AWARD NUMBER: W81XWH-12-1-0441

TITLE: Pathogen-Reduced, Platelet Additive Solution, Extended Stored Platelets (PREPS)

PRINCIPAL INVESTIGATOR: Sherrill J. Slichter, MD

CONTRACTING ORGANIZATION: Bloodworks Northwest
Seattle, WA 98104

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14. ABSTRACT
This grant pertains to finding novel approaches for storage of platelets for transfusion. Our project proposes to determine the efficacy of using a pathogen inactivation technique (Mirasol) coupled with a platelet additive solution (PAS) to extend the life of stored platelets. Our project also aims to determine how long acceptable platelet viability can be maintained in platelets stored at 4°C.

15. SUBJECT TERMS
bleeding, extended storage, hemorrhage, hemostasis, InterSol, Mirasol, pathogen inactivation, pathogen reduction technology, platelet additive solution, platelet recovery and survival, platelet storage, platelet storage solution, platelets, thrombocytopenia, transfusion, whole blood

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Pathogen-Reduced, Platelet Additive Solution, Extended Stored Platelets (PREPS)

Grant Number 11105004

Annual Report

15-SEP-2014 to 14-SEP-2015

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Pathogen-Reduced, Platelet Additive Solution, Extended Stored Platelets (PREPS)
(Previously Pathogen-Reduced, Plasmalyte-Extended Stored Platelets)
Grant Number 11105004
Annual Report
15-SEP-2014 to 14-SEP-2015

INTRODUCTION: The purpose of this project is to find better ways to store platelets for patients that need platelet transfusions. A deeper mechanistic understanding of the effects of collection and storage on platelet function could greatly aid in improving the availability and efficacy of platelets both on the battlefield and in the civilian transfusion setting. In this research study, we are interested in evaluating the novel combinations of collection, storage and pathogen reduction approaches on the structural and functional properties of platelets and on platelet viability and function following transfusion.

KEY WORDS: 4°C storage, bleeding, cold storage, extended storage, hemorrhage, hemostasis, Isoplate, Mirasol, pathogen inactivation, pathogen reduction, pathogen reduction technology, PRT, platelet additive solution, PAS, platelet recovery and survival, platelet storage, platelet storage solution, platelets, refrigerated storage, thrombocytopenia, transfusion, whole blood

OVERALL PROJECT SUMMARY: The following specific aims are described in the July 2, 2014 revised statement of work, Novel Approaches to Storage of Platelets for Transfusion.

1. Evaluation of structural and functional changes to platelets during enhanced collection, storage and pathogen reduction (enhanced platelets).
2. Evaluation of enhanced platelets in animal models of trauma and hemorrhage.
3. Evaluate enhanced autologous platelets in normal subjects.
4. Evaluation of enhanced platelet recovery and survival, bleeding time and hemostatic activity in thrombocytopenic patients with and without acute hemorrhage.

An evaluation of changes in the structural and functional properties of platelets stored as whole blood under refrigeration [Assessment of Whole Blood Cold Stored Platelets (Brrr Study)] has been completed. Enrollment for this study began in October 2013 and ended in February 2015. Data analysis was completed in April 2015. Results of this trial can be found in the attached, 'Final Report - Storage of Platelets In Whole Blood at 4°C.'

One protocol modification to the Brrr study and one Continuing Review Report (CRR) was approved by our local IRB during this reporting period.

A protocol to identify an acceptable storage bag that would allow extended platelet storage in a platelet additive solution (PAS) and to identify the maximum PAS-to-plasma ratio for extended platelet storage was submitted to TATRC and the local IRB but withdrawn at the request of the Grants Officer's Representative (GOR).

At the request of the GOR a protocol to evaluate apheresis platelets stored at 4°C in a platelet additive solution, Isoplate, was developed and submitted to the Food and Drug Administration (FDA) on September 14, 2015. The protocol, entitled Cold Apheresis Platelets in Isoplate (CAPI), is attached. Briefly, the protocol proposed to collect double hyperconcentrated apheresis units and split each unit. One half of the split unit will be stored in plasma at 4°C stored for 3 days (control), the other half will be stored in 65% Isoplate/35% plasma at 4°C for 10 - 20 days (test). Storage periods will be increased from 10 days in 2 day increments after 5 units tested and pass acceptance criteria. Each subject's Isoplate platelet recovery and survival will be considered acceptable if they are ≤20% less than the subject's corresponding 3-day stored sample measurements.

KEY RESEARCH ACCOMPLISHMENTS: The following are the key research accomplishments to date.

Brrr Study

Our study of platelets stored as whole blood at 4°C demonstrated that end-over-end rotation is required to reduce platelet adherence to the walls of the bag. Platelet yields in whole blood post-storage average 7.0 to 9.2 x 10^{10}. Thus, the FDA requirement of 5.5 x 10^{10} platelets/concentrate are easily met. At storage times between 10 to 15 days stored recoveries average 50% of fresh recoveries, stored survivals average >1 day, proposed post-storage criteria for whole 4°C stored platelets are met and based on in vitro measurements, the platelets are highly activated.

CAPI study

- Protocol, consent and other regulatory documents developed
- IND application submitted to the FDA.

CONCLUSION: We anticipate initiation of recruitment in the CAPI study in the next reporting period.

REFERENCES: None

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS: None

Slichter SJ, Fitzpatrick L, Jones MK, Pellham E, Bailey SL, Gettinger. In Vivo Viability of Platelets Stored in Whole Blood at 4°C. Submitted abstract, American Society of Hematology Annual Meeting; Orlando, FL December 5-8, 2015:184

INVENTIONS, PATENTS AND LICENSES: None

REPORTABLE OUTCOMES: None

OTHER ACHIEVEMENTS: None

REFERENCES: None

APPENDICES:

- Final Report - Storage of Platelets In Whole Blood at 4°C
- Statement of Work - Novel approaches to storage of platelets for transfusion
- Protocol - Cold Apheresis Platelets in Isoplate (CAPI)

Final Report

Award Number W81XWH-12-1-0441, EDMS 5570

Log Number 11105004

"Storage of Platelets In Whole Blood at 4°C"

April 15, 2015

Introduction:

Since the 1970's, it has been known that platelet survivals are much better maintained at 22°C compared to 4°C while platelet recoveries are not significantly different based on storage temperature (Table 1).

