purpura
vWF	=	von Willebrand factor
WBC	=	white blood cell
β-TG	=	beta-thromboglobulin

CHAPTER 1
INTRODUCTION

Since Landsteiner's blood group ABO discovery, the preparation of blood component started when sterilization techniques became available. It is widely accepted that the benefits of platelet transfusion into thrombocytopenic patients are mediated by an increase in the post-transfusion counts.

Today, platelet concentrates are produced from whole blood by differential centrifugation (buffy coat-derived platelet concentrates) or by plateletpheresis (apheresis-derived platelet concentrates). A plateletapheresis product or single donor platelet (SDP) is collected from an individual donor by apheresis. SDP contains at least $3x10^{11}$ platelets, with at least 75% of the platelets being intact, which usually contain 200 to 400 mL of plasma. The number of platelets is equivalent to a pool of five to six units of random-donor platelet concentrates in which each concentrate is prepared from whole blood donations. One unit of apheresis platelets will usually increase the post-transfusion platelet count of an adult by 30,000 to 60,000 /uL (AABB Standards, 1996).

The requirement of platelet concentrates has been increasing but the storage period of platelet concentrates is only 5 days. To extend platelet shelf-life, plastic type container and storage media have been developed. Since 1980, there has been a great interest in utilizing platelet additive solutions (PASs) for the storage of platelet concentrates (PCs). At present, such additive solutions are in use for transfusion in several countries in Europe. PAS is generally used as a substitute for plasma in order to:

1. reduce the amount of plasma transfused with platelets and to recover additional plasma for other purposes, primarily fractionation into plasma products
2. avoid transfusion of large volumes of plasma with possible adverse reactions and circulatory overload
3. improve storage conditions to increase shelf-life of the platelets while maintaining the viability and haemostatic function at a high level
4. make possible photochemical treatment for the inactivation of virus and other

pathogens in PCs.

The study of platelet additive solution (PAS) to maintain platelet quality in vitro is based on shelf life maximum allowable storage time and “storage lesion”. The platelet storage lesion is the overall changes that platelets undergo following collection, processing and storage at 22^{o}C prior to transfusion. Moreover, in vivo study includes transfusion reaction and Corrected Count Increment (CCI) following transfusion.

CHAPTER 2
OBJECTIVES

1. To compare the quality of single donor platelets in plasma with single donor platelets in additive solution in order to use additive solution instead of plasma for patient with allergic reaction and ABO incompatible platelet as indication.
2. To study the effectiveness of platelet transfusion in patients by determining Corrected Count Increment (CCI), monitor bleeding control and transfusion reactions in patients.

CHAPTER 3
LITERATURE REVIEW

Platelets are nucleate cellular fragments derived from the cytoplasm of megakaryocytes present in the bone marrow. Platelets are released into the circulation approximately 9 to12 days as small, disc-shaped cells with an average diameter of 2 to 4 uM. The normal platelet count ranges from 150,000 to 400,000/uL. In peripheral blood, approximately 30% of the platelets are sequestered in the microvasculature or in the spleen as functional reserves after they are released from the bone marrow.

The studies of platelet structure by electron microscopy show the presence of four major zones. Each zone plays a critical role in normal platelet physiology. (1)

1. The peripheral zone has an important function regarding platelet adhesion and aggregation. It is a complex region of the platelet. An exterior coat, or glycocalyx, rich in glycoprotein provides the outermost covering of the peripheral zone. A number of glycoprotein present in this area is responsible for blood group specificity (ABO), tissue compatibility (HLA), and platelet-unique immunologic antigenicity. Some components of special importance are: glycoprotein Ib, which acts as a platelet receptor for von Willebrand factor in platelet adhesion, the glycoprotein IIb-IIIa complex, which spans the platelet aggregation, glycoprotein V, a possible functional substrate of thrombin. The middle layer of the peripheral zone is a typical unit membrane. It is rich in asymmetrically distributed phospholipids which provide an essential surface for interaction with coagulant proteins. The area lying just inside the unit membrane represents the third component of the peripheral zone. It is closely linked to glycoproteins on the unit membrane by submembrane filaments serving as a cytoskeletal network to maintain the platelet's discoid shape.

2. The sol-gel zone is the matrix of the platelet cytoplasm. It contains several fiber

systems such as: microtubules, microfilaments, thrombosthenin, actin and myosin. These structures support the discoid shape of unaltered platelets and provide a contractile system involved in shape change, pseudopod extension, internal contraction, and secretion.

3. The organelle zone contains mitochondria, paroxysms, lysosomes, and storage granules of varying electron densities. The electron-dense (delta) granules are fewer in number (2 to 10 per platelet) and contain substances such as storage pool adenosine diphosphate (ADP), adenosine triphosphate (ATP), serotonin, and the major portion of the platelet's calcium. Alpha-granules are more numerous(20 to 200 per platelet) and contain substances intrinsic to the platelet, including platelet-specific proteins such as PF4, β-TG, and platelet-derived growth factor (PDGF), as well as, other proteins, including fibrinogen, fibronectin, factor V, thrombospondin, and vWF.

4. The membrane system consist of the dense tubular system (DTS) and open canalicular system (OCS). The DTS has been shown to be the site where enzymes involved in prostaglandin synthesis are localized and it is also the site where calcium important for triggering contractile events is sequestered. The surface-connected OCS provides access to the interior for plasma-borne substrates and an engross route for the release reaction.

Platelets function has an important role in bleeding control by maintaining the integrity of the vascular tree, responsible for the primary hemostatic plug. Spontaneous bleeding due to severe thrombocytopenia (5,000 to 10,000 / uL), decreased platelet production, loss from the body, and destruction and dysfunction (Tables 1 and 2) are characterized by slow diffuse oozing from venipuncture and surgical site, petechiae, ecchymosis, gingival bleeding, conjunctival hemorrhage, hematuria, and melena. On occasion, intracranial bleeding may occur.(2)

Platelet transfusion has become an essential supportive therapy for patients with severe thrombocytopenia, especially during the past two decades, a period that has seen

a growing trend toward intensive chemotherapy with or without stem cell support. Alloimmunization to histocompatibility antigens; transmission of viruses, protozoa, and bacteria; graft-versus-host disease; and immune modulation are adverse effects attributed to leukocyte admixture during transfusions.(3)

Table 1 Mechanisms underlying thrombocytopenia (4)

A. Disorders of production
  1. Decreased megakaryocytopoiesis
    a. Congenital disorder (Fanconi's anemia, TAR syndrome, intrauterine drugs or infection, etc.)
    b. Acquired hypoplasia (radiation, chemicals, alcohol, insecticides, drugs such as thiazides, chloramphenicol, or cancer chemotherapy, infections, lupus erythematosus, idiopathic, etc.)
  2. Ineffective platelet production
    a. Hereditary thrombocytopenia (autosomal dominant, May- Hegglin Anomaly, Wiskott- Aldrich syndrome, etc.)
    b. Vitamin B_{12} or folate deficiency
    c. Others (Myelodysplastic syndrome, paroxysmal nocturnal hemoglobinuria, etc.)
B. Disorders of distribution and dilution
  1. Splenic pooling (congestive infiltrative, inflammatory, infectious, hyperplastic, neoplastic etc.)
  2. Hypothermia
  3. Dilution by transfused stored blood
C. Disorder of destruction
  1. Combined consumption
    a. Snake venoms
    b. Tissue injury (surgical, trauma, anoxia, toxic necrosis, etc.)
    c. Obstertic complications (abruption placentae, retained dead fetus, amniotic fluid embolism, toxiemia, etc.)

Table 1 Mechanisms underlying thrombocytopenia (continued)

d. Neoplasms (promyelocytic leukemia, carcinoma, hemangioma, etc.)

e. Infection (bacterial, viral, rickettsial, etc.)

f. Intravascular hemolysis

2. Isolated platelet consumption

a. Thrombotic thrombocytopenic purpura

b. Hemolytic-uremic syndrome

c. Vasculitis(disseminated lupus erythrematosus, other collagen vascular disease, bacteremia, etc.)

d. Cardiopulmonary prostheses

3. Immune destruction

a. Autoimmune (acute, chronic, Transplacenta, secondary, etc.)

b. Acquired immunodeficiency disorder (AIDS)

c. Post-transfusion purpura

d. Isoimmune neonatal purpura

e. Drug-induced antibodies (gold, quinine, quinodine, sulfonamide derivatives, etc.)

f. Others

D. Combination thrombocytopenia

1. Alcoholic liver disease

2. Lymphoproliferative disorders

3. Cardiopulmonary bypass

4. Others (malignancies, infection, etc.)

Table 2 Clinical features of immunologic thrombocytopenic purpura (4)

	Children	Adults
Occurrence		
Peak age (years)	2-4	15-40
Sex (F:M)	Equal	2.6:1
Presentation		
Onset	Acute (most with symptoms < 1 week)	Insidious (most with symptoms > 2 week)
Symptoms	Purpura <10% with severe bleeding	Purpura typically bleeding not severe
Platelet count	Most <20,000/μL	Most <20,000/μL
Course		
Spontaneous remission	83%	2%
Chronic disease	24%	43%
Response to splenectomy	71%	66%
Eventual complete recovery	89%	64%
Morbidity and mortality		
Cerebral hemorrhage	<1%	3%
Hemorrhagic death	<1%	4%
Mortality of chronic, refractory disease	2%	5%

Platelets are transfused:

1. To correct severe thrombocytopenia as a prophylactic measure to prevent catastrophic hemorrhage in the central nervous system (CNS) or other vital organs, especially in leukemia and lymphoma patients undergoing high-dose myeloablative chemotherapy.
2. To bleeding patients in surgery or trauma cases with platelet counts of ≤50,000/uL.
3. To those bleeding patients with thrombocytopathy (qualitative abnormal platelet dysfunction) who may have normal platelet counts.

