A Randomized, Multi-center, Open-label, Controlled, In Vivo Study to Assess the Recovery and Survival of Radiolabeled Autologous INTERCEPT Apheresis Platelet Components Suspended in 100% Plasma Stored for up to 7 Days

Protocol CLI 00127, Version 5.0
September 16, 2020

NCT04022889

PROTOCOL

TITLE:	A Randomized, Multi-center, Multi-Stage, Controlled, *In Vivo* Study to Assess the Recovery and Survival of Radiolabeled Autologous INTERCEPT Apheresis Platelet Components Suspended in 100% Plasma Stored for up to 7 Days
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Date:	September 16, 2020

1.0 SYNOPSIS

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<tr><td colspan="2">Platelets treated with the INTERCEPT Pathogen Reduction System Suspended in 100% Plasma and stored for up to 7 days</td><td colspan="3"></td></tr>
<tr><td>Title of Study:</td><td colspan="5">CLI 00127: A Randomized, Multi-center, Open-label, Controlled, In Vivo Study to Assess the Recovery and Survival of Radiolabeled Autologous INTERCEPT Apheresis Platelet Components Suspended in 100% Plasma Stored for up to 7 Days</td></tr>
<tr><td>Investigators / Study Sites:</td><td colspan="5">Jose Cancelas-Perez, MD, PhD (Hoxworth Blood Center)
Moritz Stolla, MD, PhD (Bloodworks Northwest Research Institute)</td></tr>
<tr><td colspan="2">Studied period (years):
Date of first enrollment: TBD
Date of last completed: TBD</td><td colspan="4">Phase of development: 2</td></tr>
<tr><td colspan="6">Objectives:
The principle objective of this study is to evaluate the hypothesis that INTERCEPT Platelets in 100% plasma stored for 5 or more days (up to 7 days) after apheresis collection retain sufficient viability for therapeutic transfusion efficacy. The post-infusion recovery and survival of autologous radiolabeled 7 day INTERCEPT platelets (Test) stored in 100% plasma will be measured in comparison to “fresh” autologous radiolabeled platelets (Control) according to FDA guidance for platelet testing (FDA 1999) in Stage 2 of this study protocol.
A secondary objective is to compare the recovery and survival results for Test platelets prepared for radiolabeling using the procedures outlined by the Biomedical Excellence for Safer Transfusion Collaboration (BEST, Appendix B) or a variation of the BEST procedure (referred to as Variant 1, Appendix C) in Stage 1 of this study protocol. Cerus has demonstrated that the Variant 1 method, which does not incorporate an initial soft spin in the presence of ACD-A, results in improved in vitro platelet recovery and quality during preparation for radiolabeling compared to the BEST procedure. This comparison will evaluate the hypothesis that preparation methods prior to radiolabeling may influence in vitro quality of the radiolabeled platelets and post-infusion viability outcomes.</td></tr>
</table>

Methodology:

Study Design

The study will be performed in two stages (Table S-1). Stage 1 is a randomized, 2-period crossover design. Test platelets stored for 7 days will be radiolabeled based on either the BEST or Variant 1 methods (depending on the period and randomization scheme for the Test platelets) for 12 healthy subjects. The recovery and survival for Test platelets prepared with the BEST and Variant 1 methods will be compared with each other and against the fresh platelet Control. With agreement from the FDA (BQ200481, July 8, 2020), completion of Stage 1 is not required.

Stage 2 is a single arm design. Test platelets from 24 healthy subjects, stored for 7 days, will be prepared for radiolabeling following the Variant 1 methodology. The recovery and survival for Test platelets will be compared against the fresh platelet Control. Stage 1 subjects with evaluable Variant 1 method data will contribute to the requirement of the 24 subjects for Stage 2.

For both stages, the study population will consist of healthy subjects who meet the FDA, AABB, and site-specific research donor eligibility criteria for apheresis platelet donation. Apheresis platelets will be collected in 100% plasma on the Trima Accel® Automated Blood Collection system.

Each study apheresis collection will be processed using the INTERCEPT Blood System for Platelets. Platelet components containing 3.0 to 7.9 $x10^{11}$ platelets in 300 to 420 mL of plasma will be processed using the INTERCEPT Dual Storage (DS) set. The INTERCEPT process will begin on either the day of collection (Day 0) or the day following donation (Day 1); illumination must occur within 24 hours after the end of collection. Test platelets components will be stored for up to 7 days, from day of collection, in 100% plasma.