Table 1

EFFECT OF STORAGE TEMPERATURE ON PLATELET VIABILITY

Number of Observations	STORAGE CONDITIONS Time (Days)	STORAGE CONDITIONS Temperature (°C)	PLATELET Recovery (%)	PLATELET Survival (Days)
15	1	22	51 ± 3	8.2 ± 0.2
8	1	4	61 ± 7	1.3 ± 0.1
11	3	22	40 ± 3	7.9 ± 0.2
8	3	4	40 ± 5	1.0 ± 0.1

Legend: Platelet concentrates were prepared from normal subjects donated whole blood. After storage of the platelet concentrate, aliquots of the stored autologous platelets were labeled with ^{51}Cr, and the labeled platelets were injected into the platelet donor. Samples were obtained post-infusion to determine radiolabeled platelet recoveries and survivals. Data are reported as the average ±1 S.E.[(1)]

Approximately 80% of the platelets given in the U.S. are transfused into hematology/oncology patients where prolonged post-transfusion survivals of the stored platelets are important to decrease the need for frequent transfusions. Thus, the standard practice has been to store all platelets at 22°C regardless of the patient's clinical condition. However, for surgical/trauma patients, a short platelet life-span may be acceptable as these patients may only require immediate hemostasis until the vascular system can be repaired. The limited shelf-life of 5 days with 22°C storage severely limits platelet availability particularly at far-forward combat facilities, suggesting that we need to consider other options to support the platelet needs of these patients.

Increasingly, it has been recognized that trauma patients may be best supported with a ratio of 1 red cell, 1 plasma, and 1 platelet.[(2-3)] Therefore, the question becomes whether component therapy may be the best strategy to provide blood products for these patients or whether the transfusion of whole blood (WB) stored at 4°C would meet their needs. The major concern is the viability and function of platelets stored within WB at 4°C as platelets have never been stored in the cold for longer than 3 days and then only as platelet concentrates.

Purpose:

The purpose of this study was to determine the recovery and survival of autologous platelets that have been stored within WB for up to 22 days.

Primary Endpoint:

To determine how long autologous platelets can be stored in WB at 4°C with average post-storage platelet recoveries of ≥50% of the same donor's fresh platelet recoveries and platelet survivals of ≥1 day.

Experimental Design:

- Normal subjects donated a unit of WB.
- WB was stored at 4°C for 12 days (non-rotated) or for 10, 15, and 22 days (rotated end-over-end).
- At end of storage:
  - A platelet concentrate was prepared from the stored WB, and the platelets were labeled with ^{111}In.
  - A 50 ml blood sample was drawn from the subject, a fresh platelet sample was prepared from this blood, and the platelets were labeled with ^{51}Cr.
  - The subject was injected simultaneously with their autologous radiolabeled stored and fresh platelets.
  - Serial samples were drawn post-injection from the subject to determine the recovery and survival of the stored compared to the fresh platelets.

Results:

The first experiment was to store the WB obtained from 7 normal subjects for 12 days at 4°C without agitation until the end of storage. After storage, the WB was thoroughly mixed before a platelet concentrate was prepared (Table 2).

Table 2

12 DAY 4°C WHOLE BLOOD PLATELET STORAGE

Subject (#)	PLATELET RECOVERY (%)			PLATELET SURVIVAL (Days)		
	Fresh	Stored	% of Fresh	Fresh	Stored	% of Fresh
1	28	27	96*	8.1	2.4	30
2	50	15	30	4.9	1.9	38
4	63	19	30	9.8	1.9	19
5	73	27	37	8.7	1.3	15
6	41	35	85	8.5	2.5	30
7	53	15	28	8.9	1.5	16
8	44	14	32	8.9	1.1	12
Ave ±1 S.D.	50 ± 15	22 ± 8	48 ± 29	8.3 ± 1.6	1.8 ± 0.5	23 ± 10

*Without this result, average = 40 ± 22%.

There was a very wide standard deviation for the donors' platelet recoveries as a percentage of fresh. The first subject had an unexpectedly reduced fresh platelet recovery with a stored recovery that was 96% of the same donor's fresh recovery. Without the data from subject 1, platelet recoveries averaged only 40 ± 22% of the same donor's fresh recoveries. However, platelet survivals were all ≥1 day. In addition, 52 ± 12% of the donor's initial WB platelets were lost during storage (Table 3). These post-storage platelet results – both because of the poor platelet recoveries and platelet losses – were considered unacceptable.

Table 3

BASELINE SUBJECT DATA AND WHOLE BLOOD PLATELET AND HEMATOCRIT DATA

Subject (#)	Subject's HCT	Subject's Platelet Count (10^3/µl)	PLATELET COUNTS* Whole Blood ($x10^3$/µl) Pre-Storage	Post-Storage	Loss (%)**	TOTAL PLATELET COUNTS Whole Blood ($x\ 10^{10}$) Post-Storage	HEMATOCRITS Whole Blood Pre-Storage	Whole Blood Post-Storage
1	39	248	180	110	39	4.7	31	31
2	43	227	152	66	57	3.2	37	36
4	44	377	297	183	38	8.8	39	38
5	44	239	194	86	56	4.1	38	38
6	38	191	177	49	72	2.3	33	31
7	44	188	174	97	44	4.7	38	38
8	44	215	202	88	56	4.7	38	38
Ave ±1 S.D.	42 ± 3	244 ± 65	197 ± 47	97 ± 43	52 ± 12	4.6 ± 2.0	36 ± 3	36 ± 3

* Bag rotated end-over-end before platelet count obtained.
** Platelet loss during storage as a percent of the baseline platelet count.

Flow cytometry experiments demonstrated that the platelet loss during storage was not due to formation of either platelet aggregates or an excessive number of microparticles. Our hypothesis was that the platelets must be adhering to the walls of the bag to account for most of the platelet loss during storage. We then determined that, if the WB was rotated end-over-end during storage rather than only mixing the bag at the end of storage, 76 ± 4% of the initial platelets were maintained within the WB during 21 days of storage (Table 4).

Table 4

EVALUATION OF PLATELET LOSS DURING 4°C WB PLATELET STORAGE*

	STORAGE DAY (% of Baseline Platelet Count) 4	8	13	17	21	Ave ±1 S.D.
Rotation of WB End-Over-End During Storage	78	69	78	74	81	76 ± 4
Bench Top With Bag Rotated End-Over-End At End Of Storage	58	56	64	48	48	55 ± 7

*Two units of whole blood of the same blood type were pooled at baseline, mixed, and split into two bags for storage.

This constant end-over-end rotation of the WB during storage was then used for all future experiments. Platelet counts pre- and post-storage for up to 22 days confirmed the reproducibility of our original experiment (Table 5).

Table 5

WHOLE BLOOD ROTATED END-OVER-END DURING 4°C PLATELET STORAGE

Storage Time (Days)	N	WB PLATELET COUNTS x 10^{10} Baseline	WB PLATELET COUNTS x 10^{10} Post-Storage	Post-Storage Yield (% of Baseline)
10	10	9.5 ± 2.1	7.0 ± 1.4	74%
15	10	10.0 ± 1.0	7.6 ± 1.4	76%
22	3	12.9 ± 1.9	9.2 ± 0.7	71%

In vivo platelet recoveries and survivals were measured for platelets separated from rotated WB stored at 4°C for 10, 15, and 22 days (Table 6).