Before the availability of sterile plastic blood collection systems, it was not effectively possible to prepare blood components from whole blood. Thrombocytopenic patients were often transfused with multiple units of whole blood in attempts to raise their platelet counts and control bleeding. The development of transfusion-associated hypervolemia usually precluded transfusion of a quantity of whole blood necessary to raise platelet count to the desired levels. With the advent of plastic sterile collection systems and the ability to produce and store platelet concentrates routinely, effective transfusion therapy of thrombocytopenic patient became available. Platelets collected by apheresis are particularly useful for patients who require numerous platelet transfusions, for example cancer patients who have received chemotherapy.

Today, platelet concentrates are produced from whole blood by differential centrifugation (buffy coat-derived platelet concentrates) or by plateletpheresis (apheresis-derived platelet concentrates). Although most blood is donated as whole blood, it is also possible to donate only a portion of blood by using a technique called apheresis. Blood is drawn from the vein of a donor into an apheresis instrument, which separates the blood into different portions by centrifugation. By appropriately adjusting the instrument, a selected portion of the blood, such as the platelets, can be recovered, while the rest of the blood is returned to the donor either into the same vein or into a vein in the other arm. (Figure 1) This process takes more time than whole blood onation, but the yield of platelets is much greater. Automated cell separator devices are used for both component preparation and therapeutic application of apheresis. (5)

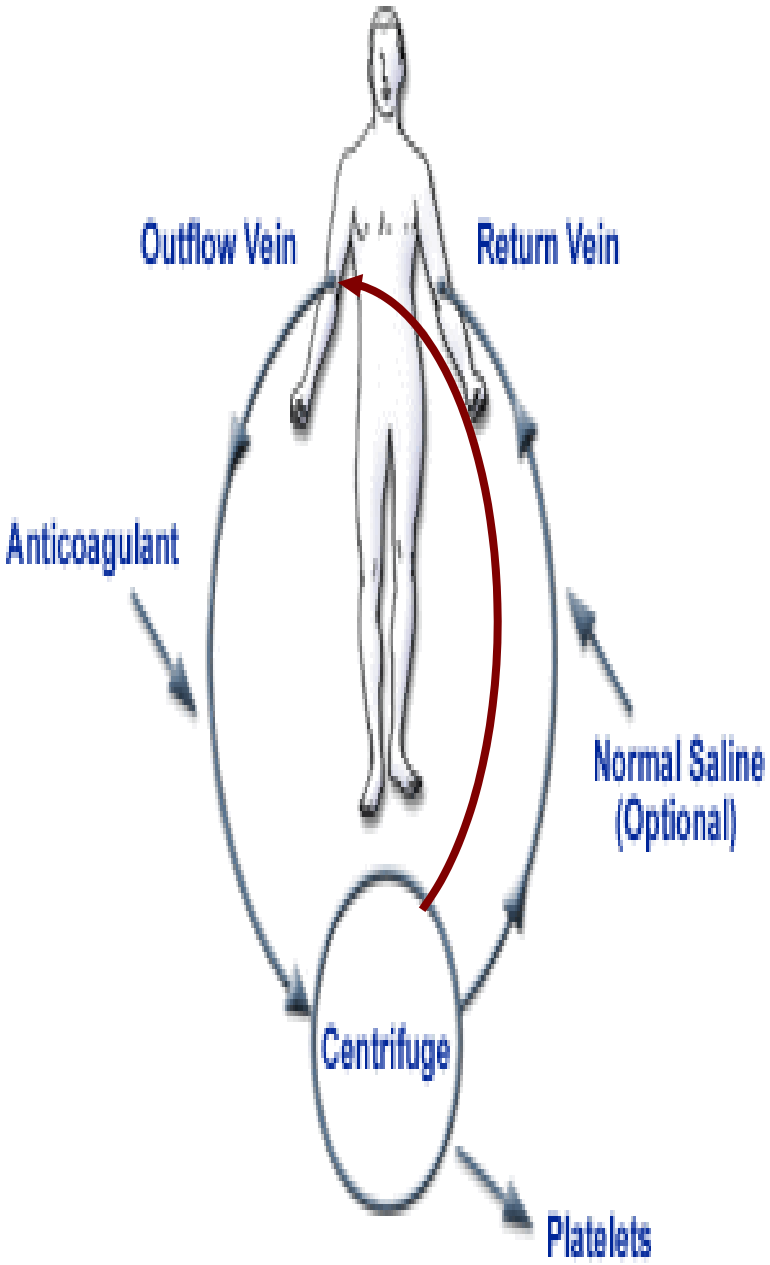

Figure1. Flow diagram of platelet concentrates produced from whole blood by plateletpheresis

Separation technique is centrifugation, the most apheresis instrument. Centrifugal force which separates blood into components is based on:(6)

1. Differences in density,
2. Membrane filtration, filtration through a membrane allows collection of plasma but does not separate specific cellular elements from whole blood and
3. Adsorption, selective removal of a pathologic material e.g. LDLs,

The data from study suggest that plateletpheresis are characterized by a superior hemostatic capacity over buffy coat-derived platelet concentrates in vitro although plateletpheresis has a very higher cost in preparation than buffy coat-derived platelet concentrates.(7, 8)

The requirement of platelet concentrates has been increasing but platelet concentrates has a short storage period of 5 days. To extend platelet shelf-life (9-11), a plastic type container (12-14) and storage media have been developed (15-17).

The difference between plasma and synthetic media as a platelet-storage environment may be less than that of red cells. Synthetic media based on additive solutions always contain a significant proportion of plasma, generally $\geq$ 20%. Previous studies indicate that plasma diluted with sodium chloride can be used for platelet storage.(18)

Since the end of the 1980s, various PASs have been developed,(18) but only few have made it to routine blood bank and clinical application as shown in Tables 4 and 5 PlasmaLyte-A, an infusion fluid, has mainly been used for research purposes, and T-sol, frequently referred to as PAS-II (produced by Baxter, Nivelles, Belgium), was the first solution applied on a large scale, mostly in European blood centers. PAS-II contains acetate as nutrient for the PLTs, citrate to prevent clumping, and saline for osmolarity of the solution.(19) (For composition see Table 3)

Table 3 Composition and use of some platelet additive solutions (components in mM)

	Plasma Lyte A	GAC/ Composol	Seto-solution	PAS	PAS-1	PAS-2 (T-Sol)	PAS-2 modified	PAS-3	PAS-3 modified	Fukushima cocktail
NaCl	90.0	90.0	90.0	110.0	70.0	115.5	115.5	115.0	69.0	90
KCl	5.0	5.0	5.0	5.1	10.0		4.5		5.0	5
$MgCl_2/Mgso_4$	3.0	1.5	3.0	3.0			1.5		1.5	3
Na_3-citrate			17.0	5.0	30.0	10.0	10.0	10.0	10.0	15
NaH2PO4			4.9	15.2	5.0				26.0	33
Na_2HPO_4			20.1				26.0	26.0		
Na-acetate	27.0	27.0	23.0			30.0	30.0	30.0	30.0	25
Na-gluconate	23.0	23.0								
D-Mannitol					30.0					
$CaCl_2$				1.7						
Glucose			23.5	35.5						25
Citric acid		3.2		0.8						2
$NaHCO_3$				2.7						
Maltose			28.8	35.0						
Glycerol										50

Table 4 In vitro results of platelets prepared using the platelet-rich-plasma (PRP) method, single donor buffy coat (single BC) or apheresis technique(AP)

Author	Method	Storage solution	Plasma/PAS ratio (vol/vol)	n	Volume (mL)	platelets x 10^{12}/l	WBC x 10^{6}/unit	pH (day 5 or 7)
Adams et al.	PRP	Plasma	100/0	4	60	0.98	>100	7.1
1986(20)	PRP	PLA + citrate	15/85	14		1.00	>100	7.0
	PRP	PLA	15/85	7		0.95	>100	6.8
Fijnheer et al	BC-PCs	Plasma	100/0	25	86±16	0.82	>5	6.9
1991(21)	BC-PCs	GAC	30/70	25	71±12	0.86	<5	6.8
Shimizu et al	AP	Seto solution	15/85	5	411	1.69	>5	7.0
1992(22)	AP	PLA	15/85	5		1.68	>5	7.3
	AP	plasma	100/0	5		1.72	>5	7.1
Gulliksson et al	BC-PCs	NaCl	35/65	12	373±35	1.01	>100	6.9
1992(23)	BC-PCs	PAS-1	35/65	6	357±25	1.05	>100	6.9
Gulliksson	BC-PCs	NaCl	35/65	8	409±31	1.09	>100	6.95
1993(24)	BC-PCs	PAS-2	35/65	8	396+35	1.17	>100	6.95
van Rhenen et al	BC-PCs	PLA	?	8	344±7	0.53	<5	7.1
1995(25, 26)	BC-PCs	PAS-2	?	8	358±7	0.74	<5	6.9
de Wildt-Eggen et al	BC-PCs	plasma	100/0	12	265±21	1.51	>5	7.1
1998 (26)	BC-PCs	PAS-2	30/70	11	299±22	1.33	>5	6.9

Data expressed as mean±SD

Table 4 In vitro results of platelets prepared using the platelet-rich-plasma (PRP) method, single donor buffy coat (single BC) or apheresis technique(AP) (continued)