During each stage samples for *in vitro* platelet testing will be collected prior to INTERCEPT treatment (Day 0/1), post INTERCEPT treatment and at the end of storage, Day 7 (see *In vitro* evaluation of platelets below).

At the end of storage, an aliquot of Test platelets will be aseptically removed from each subject's INTERCEPT platelet storage container for preparation of samples for radiolabeling using either the BEST (Stage 1) or Variant 1 (Stages 1 and 2) methodology. The *in vitro* quality of the Test platelet sample used for radiolabeling will be assessed prior to and following the pre-radiolabeling platelet sample preparations. The indices to be measured in Stage 1 are volume, $pH_{22°C}$, CD62P, platelet count, red blood cell (RBC) count and white blood cell (WBC) count. Assessments of these indices will enable the determination of platelet processing recovery for each sample preparation method and evaluation of RBC and WBC contamination in samples prior to radiolabeling. In Stage 2 platelet processing recovery during sample preparation will be calculated from volume and platelet count and $pH_{22°C}$, will be measured in the sample prior to radiolabeling.

On the day corresponding to the end of storage for the Test component, healthy subjects will return to the site, and 43 mL of whole blood (WB) will be drawn into a syringe containing 9 mL of Anticoagulant Citrate Dextrose Solution, Formula A (ACD-A). The sample will be used to prepare Control platelets following the BEST methodology. Test and Control platelets

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will be randomly radiolabeled with either ^{51}Cr (approximately10-30μCi) as sodium radiochromate ($Na_2{}^{51}CrO_4$) or ^{111}In (approximately 10-30 μCi) as indium oxine, depending upon the randomization assignment and period as applicable. Subjects will be randomized with equal probability to the radiolabeling sequences ($^{111}In/^{51}Cr$ vs. $^{51}Cr/^{111}In$) for Test/Control. The isotope labels will be assigned randomly with equal probability that Control and Test platelets will be labeled with each isotope, and the same randomization assignment of isotope labels will be utilized for both apheresis collections for the same subject in Stage 1. After radiolabeling, the autologous Control and Test platelet samples will be simultaneously infused into the subject (approximately 10-30 mL). A negative pregnancy test for females of childbearing potential is required before infusion.

Blood samples will be drawn immediately before infusion and for radioactivity measurements at 1 hour ± 15 min and 2 hours ± 15 min post-infusion (Day 0), and 6 more samples will be drawn at 1, 2, 3, 4 (or 5 or 6), 7 or 8, and 11±1 days post-infusion (DPI), at approximately the same time of day as the radiolabeled platelet infusion was administered (±4 hours). The exact time of each sample draw will be recorded.

For subjects enrolled in Stage 1, there will be a minimum washout period of four weeks between the two study periods (e.g., four weeks after the last blood sample at 11±1 DPI, in Period 1). Subjects will be monitored for safety (adverse events) from the first apheresis procedure until 24 hours after the last DPI blood sample is drawn.

Table S-1 Description of Study Stages

		Period 1			**Period 2**		
		Test		**Control**	**Test**		**Control**
Stage	**Arm**	**Storage Duration (post collection)**	**Sample Prep**	**Sample Prep**	**Storage Duration (post collection)**	**Sample Prep**	**Sample Prep**
Stage 1[a]	Arm 1	7 days	Variant 1 (n=6)	BEST (n=6)	7 days	BEST (n=5)	BEST (n=5)
	Arm 2	7 days	BEST (n=7)	BEST (n=7)	7 days	Variant 1 (n=6)	BEST (n=6)

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Stage 2	Single Arm (n=24)[b]	7 days	Variant 1	BEST	Not Applicable, Single Arm

[a] Based on correspondence with FDA (BQ200481, July 8, 2020), Stage 1 was not completed. The n represents the number of subjects which have completed the period.

[b] Stage 1 subjects with evaluable Variant 1 method data will contribute to the requirement of the 24 subjects for Stage 2.

Radioactivity measurements

Samples will be obtained from the radiolabeled Control and Test platelets before infusion and used as a radioactive standard. The total dose of radioactivity infused will be calculated based on the volume of labeled platelets infused. *In vitro* elution of the label from the transfused platelets, as well as the *in vivo* elution of radioactivity from the serial blood samples obtained post-infusion will be determined by the BEST elution assessment method (**Appendix B**).