Table 6

WHOLE BLOOD ROTATED END-OVER-END DURING 4°C PLATELET STORAGE

Storage Time (Days)	N	PLATELET RECOVERY (%) Fresh	PLATELET RECOVERY (%) Stored	PLATELET RECOVERY (%) % of Fresh	PLATELET SURVIVAL (Days) Fresh	PLATELET SURVIVAL (Days) Stored	PLATELET SURVIVAL (Days) % of Fresh
10	10	50 ± 10	26 ± 7	51%	8.0 ± 1.0	1.3 ± 0.3	16%
15	10	55 ± 11	27 ± 11	49%	8.0 ± 0.1	1.2 ± 0.4	16%
22	3	62 ± 16	15 ± 1	25%	8.4 ± 0.6	1.1 ± 0.7	12%

Both platelet recoveries and survivals met our acceptance criteria for up to 15 days of storage but not for 22 days. Data on *in vitro* platelet assays are given in Table 7. These data demonstrate that cold stored platelets are highly activated, suggesting that these platelets may provide immediate hemostasis for actively bleeding patients.

Table 7

***IN VITRO* ASSAYS OF ROTATED WHOLE BLOOD 4°C PLATELET STORAGE**

Storage Time (Days)	N	Microparticles (%) Pre	Microparticles (%) Post	Annexin V Binding (% Positive) Pre	Annexin V Binding (% Positive) Post	P-Selectin (% Positive) Pre	P-Selectin (% Positive) Post	TGT Peak (nM Thrombin) Pre	TGT Peak (nM Thrombin) Post
10	10	1.9 ± 0.9	21.7 ± 19.0	6 ± 6	32 ± 13	11 ± 11	68 ± 17	63 ± 25	177 ± 74
15	10	1.2 ± 0.9	20.0 ± 10.4	5 ± 6	51 ± 21	10 ± 11	76 ± 10	83 ± 16	162 ± 37
22	3	1.0 ± 0.8	36.3 ± 6.3	10 ± 8	56 ± 7	7 ± 2	92 ± 1	109 ± 62	171 ± 54

Conclusions:

- End-over-end rotation of WB during 4°C storage is required to reduce platelet adherence to the walls of the bag.
- Platelet yields in the WB post-storage average 7.0 to 9.2 x 10^{10}. Thus, the FDA requirement of 5.5 x 10^{10} platelets/concentrate are easily met.

- At storage times between 10 to 15 days:
  - Stored recoveries average 50% of fresh recoveries.
  - Stored survivals average >1 day.
  - Proposed post-storage criteria for WB 4°C stored platelets are met.
- Based on *in vitro* measurements, the platelets are highly activated.

Future Studies:

It will be necessary to document the hemostatic efficacy of platelets stored within WB at 4°C. This will likely require large transfusion trials monitoring bleeding outcomes in surgical or trauma patients.

References:

1. Slichter SJ, Harker LA. Preparation and storage of platelet concentrates. II. Storage variables influencing platelet viability and function. Br J Haematol 1976;34(3):403-419.
2. Borgman MA, Spinella PC, Perkins JG, *et al*. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. J Trauma 2007;63:805-813.
3. Hess JR, Holcomb JB. Transfusion practice in military trauma. Transfusion Med 2008;18:143-150.

APPENDIX A
REVISED STATEMENT OF WORK

Title: Novel approaches to storage of platelets for transfusion.

Background: Platelets are transfused to prevent bleeding and induce hemostasis, and can thus be critical in saving lives following trauma. Currently, platelets isolated from volunteers are stored at room temperature with gentle agitation for up to 5 days before transfusion. This short shelf-life severely compromises platelet inventories and creates chronic shortages for two important reasons: (1) platelets age during this period, and are functionally not as desirable as fresh platelets; and (2) storage at room temperature increases the risk of bacterial contamination. There is an urgent need to develop novel methods of storing platelets to minimize or even eliminate these issues. This need is particularly acute in the deployed military setting where platelet products are in especially short supply and are essentially unavailable far-forward, near the point of injury where they might be of greatest utility.

It is possible that manipulation of several collection and storage parameters, such as choice of apheresis systems, storage medium and temperature, and implementation of pathogen reduction technologies may improve platelet shelf life, safety and effectiveness. It is well known that platelets are sensitive to physical stimuli such as shear and contact with artificial surfaces, which may be activating and cause premature release of hemostatic and inflammatory mediators prior to or during storage. Selection of collection systems that minimize physical damage to platelets, in conjunction with storage optimization, could significantly enhance platelet product quality. Similarly, the current common practice of storing platelets in citrated plasma at room temperature may lead to significant product degradation due to the activity of endogenous proteases or other mechanisms. Use of platelet additive solutions (PAS), might reduce platelet stress. Alternatively, storage of platelets within whole blood under refrigeration may provide other factors that maintain important aspects of platelet function that need to be evaluated, as a potentially preferred product for battlefield polytrauma. This was once standard-of-care in transfusion medicine, but was abandoned once it was shown that refrigeration led to accelerated *in vivo* platelet clearance over about 48 hours rather than over one week. While not conducive to maintaining circulating platelet counts in thrombocytopenic cancer patients, this strategy might provide adequate platelet hemostatic capacity to bleeding trauma patients and improve platelet availability for such patients. This possibility has been inadequately evaluated, particularly in clinical studies. Finally, addition of a pathogen reduction technology to some combination of the preceding approaches may yield further benefit by impeding bacterial growth, which is the principal lethal transfusion risk associated with platelet transfusion. Pathogen reduction would have the greatest impact in resource-constrained settings such as the deployed military environment or the

developing world, where full transfusion transmitted disease testing is unavailable.

A deeper mechanistic understanding of the effects of collection and storage on platelet function could greatly aid in improving the availability and efficacy of platelets both on the battlefield and in the civilian transfusion setting. In this research proposal we are interested in evaluating the effect of novel combinations of collection, storage and pathogen reduction approaches on the structural and functional properties of platelets and on the functional consequences during transfusion.

We hypothesize that the *"collection of platelets in a manner that minimizes physical damage combined with an alternative storage medium and/or temperature and pathogen reduction technology will improve platelet shelf life, safety and function*." We propose the following Specific Aims to test this hypothesis.

Aim 1. Evaluation of structural and functional changes to platelets during enhanced collection, storage and pathogen reduction (enhanced platelets)

We will evaluate changes in the structural and functional properties of platelets including metabolism, protein and microRNA expression, shape changes, cytoskeletal rearrangement, membrane fluidity, receptor expression and distribution, microparticle formation, aggregation in response to agonists, whole blood clotting function, adhesion and aggregation under high shear conditions.