Author	Method	Storage solution	Plasma/PAS ratio (vol/vol)	n	Volume (mL)	platelets l x 10^{12}/	WBC x 10^{6}/unit	pH (day 5 or 7)
Gulliksson et al	AP	plasma	100/0	6	310±20	1.28	<5	7.1
2000(27)	AP	PAS-2	35/65	6	320±10	1.25	<5	6.9
Gulliksson et al	AP	plasma	100/0	6	340±10	0.87	<5	7.3
2000(27)	AP	PAS-3	35/65	6	350±10	0.90	<5	7.1
Gulliksson	BC-PCs	plasma	100/0	7	380±10	0.90	<5	7.2
2000(16)	BC-PCs	PAS-2	35/65	6	380±10	1.03	<5	7.1
	BC-PCs	PAS-3	35/65	6	390±20	1.02	<5	7.0
van Rhenen	BC-PCs	PAS-2	32/68	10	357±5	1.16	<5	6.96
2000	BC-PCs	PAS-3	34/66	10	325±3	1.33	<5	7.0
Bunescu et al	AP	plasma	100/0	5	310	1.10		>6.9
2001(28)	AP	PAS-2	?	5	360	0.84		>6.9
van der Meer et al	BC-PCs	PAS-2	35/65	10	317±9	0.97	<5	6.9
2001(15)	BC-PCs	composol	35/65	10	316±7	1.01	<5	6.9
de Wilt-Eggenet al	BC-PCs	plasma	100/0	6	242±12	1.02	<5	7.0
2002(29)	BC-PCs	PAS-2	30/70	6	308±6	0.91	<5	6.9
	BC-PCs	PAS-2 modified	30/70	6	305±6	0.89	<5	7.2

Data expressed as mean±SD

Table 5 Corrected count increments (CCI) after transfusion of platelet concentrates (PCs) in plasma or platelet additive solution (PAS)

Author	Method	Storage solution	Platelets (x 10^{11}/Unit)	Storage time (days)	Patients/ No. of transfusion	Precount platelet (x10^{9}/l)	CCI at 1-6 h	CCI at 20-24 h	Plasma vs. PAS
Bertolini et al 1989(30)	BC	PLA	3.6	1	57/259	<20	40% recovery	22% recovery	NS
	PRP	plasma	4.5	1-3	57/129	<30	31% recovery	21% recovery	NS
Gulliksson et al 1992(23)	PRP	plasma	2.82±0.06	2-5	5/9		19.0±7.9	7.2±5.4	NS
	BC	NaCl	3.16±0.03				20.4±6.6	10.9±4.0	
Oksanen et al 1994(31)	PRP	plasma	2.8	3-5	12/23	15-20	12.6±6.4		
	BC	PAS-1	2.76		24/27		12.3±6.0		
van Rhenen et al 1995(25)	BC	PLA	2.6±0.5		9/9	<40	24.0	14.2	NS
	BC	PAS-2	2.9±0.4		9/9		22.4	11.3	
Strindberg & Berlin 1996(32)	AP	plasma	3.1±0.5	1-5	32/180	<10	15.4±8.2		NS
	BC	PAS-1	3.8±1.3	0-5	32/146		15.3±9.9		NS
Eriksson et al 1996(33)	AP	plasma	3.6	0-2	36/19	<30	17	4	NS
	BC	PAS-1	3	0-2	36/12		20	7	
	AP	plasma	3.1	3-5	36/57	<30	14	4	NS
	BC	PAS-1	2.8	3-5	36/41		16	7	NS

Data expressed as mean±SD

Table 5 Corrected count increments (CCI) after transfusion of platelet concentrates (PCs) in plasma or platelet additive solution (PAS) (continued)

Author	Method	Storage solution	Platelets (x 10^{11}/Unit)	Storage time (days)	Patients/ No. of transfusion	Precount platelet (x10^9/l)	CCI at 1-6 h	CCI at 20-24 h	Plasma vs. PAS
Hogman et al 1997(34)	BC	PAS-2	2.2±0.8	0-2	20/20	?	17	14	
	BC	PAS-1	2.3±0.6	0-2	20/20	?	18	15	
	BC	PAS-2	2.2±0.8	3-5	20/20		14	7	
	BC	PAS-1	2.3±0.6		20/20		14	7	
de Wildt-Eggen et al 2000(35)	BC	plasma	3.0±0.4	<3	12/80	<20	21.6±10.3	13.1±8.8	CCI 1 h; p< 0.05
	BC	PAS-2	2.8±0.5		9/46		17.9±5.3	9.9±7.4	CCI 24 h; p< 0.05
	BC	plasma	3.0±0.4	3-5	12/97	<20	19.9±6.7	10.0±6.9	CCI 1 h; p< 0.05
	BC	PAS-2	2.8±0.5		9/62		16.5±6.7	9.1±6.7	CCI 24 h; NS

Data expressed as mean±SD

At present, such additive solutions are in use for transfusion in several countries in Europe. PAS is generally used as a substitute for plasma in order to:

1. Reduce the amount of plasma transfused with platelets and to recover additional plasma for other purposes, primarily fractionation into plasma products(36, 37)
2. Avoid transfusion of large volumes of plasma with possible adverse reactions and circulatory overload(3, 38)
3. Improve storage conditions to increase shelf-life of the platelets while maintaining the viability and haemostatic function at a high level(12, 16, 39)
4. Make possible photochemical treatment for the inactivation of virus and other pathogens in PCs.(40, 41)

Effects on platelet metabolism associated with chemical compounds in PAS

A number of effects have been observed that can be assigned to specific ingredients in PAS.(42) However, the effects on platelet metabolism associated with different factors and compounds in PAS are only partly known.

Effects of glucose

Generally in cell metabolism, glucose has a dual role as a substrate, on one hand for glycolysis resulting in the decomposition of one molecule of glucose to two molecules of lactic acid, on the other for the carboxylic acid cycle and oxidative processes with carbon dioxide and water as end products. It implies that production of lactic acid and fall in pH is a part of the effects caused by consumption of glucose during platelet storage.

Effects of acetate

Acetate is entered into platelet metabolism by the tricarboxylic acid cycle and is further oxidized in the respiratory chain. The end products are carbon dioxide from the first step and water from the second step. In parallel with glucose and fatty acids, acetate is used as a substrate for platelet metabolism. Presence of acetate in PAS has been evidenced to reduce production of lactate by platelets.

Effects of citrate

Effects on the rate of glucose consumption and lactate production related to the concentration of citrate in PAS containing media have been observed. Platelets stored in medium with a citrate concentration of 8 mmol /L produce only half the quantity of lactate as platelets in a similar medium with a citrate concentration of 14±26 mmol/ L. No negative effects on, for example, adenine nucleotide levels were observed, which can be associated with generation of energy within the platelets. These results suggest that PASs preferably should include citrate at low concentrations in order to avoid excessive lactate production and acid pH. However, a citrate concentration of about 8 mmol /L seems to be a minimum level, since clotting problems due to activation of coagulation can be expected at lower concentrations.

Effects of phosphate

Generally, phosphate has two possible roles during storage of PCs, on the one hand by stimulation of platelet glycolysis to increase production of lactic acid, and on the other by acting as a buffer to prevent fall in pH caused by excessive production of lactic acid by platelets. These two effects consequently can counteract each other. There are no indications of net utilization or production of phosphate during storage of PCs. The first situation may occur when neither anticoagulant nor PAS used in the platelet storage medium contain phosphate. ACD in contrast to CPD does not contain phosphate and is routinely used as primary anticoagulant for the preparation of apheresis platelets.

Platelet storage lesion

The storage lesion is not a single defect, but rather a group of defects that affect a wide range of platelet structures and functions, including the platelet cytoskeleton, surface membrane antigen and ligand integrity, metabolic activity, release of granule contents, discharge of cytosolic contents, morphology, recovery from osmotic stress, and in vivo post-transfusion recovery and survival.(43, 44)

The collection, processing and storage of platelet concentrate are the causative factors of platelet storage lesion (45, 46), the overall changes that platelets undergo prior to transfusion and bacterial contamination. Therefore, we should study the efficiency of collection, storage lesion, (changes in number of platelets, residual white blood cell, pH level, and platelet function), bacterial contamination and effectiveness of transfusion into patients. Moreover, the quality of platelet concentrates that are kept in platelet additive solution will be compared with platelet concentrates that are kept in plasma. (47, 48)

In vitro study

Platelet count

Nowadays, routine automated cell analyzers are employed to count platelets in platelet concentrates. The reviews show different instruments and series used. But the basic/principle one is an electrical impedance. Electrical impedance has an error in that if one more cell of platelet passes through the electrical field, the machine will count it as one platelet. Even a debris can also be counted as a platelet .(49, 50)

Residual White blood cells(51, 52)

The American Association of Blood Bank (AABB) has published standards stipulating fewer than 5 x 10^8 leucocytes per transfused unit to prevent febrile transfusion reaction, and 5 x 10^6 for prevention of cytomegalovirus infection or alloimmunization. Standards in Europe mandate leucoreduction to fewer than 1 x 10^6 leucocytes per unit. The common way to quantitate residual WBCs is a manual count, performed by using a light microscope and a large-volume hematocytometry, known as the Nageotte Method. The Nageotte method has a lower detection limit for 1 WBC/uL. Although the method is inexpensive and requires a minimum of equipment, it is extremely time-and labor-consuming. Moreover, the methods vary from laboratory to laboratory. (Table 6)

The other method is flow cytometry that can be used for the identification and quantification of residual white cells at extremely low concentrations.(53) For counting WBCs by bead-based flow cytometric methods, propidium iodide is used to stain DNA of the WBCs. The lower detection limit is 0.1 WBC/uL. This automated method has been shown to have a high degree of accuracy and precision but requires large capital outlay in the form of sophisticated equipment.

Therefore, the automated flow cytometric method is the best method for measuring residual WBCs because it is very accurate, less time-and labor-consuming, and can quantitate WBC at very low concentration.