The radioactivity of the samples will be determined by use of a gamma counter with correction for isotope spill over. Duplicate whole blood (WB) samples will be counted for each time point. Sample dilutions for enumeration of radioactivity of the standards will be calibrated to ensure that measured values are at least 10-fold above background values. The subject's WB samples will be corrected for the activity in the cellular fraction determined by simple centrifugation and non-platelet residual activity using the 11 ±1 DPI results. Data points greater than 20 hours post-infusion will be used to calculate the post infusion recovery after all radioactive corrections have been made. A multiple-hit model will be used to estimate the survival of the radioactively labeled platelets.

At defined post infusion timepoints, a portion of the subject's WB sample will be applied to a Ficoll gradient to allow for separation of the RBC/WBC fraction from the plasma/platelet/WBC fraction of the WB. The radioactivity of the non-RBC and RBC fractions will be measured by use of a gamma counter to allow for the characterization of the extent of radiolabeled RBC contamination in the WB sample. This assessment will be performed at 1, 4 (or 5 or 6), and 11±1 DPI during Stage 1 and at 11±1 DPI for Stage 2; the radioactivity will be measured alongside radioactivity measurement of the subject's cellular fraction derived from centrifugation of the WB.

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***In vitro* evaluation of platelets**

In vitro platelet function of the platelet component will be evaluated on Days 0/1 (pre-treatment) and at end of storage (Day 7). In addition, platelet count, volume, and dose will be assessed at the end of INTERCEPT treatment on Day 1/2 to assess platelet yield retention post INTERCEPT treatment.

The physical and metabolic characteristics to be evaluated at each timepoint are listed in Table S-2.

Table S-2 In Vitro Platelet Function Assays to Evaluate INTERCEPT Platelet Components

Assay	Pre INTERCEPT Day 0-1	Post INTERCEPT	End of Storage
Component weight (g)	X	X	X
Platelet count ($\times 10^3/\mu L$)	X	X	X
Platelet dose ($\times 10^{11}$ cells/unit) [a]	X	X	X
Mean platelet volume (MPV) (fL)	-	-	X
Morphology score (max 400)	-	-	X
$pH_{22°C}$	X	-	X
$pO_{2\ (37°C)}$ (mm Hg)[c]	-	-	X
$pCO_{2\ (37°C)}$ (mm Hg)[c]	-	-	X
$HCO_3^{-}{}_{(37°C)}$ (mmol/L)[c]	-	-	X
Supernatant glucose (mmol/L)[c]	-	-	X
Supernatant lactate (mmol/L)[c]	-	-	X
Supernatant LDH (U/L) [c]	X	-	X
Total LDH (U/L)	X	-	X
ATP (µmol/dL)[c]	X	-	X
Extent of Shape Change (%) (ESC)	-	-	X
Hypotonic Shock Response (%) (HSR)	-	-	X
CD62P (% P-selectin expression)	X	-	X
Platelet lysis (%)[b]	X	-	X

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Bacterial culture	-	1 - 3 days before end of storage	-

[a] Platelet dose is calculated from the platelet count and volume.
[b] Calculated from ratio of LDH activity in 1 mL of platelet concentrate supernatant to the total LDH activity in 1 mL of Triton X-100 lysed platelet concentrate.
[c] The values for these parameters will also be normalized for platelet count.

Number of subjects:

Up to a total of 36 subjects with evaluable (Test and Control) recovery and survival data may be obtained. Randomized subjects who do not have both paired (Test and Control) recovery and paired survival data for a given stage or who are unable to provide a second matched donation for Stage 1 will be replaced, unless the enrollment goal is met. Up to 45 subjects may be required to obtain a sufficient number of evaluable subjects.

Main criteria for inclusion:

Inclusion criteria:

- Age ≥18 years, of either gender.
- Normal health status (as determined by the Investigator review of medical history and blood donor physical exam).
- Meet FDA, AABB, and site guidelines for blood donation and apheresis platelet donation. Travel, tattoos/piercings and/or male to male sexual contact deferrals do not apply.
- Complete blood count (CBC) and serum chemistry values within established reference ranges or within guidelines as above.
- Pre-donation platelet count of more than 150×10^9 platelets/ L.
- Negative blood donor screening test panel for HIV, HBV, HCV, HTLV, syphilis, and WNV.
- Subjects of childbearing potential must agree to use a medically acceptable method of contraception throughout the study. A barrier method of contraception must be included, regardless of other methods.
- Signed and dated informed consent form.