Aim 2. Evaluation of enhanced platelets in animal models of trauma and hemorrhage

We will evaluate the hemostatic efficacy and inflammatory characteristics of enhanced platelets and these observations will be correlated with *in vitro* findings from Aim 1. Enhanced platelets will be optimized for collection methods, storage mediums (plasma, PAS etc.), Temperature (refrigerated, room temperature, temperature cycling etc.)and exposure to pathogen reduction technology.

Aim 3. Evaluate enhanced autologous platelets in normal subjects

As the first step in the in vivo evaluation of human platelets, autologous platelets will be obtained from whole blood or apheresis procedures. The whole blood or apheresis platelets will have been subjected to various storage conditions with or without pathogen reduction. The efficacy of the platelets obtained from these products will be assessed by determining the recovery and survival of the subjects' autologous radiolabeled platelets following re-infusion. The results of these studies will be correlated with the studies performed in Aims 1 and 2.

Aim 4. Evaluation of enhanced platelet recovery and survival, bleeding time and hemostatic activity in thrombocytopenic patients with and without acute hemorrhage.

We will evaluate the shelf life, safety, and efficacy of enhanced platelets in patients and correlate these findings with observations from Aims 1, 2, and 3 in order to optimize platelet product collection and storage conditions.

We expect our results to generate important information on how changes in platelet collection, storage medium and temperature, and exposure to pathogen reduction technologies affect stored platelet structure and function, as well as shelf life and *in vivo* efficacy.

Our collaborators at the Puget Sound Blood Center, led by Dr. Sherrill J. Slichter, have extensive experience in studying platelet biology and transfusion medicine. Dr. Slichter's laboratory and clinical study group has made a number of the seminal observations on the effectiveness of platelet transfusion strategies.

A CRADA will cover collaborative research between the Puget Sound Blood Center and the USAISR Coagulation and Blood Research Task Area. This collaborative effort is envisioned to lead to development of new platelet storage techniques. Joint authorship in publications and inventors rights will be shared by both parties.

Collaboration:

USAISR agrees to:

1. evaluate changes in the structural and functional properties of platelets following collection, storage and pathogen reduction approaches that incorporate a number of combinations of currently available technologies, or technologies in advanced development (enhanced platelets).

2. evaluate the hemostatic efficacy and inflammatory characteristics of enhanced platelets in animal models of trauma and hemorrhage, and these observations will be correlated with *in vitro* findings.

3. evaluate the shelf life, safety, and efficacy of enhanced platelets in thrombocytopenic patients with acute hemorrhage and correlate these findings with observations from Aims 1 and 2 in order to optimize platelet product collection and storage conditions.

4. engage in analysis of data and validation of findings related to changes in platelet structure, function, and viability following enhanced collection, storage and pathogen reduction.

5. write manuscripts and scientific reports, submit invention disclosures and patents.

Puget Sound Blood Center agrees to:

1. identify candidate platelet collection, storage and pathogen reduction approaches to test in model *in vitro* and *in vivo* systems with the goal of improving platelet storage life, safety and efficacy. As needed, transfer candidate technologies to USAISR for *in vitro* and *in vivo* testing as described above.

2. conduct *in vitro* and *in vivo* platelet product testing as described above.

3. conduct clinical studies of enhanced platelets such as recovery and survival experiments in normal volunteers and thrombocytopenic patients. In addition, PSBC will perform bleeding time assays and trials of hemostatic efficacy studies in thrombocytopenic patients with and without acute hemorrhage. The first collection/storage conditions to be evaluated will be platelets stored within whole blood units under refrigeration.

4. engage in analysis of data and validation of findings related to changes in platelet structure, function, and viability following enhanced collection, storage and pathogen reduction procedures.

5. write manuscripts and scientific reports, submit invention disclosures and patents.

From time to time, USAISR personnel may work in the Puget Sound Blood Center's laboratories and Puget Sound Blood Center's personnel may work in USAISR's laboratory as necessary to accomplish the goals of this collaboration.

Cold Apheresis Platelets in Isoplate (CAPI)

I. PROTOCOL INFORMATION
Title: Cold Apheresis Platelets in Isoplate (CAPI)
Phase of Study: Phase I/II. Proof of Principle.
Version/Date of Protocol: Version 1/September 14, 2015

II. SPONSOR INFORMATION
The study is being sponsored by the Department of Defense (DOD) Congressionally Directed Medical Research Program (CDMRP).

III. PRINCIPAL INVESTIGATOR'S INFORMATION
PI Name: Sherrill J. Slichter, MD
Title: Director Platelet Transfusion Research
Name & Address of Research Institution: BloodworksNW (formerly Puget Sound Blood Center)
Phone #: 206-689-6541
FAX #: 866-791-4098
Email: sherrills@BloodWorksNW.org

IV. ROLES AND RESPONSIBILITIES
Principal Investigator (PI): The PI will have overall responsibility for the study. She will ensure compliance with the protocol, institutional policies, and all applicable regulations. The PI will supervise the use of the test articles and review study data at regular intervals. The PI will permit and comply with audits and monitoring requirements. The PI will report all unanticipated problems involving risk to subjects or others to the Research Monitor, appropriate regulatory bodies, including the University of Washington Human Subjects Division and the USAMRMC, ORP, HRPO.

Study Coordinator (SC): The SC will assist in the preparation of the protocol, Institutional Review Board (IRB) applications and amendments, required quarterly reports, and other regulatory documents as needed. The SC will manage implementation of the research protocol under the supervision of the Principal Investigator. She will identify and recruit eligible subjects, review information on source documents to ensure data are complete and correct, and assist in rectifying discrepancies. She will maintain study records and logs and assist in evaluating study results. In addition the SC may perform all tasks ascribed to the Clinical Research Staff (below).

Clinical Research Staff (CRS) will perform research-related interventions under the direction of the PI and/or the SC. CRS will ensure that subjects have read and understand the informed consent document and have all questions appropriately answered and that informed consent documents are properly signed and dated. CRS will schedule study subject visits; explain study procedures; assess and document study subject's clinical status as required by research protocol; collect apheresis units; obtain subject blood samples; administer radiolabeled platelets as required by the protocol (only trained Registered Nurse CRS will perform this task); monitor study subject's progress and report adverse effects to the PI.

Laboratory Research Staff will perform research-related laboratory testing and platelet radiolabeling in accordance with the study protocol. Laboratory Research Staff will perform data entry into the study data base. Upon occasion Laboratory Research Staff may also collect blood samples from subjects.

BloodworksNW Staff (either research or non-research) will collect follow-up blood samples and hold them for pick up and processing by Laboratory Research Staff.

Research Monitor: The Research Monitor will act as the safety advocate for study subjects. The Research Monitor will review all unanticipated problems involving risk to subjects or others and will provide an unbiased written report of the event to appropriate regulatory bodies, including the University of Washington Human Subjects Division and the USAMRMC, ORP, HRPO.