Table 6 Quality Control (QC) of platelet component

Component	Specifications and standard	AABB standard(54)
Platelets	≥5.5 x 10^{10} per unit and pH ≥ 6.2 in 90% of units tested	5.7.5.16
Platelets leucocyte reduced	above, also < 8.3 x 10^{5} leucocytes in 95% of units tested	5.7.5.17
apheresis	≥ 3.0 x 10^{11} platelets in final container of components tested and pH ≥ 6.2 in 90% of units tested	5.7.5.19
Platelets leucocytes reduced apheresis	< 1.0 x 10^{6} leucocytes(55) or < 5.0 x 10^{6}(54) leucocytes in 95% of components tested and ≥ 3.0 x 10^{11} platelets in the final container and pH ≥ 6.2 in 90% of tested units	5.7.5.20

Platelet morphology

The swirling phenomenon

The swirling is a simple and inexpensive way to check viability of platelets. This method employs the visible light to pass through platelet concentrates to see the swirling of platelets. Then, they are manually graded. The absence of swirling is responded by quick elimination of platelets from circulation.(56)

Kunicki morphology score

The method employs a phase microscope to evaluate morphological of platelets. The percentage of each morphologic type was multiplied by a series of arbitrary factors as follows: discs x 4, spheres x 2, dendrites x 1, and balloons x 0. This method is easy but requires experience of laboratory staff to identify morphology of cell.

Extent of shape change (ESC) and Hypotonic shock response (HSR)

A review found that the platelet discoid shape as measured photometrically by the ESC and HSR correlated with in vivo viability. But the study conducted by Vanden Broeke T. 2004 shows the lack of accuracy of test when storing platelet concentrates in platelet additive solutions.(57, 58)

Image analysis

This method uses special instruments and software to evaluate the photos of platelet cells from electron microscope. Laboratory personnel should have appropriate training so that they have experience in evaluating cells' images.

Platelet function(59)

Platelet activation

The p-selectin or CD62p antigen, also known as platelet activation-dependent granule-external membrane (PADGEM) protein or granule membrane protein (GMP-140), is a member of adhesive molecules and mediates the adhesion of activated platelets to neutrophils and monocytes in hemostasis. The CD62p antigen is an internal membrane protein associated with α-granules of platelets, Weibel-Palad bodies of endothelial cells, and megakaryocytes. The CD62p antigen is expressed on the internal α-granule membrane of resting platelets. Upon platelet activation and granule secretion, the α-granule membrane fuses with the external plasma membrane and the CD62p antigen is expressed on the surface of the activated platelet.(60, 61)

The CD 41 antigen (platelet GP IIb, IIb integrin) is a transmembrane glycoprotein composed of two chains GPIIbα (120kDa) and GPIIbβ (123 kDa) linked by one disulfide bond. CD41 is always non-covalently associated with CD61 (platelet GPIIIa,β3 integrin, to form the GPIIb-IIIa (CD41/D61) complex.CD41 is expressed by platelets, megakaryocytes and a small subset of CD34+cells, suggesting that CD41/CD61 is one of the earliest markers of the megakaryocytic lineage. The resting form of the CD41/CD61 complex binds to immobilized fibrinogen and upon platelet activation, the complex becomes a receptor for soluble fibrinogen, fibronectin, vWF, vitronectin and thrombospondin.It is involved in platelet aggregation

Flow cytometry is a system for sensing cells or particles as they move in a liquid stream through laser(*l*ight *a*mplification by *s*timulated *e*mission of *r*adiation)light beam past a sensing area. The relative light scattering and color-discriminated fluorescence of the microscopic particles is measured. Analysis and differentiation of the cells based on size, granularity and whether the cell is carrying fluorescent molecules in the form of either antibodies or dyes. As the cell passes through the laser beam, light is scattered in all directions and that scattered in the forward direction at low angles (0.5-10°) from the axis in proportional to the sequence of the radius of a sphere and so the size of the cell or particle.(62)

Bacterial contamination

The incidence of bacterial overgrowth increases exponentially during platelet storage at 22^{o}C, and it is estimated that 0.3% to 1.6% of platelet component are bacterially contaminated.(51) There are several possible causes of bacterial contamination example: failure to adequately reduce the bacterial contamination of the donor's skin at the

1. Time of the venapuncture
2. Inapparent donor bacteremia
3. Contamination during collection and processing of platelets

The methods of correcting this problem are to:

1. Improve skin preparation/disinfection
2. Remove the first aliquot of blood
3. Utilize a method of pre-transfusion detection of bacterial
4. Decontaminate the platelets prior to transfusion

Bacterial screening is being considered because contamination of platelet concentrates with bacteria causes significant morbidity and mortality. A major problem with platelets stored at room temperature (22 ^{o}C in platelet incubator with agitator) is the risk of bacterial contamination during the platelet's 5 days storage, which allows for the proliferation of even small bacterial inoculums. Among the common platelet contaminants are skin contaminants (*Staphylococci* spp), other commensals (*Corynebacterium* spp), environmental contaminants (*Bacillus cereus* and *Pseudomonas* spp), and enteric organisms (*Escherichia coli* and *Salmonella* spp). The potential sources of platelet contamination come from donor skin organisms, contamination occurring during the collection or processing of the unit, or from transience infections in the donor. The automated blood culture system, 3D BacT/ALERT, has the potential to screen platelet concentrates for the presence of bacterial,(63). However, some studies report on the false-positive of these tests.

In vivo study

Indications for prophylactic platelet transfusion are more controversial than for therapeutic rationales. A threshold of 20,000/uL or less for patients with chemotherapy-induced thrombocytopenia has been used by many physicians, but prospective randomized trials have shown that a threshold of 10,000/uL in stable patients is equally safe and results in significant decreases in platelet usage. In vivo study of additive solution study is the study about effectiveness of transfusion in patients, that is very important.(17, 20, 35) The response to platelet transfusion is best assessed by observing whether bleeding stops and by measuring the post-transfusion platelet increment.(64) Expected recovery is usually 7,000-10,000 platelets/uL/m^2. A higher transfusion trigger is often used for patients with fever, evidence of rapid consumption, high white cell counts, coagulation defects, and intracranial lesions. Patients with cerebral leukostasis are at high risk for fatal intracranial hemorrhage. In contrast, many stable thrombocytopenic patients can tolerate platelets. The past studies concentrate on:

Transfusion reaction

The physician observed and recorded reaction during and after transfusion of platelet and compared it with platelet kept in plasma

Corrected Count Increment (CCI)

CCI was calculated from pretransfusion, posttransfusion platelets and body surface area divided by the number of transfusion platelets. CCI was studied at 1hr and 24hr after transfusion.

Radiolabelled platelet

The platelets were labeled with radioisotope and transfused into volunteers to study platelet recovery and survival. But the responses to platelets in patients were different due to health conditions. Moreover, the use of radiolabelling is very difficult. Currently, there has been no standard procedure. Each laboratory adopts its own procedure.

CHAPTER 4
MATERIALS AND METHODS

Material

1. Instrument
   1.1. Automated blood cell separator (Amicus, Baxter)
   1.2. Automated cell counter (Cell Dyne 3700)
   1.3. Automated flow cytometer (FACSort) and software (CellQuest)
   (All: Becton Dickinson, San Jose, CA).
   1.4. Sterile connection device (TSCD SC-201A, Terumo Corp., Tokyo, Japan)
   1.5. Platelet incubator with agitator
   1.6. pH meter
   1.7. Centrifuge with swinging-bucket rotor
   1.8. Digital scale
   1.9. Vortex mixer
2. Reagent
   2.1. PE-conjugate monoclonal antibodies to human CD62p antigen.
   (BD Biosciences, San Jose, CA)
   2.2. FITC-conjugate monoclonal antibodies to human CD41a antigen
   (BD Biosciences, San Jose, CA)
   2.3. LeucoCOUNT (BD Biosciences, San Jose, CA)
   2.4. 1x phosphate-buffered saline (PBS)
   2.5. 1% paraformaldehyde.
3. Equipment
   3.1. Disposable single needle apheresis kit (Amicus, Baxter)
   3.2. Disposable 12 x 75 mm polystyrene tubes with cap.
   3.3. Micropipette with tips
   3.4. Plastic transfer pipettes

Method

1. Collected 240 units of Single donor platelets from repeated donors in Blood Bank, Ramathibodi Hospital.

2. Followed the donation's work instruction in that donors were checked for weight, height, blood pressure, pulse, medical history and hemoglobin concentration before donation.

3. Collected Single donor platelets from donors by Automated Blood Cell Separator

Blood was drawn from the vein of a donor into an instrument (Figure 2), which separated the blood into different portions by centrifugation. The instrument was appropriately adjusted so that the platelets were recovered, while the rest of the blood was returned to the donor into the same vein (Figure 3). The whole blood from donors was sampled for:

3.1. Pre-donation platelet count

Sampling whole blood from blood sampling bag to EDTA tube to count platelets by using automated cell counter

3.2. Post-donation platelet count

Sampling whole blood from return tube of apheresis kit to EDTA tube to count platelets by using automated cell counter

At the end of procedure, platelet concentrates were transferred to storage bags. 35%plasma and 65% additive solution were added for single donor platelet in PAS, while only autologousplasma was added in Single donor platelet in plasma.

4. Placed single donor platelets in platelet incubator 22^{o}C without an agitation for 2 hours and sampling single donor platelets for in vitro studies. The samples for in vitro tests were kept in storage bags in platelet incubator 22^{o}C with agitation.

5. Calculated product volume (mL) by weighing a bag of single donor platelet and dividing it by specific gravity(65)

$$\text{Volume} = \frac{\text{Net weight (g)}}{1.030\ (\text{g/mL})}$$

6. Calculated platelet product yield and collection efficiency

6.1. Platelet product yield(65)

Platelet yield = product volume (mL) x product count (platelets /uL) x conversion factor (1000 uL/mL)

6.2. Collection efficiency

Collection Efficiency = (Platelet yield / Total platelets processed∗) x 100

∗Total platelets processed = [pre+ post count (platelets /uL) / 2] x total blood volume processed mL x conversion factor (1000 uL/mL)

7. Observed the donor reaction

8. Studied the quality of 240 units single donor platelets

8.1. 120 units' single donor platelets in plasma were called "Control group".