Exclusion criteria:

- For participation in Stage 2, received any previous infusion in this study.
- Clinically significant acute or chronic disease (as determined by the Investigator).
- Pregnant or nursing females.

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<tr><td>Platelets treated with the INTERCEPT Pathogen Reduction System Suspended in 100% Plasma and stored for up to 7 days</td></tr>
<tr><td colspan="4">• Subjects of childbearing potential not using effective contraception.
• Disease states or conditions that preclude apheresis platelet donation per AABB reference standards.
• Treatment with aspirin or aspirin-containing medications within 7 days of apheresis or treatment with non-steroidal anti-inflammatory drugs (NSAID), anti-platelet agents (or other drugs affecting platelet viability within 3 days of apheresis (e.g., ibuprofen or other NSAIDs).
• Subject received platelet inhibitors within 14 days of donation (e.g., clopidogrel, ticlopidine, amphetamines (e.g., Adderall, Dexedrine)).
• Subjects with positive cocaine and/or amphetamine results from urine drug screen.
• Splenectomized subjects.
• History of known hypersensitivity to indium or chromium.
• Has received an investigational drug within the past 28 days.</td></tr>
<tr><td colspan="4">Test product and mode of administration:
Apheresis platelet components in 100% plasma collected using the Trima separator, prepared with the INTERCEPT Blood System for Platelets (Test Platelets) and stored for 7days at 20-24°C with continuous agitation. Samples from the Test component will be processed with either the BEST or the Variant 1 procedure prior to radiolabeling. The radiolabeled autologous Test and Control platelets (approximately 10-30 mL) will be simultaneously administered intravenously into the subject.</td></tr>
<tr><td colspan="4">Duration of treatment:
Within 15 days prior to participating in any study stage, subjects will be screened for eligibility. For each enrolled subject, study participation will include 17-19 days following each apheresis platelet procedure depending on day of the last blood sample, with an initial screening window of up to 15 days, or approximately 77-81 days (including wash out period) for a Stage 1 or approximately 32-34 days for Stage 2.</td></tr>
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Reference therapy and mode of administration:

Autologous fresh, whole derived platelets isolated from platelet rich plasma (Control platelets) processed and radiolabeled using the BEST protocol will be administered simultaneously with the radiolabeled Test platelets into the subject.

Criteria for evaluation:

Efficacy:

Primary endpoints

The primary efficacy endpoints for Stage 1 and Stage 2:

- Post-infusion recovery at end of storage
- Post-infusion survival at end of storage

Using the FDA standard on platelet viability by radiolabeling (i.e., recovery and survival compared to a "fresh" autologous platelet sample as control, drawn and prepared on day of reinfusion of radiolabeled test samples), the acceptance criteria are:

- Lower bound of a two-sided 95% confidence interval (CI) for the mean treatment difference (Test-0.66×Control) in post-infusion recovery is greater than or equal to zero.
- Lower bound of a two-sided 95% CI for the mean treatment difference (Test-0.58×Control) in survival is greater than or equal to zero.

For Stage 1, the above criteria for recovery and survival of INTERCEPT components will be evaluated separately for INTERCEPT platelets (Test) labeled with the BEST and Variant 1 procedures.

Secondary endpoints (product parameter evaluation of platelet components)

For each stage, the secondary efficacy endpoints will be summarized descriptively and are based on product parameters post-INTERCEPT treatment and at end of storage.

Product Parameters at the end of INTERCEPT Treatment

- Platelet count, volume, and dose ($\geq 3.0\times10^{11}$)
- Platelet yield retention ($\geq$80%)

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Product Parameters at the end of storage

- pH $_{22°C}$ (≥6.2)

Additional endpoints

Additional analyses will include the *in vitro* function parameters of the stored INTERCEPT platelet component (before radiolabeling preparation process) and the platelet samples (after radiolabeling preparation process) prior to radiolabeling.