V. SITE INFORMATION

All study activities with the exception of the laboratory tests noted below will occur at the BloodworksNW (formerly Puget Sound Blood Center) under the direction of Dr. Sherrill J. Slichter. Bacterial testing and Gram Staining will be conducted by the University of Washington (UW) Microbiology Laboratory in Seattle, or by LabCorp in Seattle. All samples sent to outside microbiology laboratories will be stripped of all personal identifiers and labeled with a study ID number only.

VI. STUDY INFORMATION

Type of Research: Biomedical

VII. STUDY DESIGN

Background

Platelets are transfused to prevent bleeding and induce hemostasis, and can thus be critical in saving lives following trauma and in supporting thrombocytopenic cancer patients. Currently, platelets collected from volunteers are stored at room temperature. Room temperature storage has been demonstrated to maximize platelet recovery and survival in transfused patients; however it also increases the opportunity for bacterial growth in the platelet unit. The FDA limits the shelf life of platelets to 5 days or less to minimize this bacterial risk. Recently, the FDA has allowed 7 day storage with additional point-of-release bacterial testing. Nonetheless, transfusion associated sepsis remains the principal lethal risk associated with platelet transfusion.

Cold storage (4°C) is known to reduce post transfusion platelet recoveries but the effect is no more than 10% to 20% after 3 days of platelet concentrate storage. However, survivals are reduced to 1 to 2 days compared to an average survival of 4 to 5 days at 22°C storage[1-3]. In addition, there is controversy regarding the ability of 4°C stored platelets to correct bleeding times in thrombocytopenic patients compared to 22°C stored platelets[1]. However, we have demonstrated, in preliminary studies that platelets stored within whole blood for 15 days have radiolabeled autologous recoveries of 27±11% (49% of the same donor's fresh autologous recoveries) and survivals averaging 1.2±0.4 days (16% of the same donor's fresh autologous survivals)[4]. These data suggest that 4°C storage of apheresis platelets, as proposed in this study, may clearly show similar or even better platelet viability as platelet storage within whole blood.

Platelet Additive Solution

Based on our preliminary studies using 22°C storage, apheresis platelets stored in a platelet additive solution allow the longest reported storage time of platelets[5]. Post transfusion platelet recovery and survival meet FDA guidelines for 13 days of storage. It is anticipated that storage at 4°C will also benefit by storage in an additive solution.

Cold Stored Platelets

While not conducive to maintaining circulating platelet counts in thrombocytopenic cancer patients, transfusion of refrigerated platelets for deployed military medical units might provide adequate platelet hemostatic capacity to bleeding trauma patients and improve platelet availability for such patients. Based on recent in vitro studies of 4°C versus 22°C stored platelets, clot strength, platelet aggregation and sheer induced platelet aggregation are all better maintained at 4°C[6-8]. Furthermore, there is much less aggregation of platelets when the platelets are stored at 4°C in an additive solution and not agitated during storage. This possibility has been inadequately evaluated, particularly in clinical studies.

A deeper understanding of the effects of cold storage on platelet function could greatly aid in improving the availability of platelets on the battlefield and in the civilian transfusion setting. In this research proposal, we are interested in evaluating metabolic, functional and viability changes to apheresis platelets preserved in an additive solution and stored at 4°C. We will also determine the recovery and survival of these platelets by radiolabeling an aliquot of the apheresis platelets and re-infusing it into the donor/subject.

Current Research Approach

A double hyperconcentrated apheresis platelet unit will be collected from a healthy adult volunteer subject using the Trima Accel® Automated Blood Collection System. Concurrent plasma will be collected. After collection the unit will be split into two equal portions and will be re-suspended in 65% Isoplate (a platelet additive solution) and 35% plasma while the other half will be suspended in plasma. Both units will achieve a final platelet concentration of ~1500 x 10^3 platelets/µL. Both units will be stored, without agitation, at 4°C. The plasma suspended unit (control) will be stored for 3 days, the maximum platelet storage time allowed for refrigerated platelets by the FDA. The Isoplate/plasma unit (test) will be stored for up to 20 days.

On Day 3 the subject will receive an Indium 111 (In-111) radiolabeled aliquot of their control plasma 4°C stored platelets. Follow-up samples from the subject will be collected approximately 2 hours post-infusion and on Days 1 (2X), 2, 3, 4, and 5 to calculate recovery and survival of the subject's 3 day stored platelets. On Day 1 the sample draws will be 2 - 10 hours apart.

The Isoplate 4°C stored platelets will initially be stored for 10 days. The subject will receive an infusion of In-111 aliquot of their 4°C Isoplate stored platelets. Follow-up samples, as above, will be collected to calculate platelet recovery and survival of the test unit.

These studies will allow comparison of the 3 and 10 day stored platelets. Indium will be used to label both control and test platelets because the other available isotope, chromium, is not taken up by refrigerated platelets. The In-111 administered on Day 3 will be largely undetectable by Day 10 and therefore re-use of the same isotope to measure both control and test cold stored platelets is valid. Additionally, we will collect a pre-infusion radioactivity sample to account for any residual In-111, and adjust our calculations accordingly.

Each subject's Isoplate platelet recovery and survival will be considered acceptable if they are ≤20% less than the subject's corresponding 3-day stored sample measurements. Acceptance criteria are met for a given storage time if samples from all subjects in that group meet acceptability threshold.

So long as our acceptance criteria are met with 5 split paired units stored for 10 days we will progressively increase the storage period in two day increments until acceptance criteria are no longer

met (i.e., when at least one subject has >20% reduction in either recovery or survival compared to the same subject's 3-day 4°C plasma stored platelets). We will evaluate 12, 14, 16, 18 and 20 day storage so long as test platelets for all 5 subjects in the group demonstrate ≤20% reduction in recovery and survival as compared to their own 3-day plasma 4°C stored platelets. Once we identify the first storage period that fails to meet acceptance criteria, we will fall back by one day to evaluate the mid storage period between that which met and that which failed criteria. If that mid-storage period fails, then we will evaluate the prior storage period again with 5 additional samples, continuing to step back down until a group meets acceptance criteria for all 10 subjects. Thus, once the maximum acceptable storage time has been determined, a total of 10 subjects satisfying acceptance criteria will have platelet viability data collected at that storage time.

In addition to radiolabeled platelet recovery and survival measurements, various in vitro assays (see "In Vitro Tests Performed on Test Units") will be performed at the end of each storage condition (3 day and extended stored; i.e. 10 to 20 day).

VIII. INCLUSION / EXCLUSION CRITERIA

Inclusion Criteria

The subject is in good health, is taking no excluded medications and meets platelet donor suitability requirements aimed at assuring donor safety. Recipient safety restrictions (e.g. travel and sexual contact) do not apply for this study. No infectious disease testing will be performed.