8.2. 120 units' single donor platelets in 35% plasma and 65% additive solution were called "Study group". (Composite: 115.0 mmol NaCl, 10.0 mmol Na_3-citrate, 26.0 mmol, H_2PO_4/Na_2HPO_4 and 30.0 mmol Na-acetate)

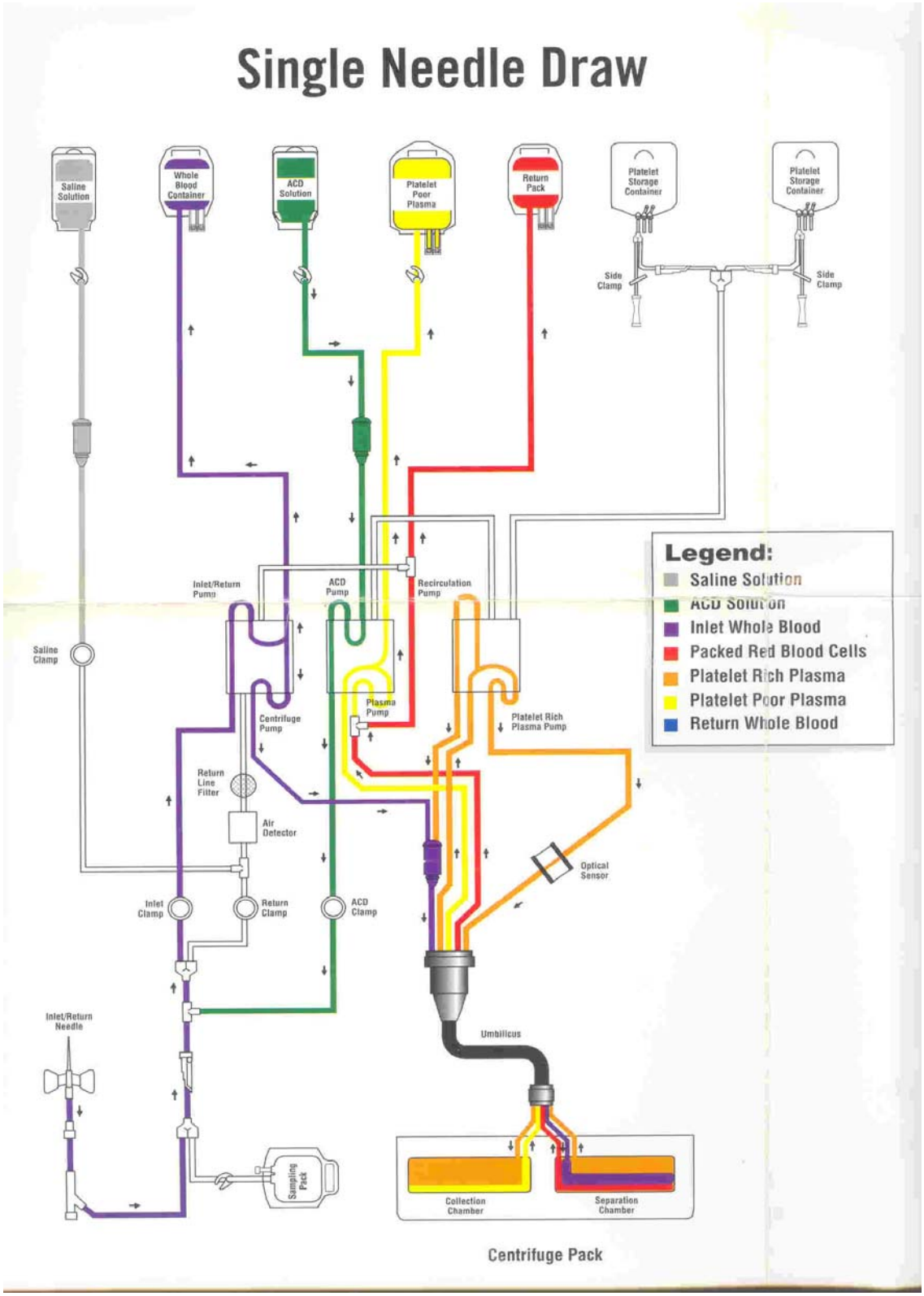

Figure 2. Flow diagram (draw) of plateletpheresis with the Amicus blood cell separator (Courtesy of Baxter Biotech)

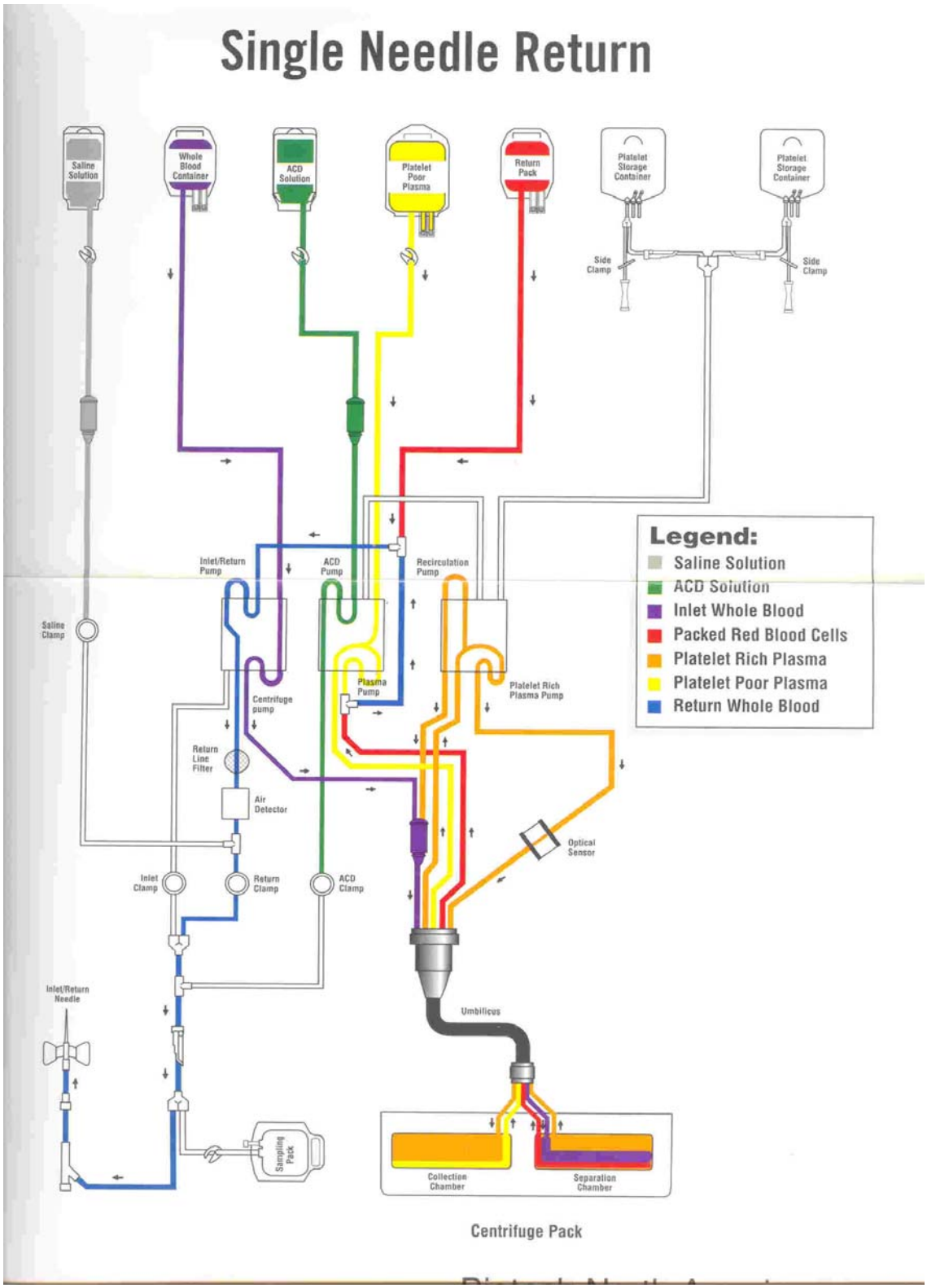

Figure 3. Flow diagram (return) of plateletpheresis with the Amicus blood cell separator (Courtesy of Baxter Biotech)

In vitro study

1. Studied at phlebotomy day (day 0) and repeated tests on day 3, 5 in "control group" and repeated tests on day 3, 5and 7 in "study group". All tests were repeated immediately at the same time as previous day.

2. Platelet yield

Platelets were measured with the automated cell counter and were diluted with 1:10 before being counted.

$$\text{Platelets yield/unit } (x10^{11}) = \frac{\text{Platelet count } (x10^{9}/L) \text{ x volume (mL)}}{1000}$$

3. Residual white blood cell used LeucoCOUNT and Flow cytometer

3.1. The test tubes that contain the beads were labeled

3.2. 100 μL of SDP and 400 μL of LeucoCOUNT reagent were pipetted

3.3. Samples were mixed and incubated in the dark for 5 minutes

3.4. They were analyzed with FACScan flow cytometer

3.5. For each sample a fixed number of 10,000 bead events were counted

4. Platelet metabolism via pH level by pH meter

The pH of SDP was determined at room temperature by pH meter.(66)

5. Platelet morphology by observing the swirling phenomenon

Examination for swirling was accomplished by gently rotating or tapping a PLT bag in front of a light source.

6. Bacterial contamination by automated blood culture that tested at phlebotomy day (day 0) and repeated test at day 5

7. Platelet activation (CD62p) used monoclonal antibody to CD62p and flow cytometer

7.1. Preparation of platelet suspensions

7.1.1. Within 30 minutes of collection, placed 100 μL aliquot of sample into a 12 x 75 mm test tube containing 1 mL of cold (2^{o}-8^{o}C) 1% paraformaldehyde. Mixed well by vortex

7.1.2. Fix platelets at 2^{o}-8^{o}C for a minimum of 2 hours. Fixed platelets are stable up to 5 days. Stored at 2^{o}- 8^{o}C.

7.2. Immunofluorescence staining

7.2.1. Prior to staining, centrifuged the fixed sample at 1200 g for 5 minutes at room temperature (20^{o}-25^{o}C).

7.2.2. Aspirated the supernatant, and added 1 mL of room temperature (20^{o}-25^{o}C) 1xPBS with 0.1% azide.

7.2.3 Resuspended the pellet by vortex thoroughly. Centrifuged at 1200 g for 5 minutes at room temperature (20^{o}-25^{o}C).

7.2.4. Aspirated the supernatant and resuspended the pellet in 1 mL room temperature (20^{o}-25^{o}C) PBS

7.2.5. Labelled clean 12 x 75 mm test tube, and added 5 μL of PE-conjugated monoclonal antibodies to human CD62p antigen

7.2.6. Labelled clean 12 x 75 mm test tube, and added 5 μL of FITC-conjugated monoclonal antibodies to human CD41a antigen

7.2.7. Used a new micropipettor tip each time, carefully added 50 μL of fixed sample suspension to the bottom of each tube.

7.2.8. Vortex tubes. Incubated for 15-20 minutes at room temperature (20^{o}-25^{o}C). Protected samples from light during this procedure.

7.2.9. Added 1 mL of room temperature (20^{o}-25^{o}C) PBS with 0.1% azide to each tube and vortex. Centrifuged the fixed blood at 1200 g for 5 minutes at room temperature and aspirated supernatant.

7.2.10. Resuspended in 1 mL of room temperature (20^{o}-25^{o}C) PBS with 0.1% azide. Stored in the dark at 2^{o}-8^{o}C and will be analyzed within 3 hours.

7.2.11. If samples will not be analyzed within 3 hours, add 1 mL of cold (2^{o}-8^{o}C) 1% paraformaldehyde to each tube and vortex thoroughly. Stored in the dark at 2^{o}-8^{o}C for analysis.

7.2.12. Analyzed with FACScan flow cytometer.

In vivo study

1. Single donor platelets were kept in platelet incubator 22^{o}C with agitation and were transfused into patients on day 3 of collection.

2. Studied platelet count in patient

Collected 1 cc. EDTA blood from patient 3 times to count the number of platelets

2.1. Pretransfusion platelet count

2.2. 1-hour posttransfusion platelet count

2.3. 24-hour posttransfusion platelet count

3. Calculated Corrected Count Increment (CCI) by

$$CCI = \frac{(\text{Posttransfusion} - \text{Pretransfusion Platelet count}) \times \text{Body surface area}}{\text{Number of platelet transfused}}$$

4. Observed the transfusion reaction

4.1 Fever

4.2 Chill

4.3 Nausea

4.4 Hypotension

4.5 Shock

4.6 Hemoglobinuria

4.7 Urticaria

4.8 Dyspnea

Observed the transfusion reaction during and 15 minutes after transfusing single donor platelets, then recorded and sent data to blood bank.

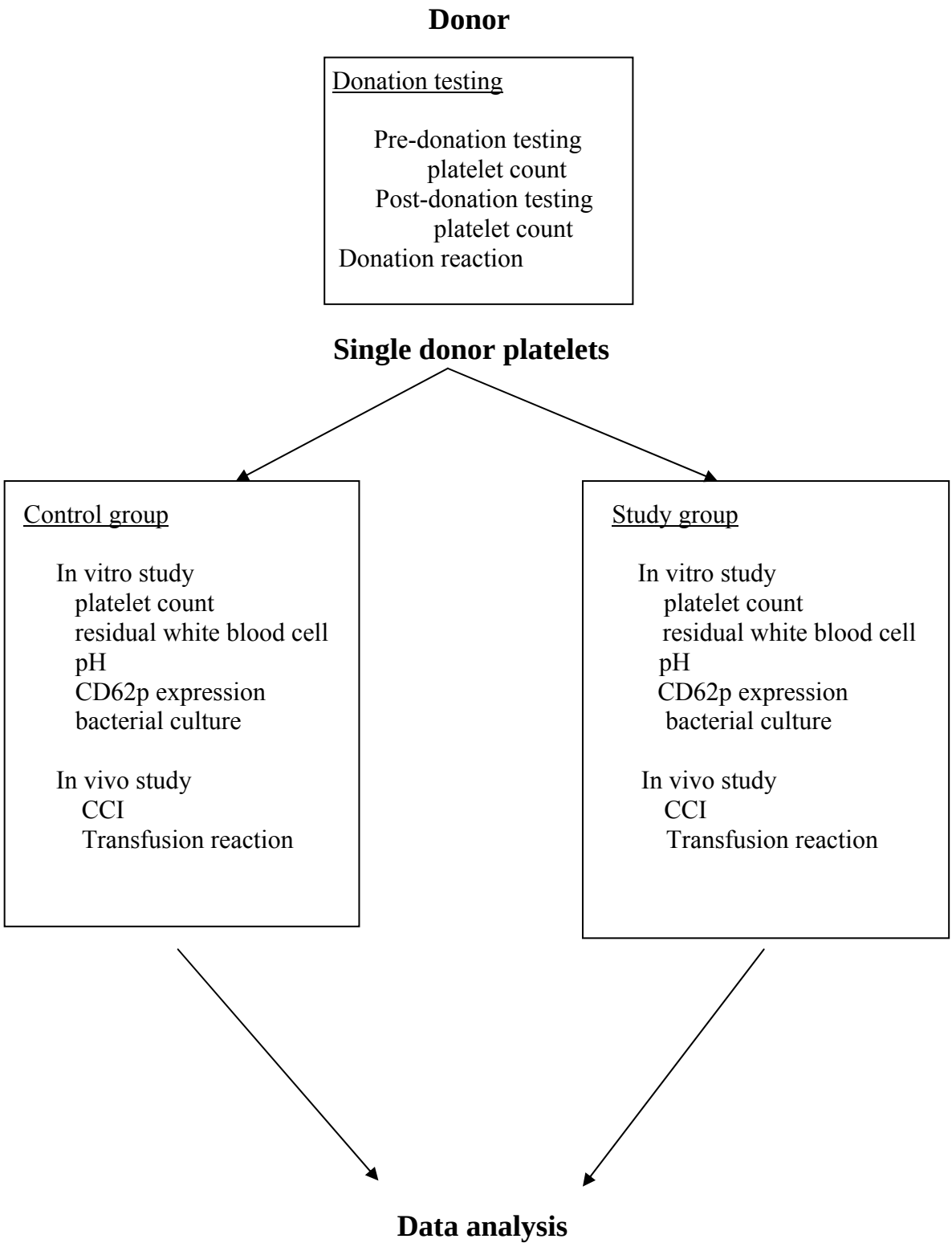

Figure 4. Protocol flow chart of in vitro and in vivo studies

Statistical analyses

All data were compared by using software (SPSS/PC+ for window, version12; Chicago. IL). The results were tested for normal distribution by using the Kolmogorov -Smirnov test. For statistical analysis, the t-test was used to compare the quality of single donor platelets in plasma with single donor platelets in additive solution.

The expected outcome and benefit of this thesis

1. The quality of single donor platelets in additive solution is the same as that of single donor platelets in plasma.
2. The additive solution can be substituted for plasma when preparing single donor platelets and it should be routinely used.

CHAPTER 5
RESULTS

Two hundred and forty units of SDP were collected from repeated apheresis donors who came to donate blood at the division of Blood Bank, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Manidol University. One hundred and twenty donors (117 males and 3 females) were enrolled in this study. Donors' preprocedure platelet count was $\geq 150 \times 10^3$ /μL and venous accesses were available. The mean ±SD of processing time was 72±12 minute (range = 41-91 minute), and collection efficiency was 84.9±13.6 (range = 40.4 – 100.0).

In vitro study

1. Platelet yield

Platelet yield of SDP in PAS and in plasma were shown in Table 7. 116 units of SDP in PAS and in plasma passed the standard platelets ($\geq 3.0 \times 10^{11}$), but 4 units of SDP in PAS and in plasma failed the standard platelets yield. SDP in PAS and in plasma were not significantly different (p-value > 0.05.**)**

2. Residual WBC

The data of residual WBC were presented in Table 8. Either SDP in PAS or SDP in plasma passed the standard of AABB standard $< 5.0 \times 10^6$ leucocytes, and the standard Council of Europe $< 1.0 \times 10^6$ leucocytes. All of them were not significantly different (p-value > 0.05**.)**

3. pH level

As shown in Table 9, pH level of SDP in PAS was very stable and slightly less than that of SDP in plasma. SDP in PAS, pH > 6.2 was 100% on day 0 and 3, 98.33%

on day 5 and 95.83% on day 7. SDP in plasma pH > 6.2 was 100% on day 0, 3 and 5. During the storage period, SDP in PAS and in plasma showed significant difference with p-value < 0.05.

4. Platelet activation

The study of platelet activation via % CD62p expression showed that SDP in PAS has higher expression level than SDP in plasma on day 0 (30.80±14.02 vs. 10.35±6.93), day 3 (49.01±23.15 vs. 21.94±8.27), and day 5 (58.00+24.31 vs. 62.41+10.91). However, there were not statistically significant difference with p-value > 0.05. The comparative data are shown in Table 10.

5. Swirling phenomenon

On day 0 and day 3, the swirling was 100% positive in both groups. On the expired date of SDP in plasma, it showed 95% positive, which was similar toSDP in PAS (95.9% and 92.5% positive on day 5 and day 7, respectively) as shown inTable 11.

6. Bacterial contamination

On day 0 and 5, neither SDP in PAS nor SDP in plasma showed an evidence of bacterial contamination.