Specific inclusion criteria are:

- Weight: ≥125 pounds
- Hematocrit: ≥38%
- Platelet count ≥225X10^3/mm^3
- Temperature: ≤99.5°F
- Resting blood pressure: systolic ≤ 180 mmHg; diastolic ≤100 mmHg
- Resting heart rate: 40 to 100 beats per minute
- Subjects must be ≥ 18 years old, of either sex
- Subjects must be able to read, understand and sign the informed consent document and commit to the study follow-up schedule. The ability to read and speak English is required for participation.
- Subjects must have good veins for apheresis platelet collection and drawing blood samples.
- Subjects of child-bearing potential (either male or female) must agree to use an effective method of contraception during the course of the study. The following methods of contraception will be considered 'effective' when self-reported by subject; abstinence, intrauterine contraception devices, hormonal methods, barrier methods or history of sterilization.

Exclusion Criteria

Healthy subjects will be excluded from the study for any of the following reasons:

- Ever received radiation therapy.
- Already participated in 4 research studies involving radioisotopes within the contemporaneous calendar-year.
- Taken aspirin, non-steroidal anti-inflammatory, or other platelet affecting drugs within 72 hours of collection or infusion.

- Currently pregnant or nursing as assessed during interview. A urine pregnancy test prior to radioisotope infusion is required for women of childbearing potential.
- Unable to comply with the protocol in the opinion of the investigator.
- Donated granulocytes within the last 2 days.
- Donated whole blood within the last 7 days.
- Donated platelets or plasma within the last 28 days.

IX. SUBJECT RECRUITMENT & SCREENING

The study will advertise for healthy adult volunteers on websites, newspapers and/or bulletin boards. Prospective subjects will be asked to contact the Study Coordinator by email or phone. Email inquiries will be answered, by the Study Coordinator, with a summary email along with attachments of study documents (study consent, HIPAA policy, directions to BloodworksNW and a schedule of study visits). The subject will be encouraged to call the Coordinator to discuss the study by phone before making a screening appointment. The Study Coordinator may reference *Talking Points for Volunteer Inquiries* during the phone conversation.

Prospective subjects responding by phone will speak with the Study Coordinator, as described above, and will be offered an email with attached study documents.

Individuals who wish to make an in person appointment for consent and screening will make those arrangements by phone or email with the Study Coordinator. An email confirmation and reminder will be sent by the Study Coordinator. Contact information from people who do not make appointments will not be retained.

A total of 80 subjects may be enrolled to achieve 40 complete data sets, which is the maximum number of evaluations our study design would demand.

X. INFORMED CONSENT PROCESS

At the time of the recruitment visit, Clinical Research Staff, usually the Study Coordinator will review the consent with the study subject in a private space at the BloodworksNW. The purpose of the study, the study procedures, the risks and options to not participate or to withdraw will be discussed. The number of venipunctures, the radioisotope exposure and the time demands of multiple blood draws will be emphasized. Throughout the process the subject will be encouraged to ask questions or make comments.

Subjects will sign the consent form in the presence of the staff administering the consent and that person will also sign the consent. The subject will be given a copy of the consent and HIPAA document.

Screening questions are related to establishing that the subject is in good health. See Section 8, Inclusion/Exclusion Criteria.

After the subject has given informed consent and passed screening a double apheresis platelet collection will be performed. See Study Procedures section below.

All Clinical Research Staff have been trained and are certified in the Protection of Human Research Subjects.

XI. STUDY PROCEDURES

Screening

An abbreviated version of blood donor screening will be performed including completion of a study specific health history questionnaire, check of vital signs and a blood draw to obtain a 2 mL sample for a complete blood count (CBC) to obtain the hematocrit and platelet count. Only criteria aimed at assuring donor safety will apply. Recipient safety restrictions (e.g. travel and sexual contact) do not apply for this study. No infectious disease testing will be performed.

Apheresis Platelet Collection

The subject's platelets will be collected using the Trima Accel Automated Blood Collection System which is licensed by the FDA for this purpose. A venipuncture site will be selected and cleaned using standard BloodworksNW procedures. A needle will be placed in one of the subject's arms at the antecubital area. Whole blood is drawn into the apheresis machine and the blood components are separated by centrifugation. Platelets and plasma are collected into storage bags and the red blood cells are returned to the subject. Along with the return of the subject's red blood cells the subject receives approximately 350 mL of ACD (citrate) anticoagulant during the collection process. The platelet apheresis collection lasts 2-3 hours. Subjects are observed throughout the collection by a nurse or technician specifically trained in apheresis.

Suspension in Additive Solution and Storage

Immediately after apheresis collection, using sterile technique, research laboratory staff will split the hyperconcentrated platelet unit into two equal portions and complete the processing and storage procedures.

Isoplate will be added to half of the collection to achieve a 50% plasma/50% Isoplate solution by weight. The platelet unit will rest as a 50/50 mixture, at room temperature, on a bench for one hour. Albumin concentrations will be performed to calculate how much additional Isoplate is required to achieve a 65% Isoplate/35% plasma suspension. The required amount of Isoplate will be added to the platelet product by weight.

Isoplate™ Solution Platelet Additive Solution [PAS-F] is an isotonic solution used to replace a portion of the plasma to store leukocyte reduced apheresis platelets collected on Terumo BCT's Trima Accel® System. Platelets in Isoplate™ Solution are FDA approved for storage in a 65% Isoplate™ and 35% plasma mixture in a concentration range of 700-2100 x 10^6/mL for up to 5 days at 20-24°C with continuous agitation in the EXCEL® container.

The second unit will be suspended with plasma so that it equals the final weight of its paired 65% Isoplate/35% plasma suspended unit.

Both units will be placed in a refrigerator at 4±2°C. The plasma resuspended platelet unit will be and stored for 3 days. The other unit will be resuspended in 35% plasma/65% Isoplate and stored for 10 to 20 days. The units will not be agitated during storage. Both units will be stored in the EXCEL® container.

Temperature monitors will record temperatures and trigger alarms for out of range conditions. End of storage will be defined as the date and time when the aliquot for radiolabeling and infusion is removed from the stored unit.

Autologous infusion of radiolabeled platelets

Three days after the apheresis collection the subject will receive an infusion of radiolabeled platelets. The radiolabeled platelets administered on Day 3 will be extracted from the ½ split unit, suspended in plasma, and stored in a refrigerator at 4±2°C for 3 days and labeled with Indium 111.

Ten to 20 days after the apheresis collection the subject will receive another radiolabeled platelet infusion. An aliquot of platelets from the other ½ split unit, suspended in 65% Isoplate/35% plasma, and stored in a refrigerator at 4±2°C for 10 to 20 days will also be labeled with Indium 111.