Table 7 Comparative results of platelet yield ($x10^{11}$ /unit)

		SDP in PAS N=120	SDP in Plasma N=120	p-value
Day 0	Range Mean±SD	2.44-5.78 4.39±0.74	2.77-5.87 4.50±0.72	0.37
Day 3	Range Mean±SD	2.04-6.06 4.22±0.79	2.64-5.95 4.17±0.70	0.49
Day 5	Range Mean±SD	2.20-5.95 4.13±0.76	2.56-5.87 4.09±0.70	0.51
Day7	Range Mean±SD	2.26-5.56 3.85±0.71		

Table 8 Comparative results of residual WBC ($x10^6$ /unit)

		SDP in PAS N=120	SDP in Plasma N=120	p-value
Day 0	Range Mean±SD	0.00-0.20 0.06±0.04	0.00-0.20 0.06±0.04	0.70
Day 3	Range Mean±SD	0.00-0.14 0.03±0.03	0.00-0.14 0.03±0.03	0.57
Day 5	Range Mean±SD	0.00-0.18 0.03±0.03	0.00-0.19 0.03±.003	0.73
Day7	Range Mean±SD	0.00-0.12 0.02±0.02		

Table 9 Comparative results of pH

		SDP in PAS N=120	SDP in Plasma N=120	p-value
Day 0	Range Mean±SD	6.43-7.12 6.88±0.13	6.87-7.35 7.12±0.12	<0.05
Day 3	Range Mean±SD	6.20-7.19 6.77±0.16	6.48-6.98 6.73±0.12	<0.05
Day 5	Range Mean±SD	6.05-6.87 6.61±0.16	6.33-6.88 6.58±0.12	<0.05
Day7	Range Mean±SD	6.06-6.82 6.48±0.17		

Table 10 Comparative results of platelet activation (% CD62p expression)

		SDP in PAS N=120	SDP in Plasma N=120	p-value
Day 0	Range Mean±SD	7.47-83.66 30.80±14.02	1.28-29.42 10.35±6.93	<0.05
Day 3	Range Mean±SD	12.83-98.75 49.01±23.15	11.65-53.09 21.94±8.27	<0.05
Day 5	Range Mean±SD	19.60-97.21 58.00±24.31	32.04-88.07 62.1±10.91	0.06
Day7	Range Mean±SD	17.09-98.51 58.77±23.32		

Table 11 Comparative results of swirling phenomenon

		SDP in PAS N=120	SDP in Plasma N=120
Day 0	Swirling-positive Swirling-negative	100% 0%	100% 0%
Day 3	Swirling-positive Swirling-negative	100% 0%	100% 0%
Day 5	Swirling-positive Swirling-negative	95.90% 4.10%	95.00% 5.00%
Day7	Swirling-positive Swirling-negative	92.50% 7.50%	

In vivo study

Nine units of SDP in PAS and in plasma from the same donor were transfused to thrombocytopenic patients at Ramathibodi Hospital. 9 units of SDP in PAS were transfused to 4 patients (chemotherapy, BMT, fungal infection and lung biopsy) and 9 units of SDP in plasma were transfused to 7 patients (thrombocytopenia, BMT, leukemia and preoperative). One-hour and 24-hour corrected count increment (CCI) were not significantly different (p-value > 0.05). The expected recovery is usually 7,000-10,000 platelets/uL/m^2 (Table 12). All patients do not have any adverse reaction during and 15 minutes after transfusion. (Table 13)

Table 12 Comparative results of CCI

	Mean±SD		
	SDP in PAS N = 9	SDP in Plasma N = 9	p-value
CCI 1h	19,668.00±12,484.49	25,067.67±16,385.51	0.44
CCI 24h	17,332.89±17,093.18	18,210.89±16,385.51	0.91

Table 13 Data of patients (in vivo study)

Donor No.	Type of SDP	Patient		
		Age (years)	Diagnostic	Bleeding
1	PAS	12	Chemotherapy	No
	plasma	11	Thrombocytopenia	No
2	PAS	9	Fungal infection	No
	plasma	8	BMT	No
3	PAS	16	Lung biopsy	No
	plasma	8	BMT	No
4	PAS	16	Lung biopsy	No
	plasma	11	BMT	No
5	PAS	10	BMT	No
	plasma	12	Leukemia	No
6	PAS	10	BMT	No
	plasma	9	BMT	No
7	PAS	10	BMT	No
	plasma	10	BMT	No
8	PAS	10	BMT	No
	plasma	17	Lympnode biopsy	No
9	PAS	10	BMT	No
	plasma	11	Thrombocytopenia	No

CHAPTER 6
DISCUSSION

In this study, we have stored SDP for 5 days at 22^{o}C in either PAS or plasma. The platelet yields of SDP in PAS and in plasma have 4 units, from the same donor, that fail the standard (< 3.0 x 10^{11}/unit). The first donor preprocedure' platelet count was 167 x 10^{3} /μL, while the other had higher plasma lipid level. During storage period, platelet yield in PAS and in plasma had slightly decreased, as shown in Figure 4.

The comparison of white cell content in plateletpheresis showed that the number of WBCs of SDP in PAS was not significantly different from that of SDP in plasma.(67) The WBC contamination in SDPs can cause several adverse effects. The transfusion of passenger leukocytes during blood component therapy may be associated with a variety of adverse effects such as alloimmunization to leukocyte antigens, febrile nonhemolytic transfusion reactions, and the transmission of cytomegalovirus.(68)

The measurement of pH in platelet concentrates is widely used as a parameter to ensure the quality of stored platelets. The acidification of the medium is accompanied by a change of the platelet morphology from discoid to spherical. Below pH 6.8, most platelets become spherical, and below pH 6.2 this shape change seems to become irreversible. This shape change is strongly correlated to a decreased function and survival of the platelets in vivo. Our data show that the pH levels of SDP in PAS are very stable but significantly lower than those of SDP in plasma.(69) (Figure 5)

CD62p expression was recorded as high at later stages of storage (day 5). It indicates that our test is able to pick up higher levels of CD62p, should this occur. The usually low CD62p expression may be due to a different separation process combining centrifugation to the removal of activated platelets that selectively adhere to WBC, or to the low WBC content of the apheresis resulting in less nutrient consumption, less

cytokine secretion, and less platelet activation. Platelet activation (% CD62p expression) in PAS was higher than that in plasma. (Figure 6) But at 1h and 24h CCI show no significant difference in either SDP in PAS or SDP in plasma.

On the expired date of SDP in plasma, the swirling was 5% negative, the same as that of SDP in PAS which was 4.1% and 7.5% negative on day 5 and day 7, rspectively. The comparison of pH level shows that all negative swirling units have the pH levels close to or equal to 6.2. Since the pH levels are about 6.2, platelets become spherical.

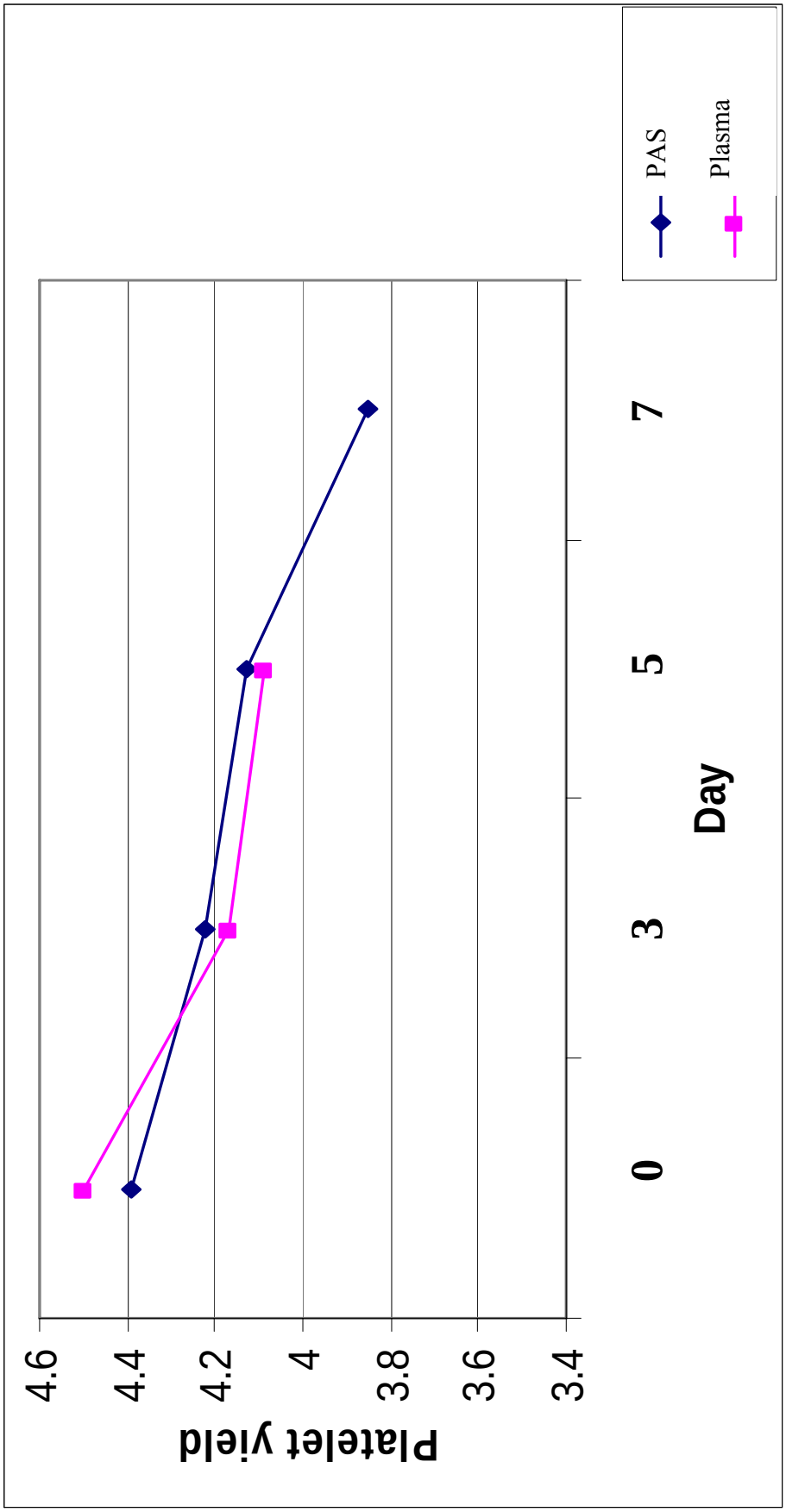

Figure 5 Comparative results of platelet yield ($x10^{11}$ /unit)

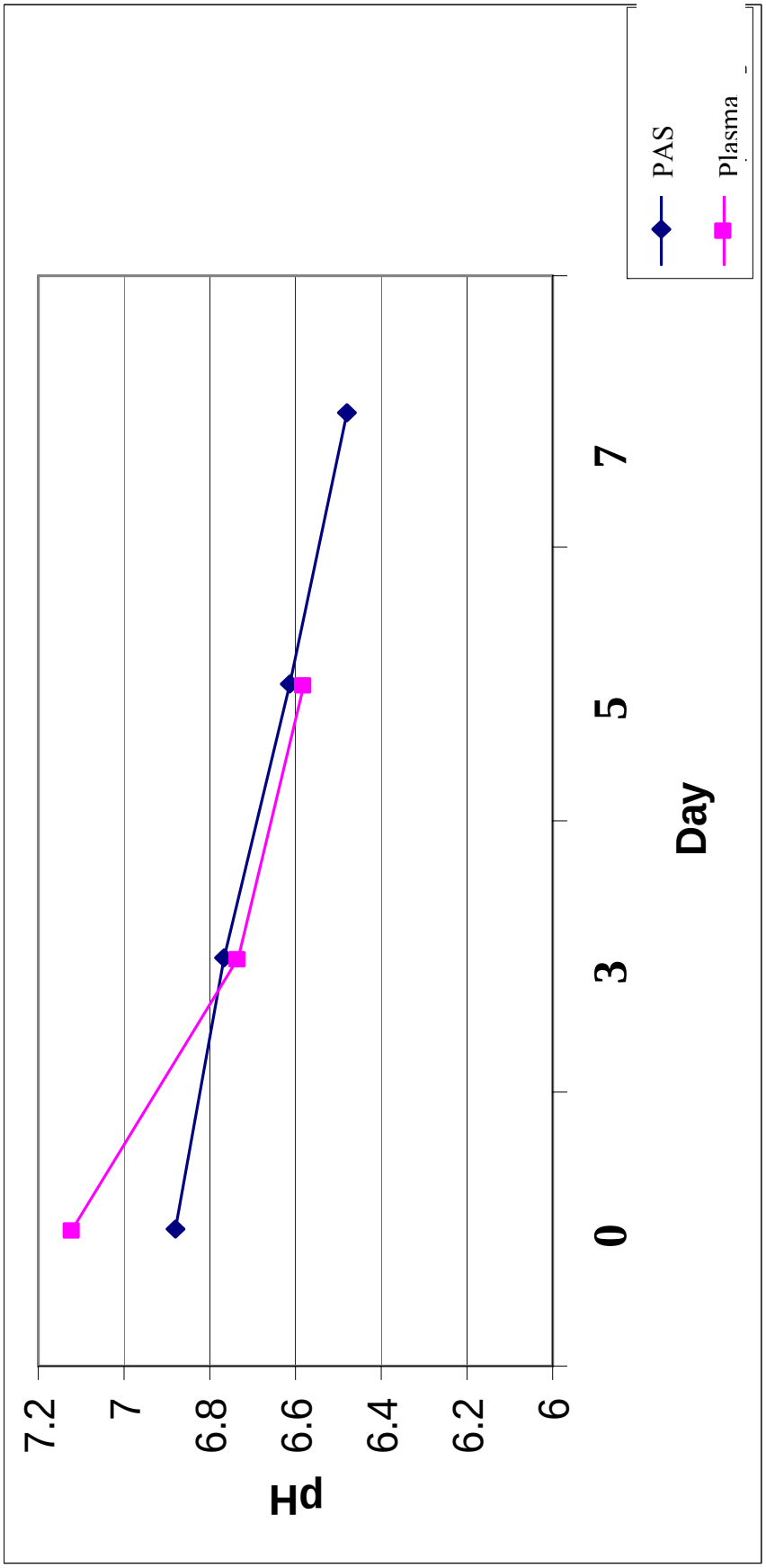

Figure 6 Comparative results of pH

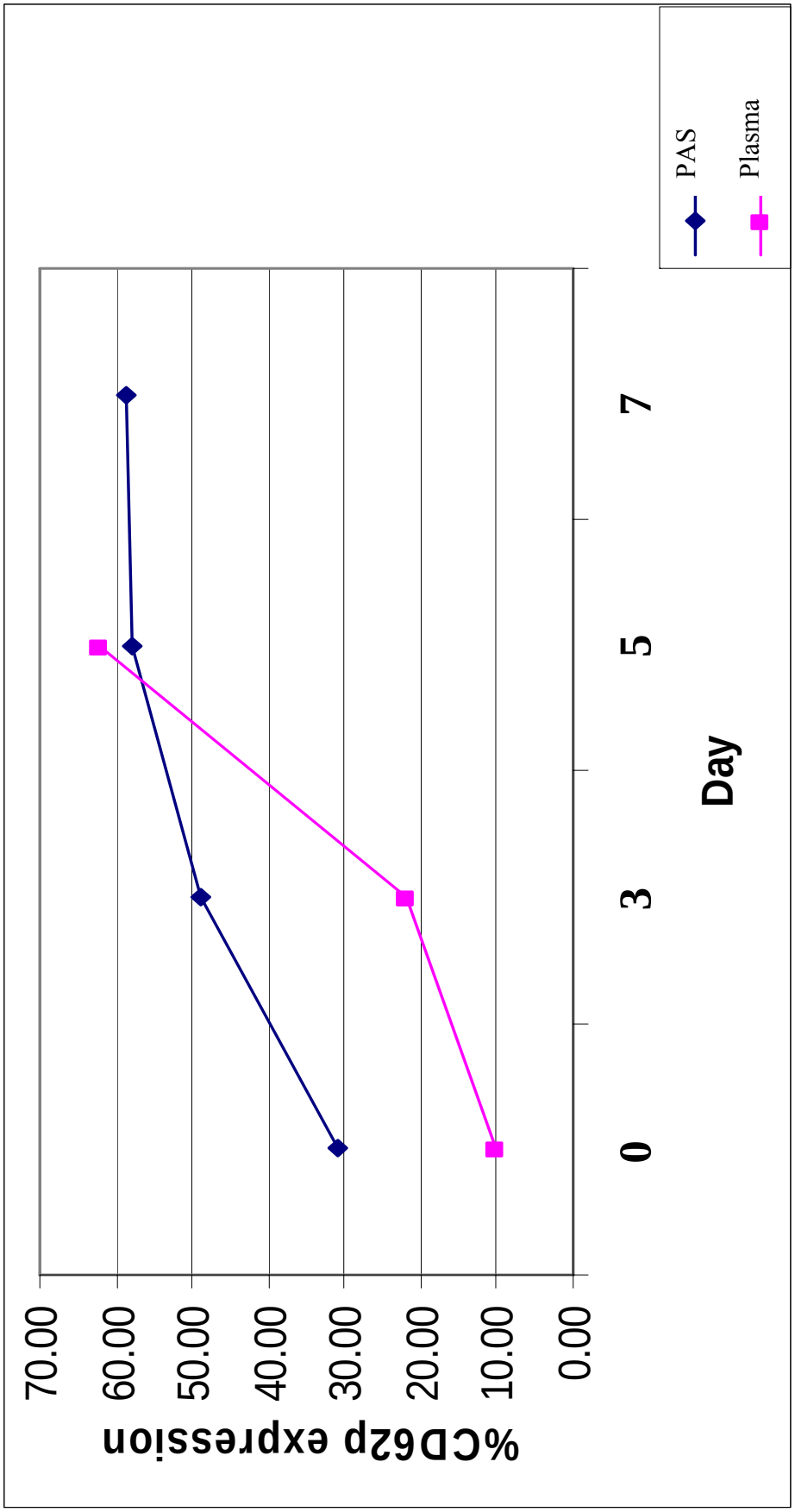

Figure 7 Comparative results of Platelet activation (% CD62p expression)

CHAPTER 7
CONCLUSION

The knowledge and experience gained from experimenting with PAS suggest that such solutions used to store platelets have a great potential for transfusion medicine. In particular, the use of PAS in combination with PCs prepared from pooled buffy coat and from apheresis is of interest.

The knowledge of the effects associated with different possible components discussed above suggests that platelet storage media should be based on three components, namely citrate, acetate and phosphate.

Further studies may supplement this list with additional components that improve platelet quality after storage or alternatively reduce the percentage of plasma included in the storage medium. Future use of PAS in association with photochemical inactivationof viruses and bacteria in PCs may accelerate the introduction of PAS on a large scale.

We conclude from this study that single donor platelets in additive solution have the comparable quality as single donor platelets in plasma. The additive solution can be substituted for plasma when preparing single donor platelets and it should be routinely used.

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APPENDIX

APPENDIX

Preparation of buffers and reagents

1. Phosphate buffer saline (PBS) pH 7.2

K_2HPO_4	0.752 g
NaH_2PO_4	0.132 g
NaCl	0.72 g

Dissolved in 90 mL of distilled water then adjusted the volume to 100mL and adjusted pH to 7.2 with 1N NaCl. The PBS should be filtered through a 0.2-uM filter prior to use. Store at 2^o- 8^oC

2. 1% paraformaldehyde in PBS pH 7.4

Paraformaldehyde	1 mg

Dissolved in 100 mL of PBS pH 7.4. Filtered through a 0.45-uM filter prior to use. Store in a glass container at 2^o-8^oC for up to 1 week.

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