Prior to infusion the subject's health will be reassessed via interview. If the subject feels unwell, has flu-like symptoms, or has any significant negative change to his or her health status, then he/she will be considered ineligible for the radiolabeled infusion and will exit the study. Pre-menopausal female subjects will have a urine pregnancy test to confirm that they are not pregnant prior to both infusions. Any subject with a positive pregnancy test will be ineligible to continue with the infusion and will exit the study. Prior to infusion, microbiological tests (bacterial testing and Gram stain) of the platelet unit will be verified as negative.

Height and weight will be measured at the time of infusion. After venous access has been established, a blood sample (30 mL) will be obtained to determine baseline radioactivity. Approximately 10 mL (2-10 mL) of autologous radiolabeled platelets will be infused back into the subject. During each platelet infusion, the subject will be carefully monitored for adverse reactions; i.e., fever, chills, dyspnea, urticaria or pain (infusion site, chest pain or other). Any adverse reactions will be recorded and reported to the study investigator.

After infusion, the line will be flushed with saline and removed. The subject will remain at, or return to, BloodworksNW for the Day 0 post-infusion blood sample, which will be collected ≥2 hours after the infusion.

Follow-up

The subject will return to BloodworksNW for sample collection (10 mL of blood) for measurement of radioactivity to calculate platelet-survival curves; Day 1 (twice, 2-10 hours apart), Day 2, Day 3, Day 4, Day 5 for each radiolabeled platelet infusion. (See Schedule of Events below). These samples will be used to determine platelet recovery and survival using computerized modeling of a multiple hit decay function.

Schedule of Events

Study Day	Study Procedures
Day 0	Informed consent process
	Screening and enrollment
	Apheresis platelet collection
	Apheresis unit split into two equal volumes in separate bags • ½ platelets suspended in plasma • ½ platelets suspended in 65% PAS/35% plasma
	Both platelet bags put into storage, unagitated, at 4±2°C
Day 1	Bacterial culture sample collected from both platelet units (4°C)
Day 3	3 day 4°C platelet storage ends. End of storage in vitro testing.
	Platelets processed for In-111 radiolabel
	Pre-infusion vital signs and health assessment
	30 mL blood sample from subject for baseline radioactivity
	Infusion of In-111 radiolabeled platelets
	Post infusion R&S sample from subject (≥2 hours post infusion)
Day 4	Post infusion R&S sample from subject (twice, 2 - 10 hours apart)
Day 5	Post infusion R&S sample from subject
Day 6	Post infusion R&S sample from subject
Day 7	Post infusion R&S sample from subject
Day 8	Post infusion R&S sample from subject
Day 10 *	10 day 4°C platelet storage ends. End of storage in vitro testing.
	Platelets processed for 2nd In-111 radiolabel.
	Pre-infusion vital signs and health assessment
	30 mL blood sample from subject for baseline radioactivity
	Infusion of 2nd aliquot of In-111 radiolabeled platelets
	Post infusion R&S sample from subject (≥2 hours post infusion)
Day 11	Post infusion R&S sample from subject (twice, 2 - 10 hours apart)
Day 12	Post infusion R&S sample from subject
Day 13	Post infusion R&S sample from subject
Day 14	Post infusion R&S sample from subject
Day 15	Post infusion R&S sample from subject
	Subject exits study

* The second In-111 labeling will be done at the end of storage for the Isoplate/plasma stored platelets which may be from 10 – 20 days after the apheresis collection.

Total Volume of Blood Collected

The total amount of blood loss during the course of the study is approximately 287 mL. This includes CBC (2 mL), diversion pouch sample (~25 mL), apheresis platelets (~60 mL residual in disposable kit), immediate pre-infusion for baseline radioactivity (30 mL X2), and post infusion blood samples (10 mL each X 14) to determine circulating radioactivity.

In addition to the above volumes, approximately 170 mL of apheresis platelets and up to 300 mL of concurrent plasma will be collected.

In Vitro Testing Schedule

In addition to the in vivo platelet viability measurements after re-infusion, a number of in vitro laboratory measurements will be performed. Samples for these experiments will be obtained from the apheresis unit at the end of storage for both the plasma and Isoplate resuspended units. These tests will be performed using standardized methods.

A sample from each platelet product will be sent for bacterial culture to an outside microbiology laboratory one day after the platelet collection. At the end of the storage period a sample from the stored platelet unit will be sent to the University of Washington Microbiology Lab for a Gram stain. If either test is positive, the subject's stored platelets will not be reinfused and the subject will be withdrawn from the study.

The following table provides a list of the tests that will be performed on the apheresis platelet unit at the beginning and end of storage. These are the standard in vitro assays that the FDA requires for platelet licensing.

In Vitro Tests Performed on Stored Apheresis Units at the End of Storage

Test Type	End of storage testing	
	Plasma suspended, 3 day stored unit.	Plasma/Isoplate suspended, 10 – 20 day stored unit
Platelet Concentration	✓	✓
Volume	✓	✓
Platelet yield	✓	✓
Blood Gases (pH and pCO2, PO2, HC03)	✓	✓
Glucose and Lactate	✓	✓
P-selectin*	✓	✓
Morphology	✓	✓
Annexin V binding	✓	✓
Extent of Shape Change	✓	✓
Hypotonic Shock Response	✓	✓
Platelet Microparticles	✓	✓
Swirling	✓	✓
Mean Platelet Volume (MPV)	✓	✓
Bacterial Culture**	✓*	✓*
Gram stain	✓	✓

All samples will be discarded once testing is complete and no residual radiation is detectable.

*P-selectin samples will be prepped on end of storage day and batch tested.

**Bacterial Culture sample removed from unit 1 day after collection and evaluated at end of storage.

Adverse Event (AE) Assessments

During apheresis collection and infusion of platelets, the subject will be carefully monitored for adverse reactions; e.g., fever, chills, dyspnea, urticaria, or pain (infusion site, chest pain or other). Adverse reactions will be recorded in the study file and reported to the study investigator. Subjects will be instructed to report changes in health condition over the course of the study to the study coordinators. Minor AEs that are associated with venipuncture and blood collection, such as minor bruising at the needle site, will not be recorded as AEs, unless they worsen over time (e.g., become infected, etc.).

XII. DATA and ANALYSIS

Laboratory and other evaluable results will be transcribed from source documents (e.g. lab result print-outs) into an electronic database.

Summary statistics (means, medians, standard deviations, interquartile range) will be calculated for all in-vitro assays. A table of in vitro summary statistics will be presented to facilitate comparison of assay values between the 3 day and extended stored platelets from the same subjects.

Tables of recovery and survival summary statistics will display values by group from 3 day and extended stored platelets. Recovery and survival of extended stored platelets as percentage of corresponding 3-day 4°C stored platelets will be plotted against days stored. Regression methods will be used to determine if there is evidence of any trend in the mean storage or recovery as percentage of corresponding 3-day 4°C stored platelets with respect to storage time. Histograms of recovery and survival as percentage of corresponding 3-day 4°C stored platelet measurements will be plotted, and corresponding confidence intervals will be calculated. Counts and percentages of the number of subjects at each storage interval whose corresponding 3-day 4°C stored platelets have >20% loss will be tabulated to determine storage intervals for which the stored platelets meet performance criteria.

XII. LABELING & STORAGE OF DATA & SPECIMENS

Study records, samples, and test results will be identified with a unique identifier and access will be limited to sponsor authorized personnel, the investigator, site research staff, and authorized regulatory authorities, including representatives of the FDA.

An alpha-numeric code that is unique to this study will be used as study identifiers. The study ID number will be associated with the subject's name on a study ID log. That log and the study database will be kept in separate folders on an electronic network at BloodworksNW. BloodworksNW uses Active Directory NT Authentication along with Access Control Lists (ACL's) for all network folders. File and folder access is logged on network shares. Security is enforced by the Information Technology Department. A network firewall is used to prevent unauthorized access to the network from outside entities.

Source paper documents will be kept in the Study Coordinator's office at BloodworksNW which is a security-card-restricted-access-building. The door to the coordinator's office is kept locked. Any documents not needed for source documentation will be shredded using a secure records-destruction service.

The link between the subject's identify and their study data will be destroyed/deleted when the research ends and any required monitoring of the study is finished, which will be no later than December 31, 2025. Consents will be destroyed six years after the conclusion of data analysis.

BloodworksNW utilizes an independent waste management contractor to dispose of research samples. The waste management contractor is contractually obligated to be in compliance with all applicable regulations regarding the pick-up, transport and treatment of regulated medical waste.

Subject samples that are radioactive at the time of collection are stored in a lead box in a secure-access temperature controlled room until such time as they have no detectable residual radiation. This is generally about 3-4 months. At that point they are disposed of as described above.

XIII. RISK AND INJURY

Apheresis-Related Risks and Precautions

Risks associated with standard platelet-product apheresis procedures are listed below. A single apheresis procedure typically lasts about 3 hours.

- Venipuncture-related risks: Venipuncture may lead to apprehension, discomfort, pain, bruising or infiltration at the venipuncture site. A vasovagal response, such as lightheadedness or fainting, nausea, or vomiting may occur. There is a very small risk of infection at the venipuncture site.
- Citrate infusion related risks (hypocalcaemia): Citrate (Acid-Citrate-Dextrose) is added to the apheresis circuit as an anticoagulant. This may result in perioral tingling or paresthesias. Non-specific mild symptoms of hypocalcaemia include headaches, nervousness, irritability, lightheadedness, flushing, shivering, nausea, vomiting, chest discomfort and abdominal cramping. Slowing the collection rate, pausing the collection and/or administering oral calcium (TUMS) will effectively address these symptoms. Rarely, intravenous calcium is administered when symptoms do not resolve. If allowed to progress citrate toxicity could potentially manifest as muscle cramps, tremors, tetany, laryngospasm, seizures and life threatening cardiac arrhythmias.
- Blood Loss: In rare and unusual circumstances, blood loss has occurred due to inability to complete the procedure.

The following precautions will be taken: The subject's pre-apheresis vital signs (blood pressure, heart rate, temperature) and pre-apheresis hematocrit will be determined. Subjects will be visually monitored for signs of distress during all procedures by trained and experienced staff. Citrate reactions will be treated according to the standard treatments at the site, which includes oral or, rarely, intravenous calcium supplementation, and/or slowing, pausing or stopping the procedure.

Radioisotope Infusion-Related Risks and Precautions

The radiation dose in this study is less than annual background radiation (3 mSv) and is not known to be associated with any health hazard. The amount of the isotope that will be infused is ≤30 μCi of indium. The total radiation activity infused is ≤30 μCi. The total body effective dose is approximately 0.8 mSv. The total absorbed dose to the spleen is approximately 8.3 mGy. The risks of radiation exposure to a fetus are unknown. Therefore, women of childbearing potential will have a pregnancy test performed prior to the radiolabeled platelet infusion.

BloodworksNW's Platelet Transfusion Research Department will maintain a record of each subject's participation and will limit the number of studies any one individual can participate in to four studies in a calendar year. Patients who have received radiation therapy will be excluded from the study.

Platelet Transfusion-Related Risks and Precautions

Risks associated with receiving any blood product include chills, fever, hives, itching, immune response against blood cells, and/or blood infection from bacterial contamination. There is a rare risk of receiving the wrong subject's cells upon infusion, which could cause symptoms similar to those listed above.

The following precautions will be taken: In this study, subjects will be infused with their own cells; confirmation of identification will be done by two person verification of the infusion material. To prevent bacterial contamination, the product will be bacterially screened before infusion and sterile technique will be used for all manipulations of the study platelets.

Venipuncture-Related Risks and Precautions

Risks associated with venipuncture for blood sampling are apprehension, pain, discomfort, venospasm, fainting, bleeding, or bruising or infiltration at the venipuncture site.

The following precautions will be taken: Trained and experienced phlebotomists will perform the venipuncture procedures so that discomfort of the subject should be minimal.

XIV. BENEFIT(S)

There is no direct benefit to the study subject. Real benefits are altruistic in nature: subjects participating in this study will assist the scientific and medical communities in gathering important information to improving the availability of platelet transfusions.

XV. COMPENSATION

Subjects will receive $1,000.00 at the conclusion of the study for their time involved in study participation. If the subject is unable to complete the entire study or has to be withdrawn from the study, they will receive partial payment for their time involved in the study. The partial payment scale is the following (number in parentheses equals the number of times each procedure occurs during the course of the study):

Initial screening (Day 0)		$30
Apheresis collection (Day 0)		$200
Infusion of radiolabeled platelets, including pre-infusion sample draw		
(Day 3 and Day 10 – 20. Two separate infusions)	$100 (x2) =	$200
Follow-up blood sample, platelet recovery and survival calculation	$35 (x14) =	$490
End of study exit		$80
Total for completing all study procedures		$1,000

XVI. CONFIDENTIALITY

BloodworksNW considers all data and information collected during this study confidential. All data used in the analysis and summary of this study will be anonymous, and without reference to specific subject names. Study records, samples, and test results will be identified with a unique identifier and access will be limited to sponsor authorized personnel, the investigator, site research staff, and authorized regulatory authorities, including representatives of the FDA.

XVII. USAMRMC REPORTING REQUIREMENTS FOR SAE

All unanticipated problems involving risk to subjects or others will be promptly reported by telephone (301-619-2165), by email (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil), or by facsimile (301-619-7803) to the Human Research Protection Office (HRPO). A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army

Medical Research and Materiel Command, ATTN: MCMR-RP, 810 Schreider Street, Fort Detrick, Maryland 21702-5000.

XX. LITERATURE REVIEW

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