Assessments of the stored INTERCEPT platelet components (end of Day 7)

Product Parameters	**Biochemical**	**Functional**
• component volume	• glucose & normalized consumption per platelet count	• hypotonic shock response (HSR)
• platelet count	• Lactate & normalized production per platelet count	• extent of shape change (ESC)
• platelet dose	• pO_2 & normalized consumption per platelet count	• CD62P (p-selectin) expression
	• pCO_2 & normalized production per platelet count	• morphology score
	• bicarbonate	• mean platelet volume
	• lactate dehydrogenase normalized per platelet count	
	• ATP & normalized per platelet count	
	• Lysis and % of baseline adjusted lysis	

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In Vitro Evaluation of Processed Platelet Samples Prior to Radiolabeling

For Stage 1, the *in vitro* platelet processing recovery (≥ 80%), pH $_{22°C}$ (≥ 6.2), volume, platelet count and CD62P, as well as RBC and WBC contamination, will be summarized descriptively for Test samples processed using both the BEST and Variant 1 procedures.

During Stage 2 the *in vitro* platelet recovery (≥ 80%), pH $_{22°C}$ (≥ 6.2), volume and platelet count will be summarized descriptively for Test samples processed using the Variant 1 procedure.

Safety

Subjects' vital signs, hematology and chemistry evaluations, and treatment-emergent adverse events (AE) will be collected from the initiation of apheresis collection through 24 hours after the last DPI blood sample is drawn.

Analysis sets:

Safety Analysis Set:

All randomized subjects who successfully initiate an apheresis collection.

Evaluable Analysis Set (EAS):

All randomized and infused subjects who have both paired (Test and Control) recovery and paired survival data for the relevant study stage and/or radiolabeling method, and otherwise complied with the protocol without any other major protocol deviation.

Summaries for the efficacy endpoints will be presented using EAS. All other summaries will be presented using the safety analysis set unless stated otherwise.

Statistical methods:

Data will be summarized descriptively by mean, standard deviation, median, and range (minimum, maximum) for continuous parameters, and by frequencies and percentages for categorical data variables. Summaries will be presented by radiolabeling method across all study sites and within each study site.

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Primary endpoints

Stage 1's recovery and survival data with 7-day platelet storage will be assessed separately by the pre-radiolabeling platelet sample preparation (BEST or Variant 1) and compared with its paired control descriptively.

For Stage 2, the following acceptance criteria for recovery and survival will be used to demonstrate non-inferiority of Test (stored INTERCEPT treated) platelets against Control (fresh) platelets:

(1) For post-infusion recovery:

H_0: Test - 0.66×Control <0 vs. H_1: Test - 0.66×Control ≥0

The null hypothesis will be rejected if the lower bound of a two-sided 95% CI for the mean treatment difference (Test - 0.66×Control) is greater than or equal to zero.

(2) For survival:

H_0: Test - 0.58×Control <0 vs. H_1: Test - 0.58×Control ≥0

The null hypothesis will be rejected if the lower bound a two-sided 95% CI for the mean treatment difference (Test - 0.58×Control) is greater than or equal to zero.

The primary analysis population for the primary efficacy endpoints will only include subjects who have both paired (Test and Control) recovery and paired survival data for the relevant study stage and/or radiolabeling method. The two-sided CIs will be computed using the variance estimated from a paired t test.

In the event that non-inferiority cannot be declared for the primary endpoints for Stage 2 with a minimum of 24 evaluable subjects, additional exploratory analyses may be conducted to quantify the proportions of subjects whose recovery and survival data meet the 0.66 and 0.58 cutoff, respectively (e.g., platelet recovery from a Test platelet component greater than or equal to 66% of platelet recovery from the paired fresh control platelets).

Secondary endpoints

Data will be summarized descriptively for each stage. The proportions of Test platelet components with platelet dose ≥3.0×10^{11} (end of INTERCEPT treatment), physical platelet recovery ≥80% (end of INTERCEPT treatment), and $pH_{22°C}$ ≥6.2 (end of storage) will be summarized, with the corresponding lower bound of a one-sided 95% CI for the proportion provided.

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<tr><td colspan="4">Correlations between the primary and secondary efficacy endpoints may also be explored using graphs and/or regression analysis.
Additional endpoints
Evaluation of the BEST (Stage 1) and Variant 1 (Stages 1 and 2) procedures for processing Test platelet samples prior to radiolabeling will also include the following assessment criteria:
• In vitro physical platelet recovery ≥ 80%
• pH $_{22°C}$ ≥ 6.2
The proportions of processed samples meeting each of the above assessment criteria will be summarized separately for the BEST and Variant 1 procedures as applicable.
Additionally, RBC and WBC contamination in Test samples prior to radiolabeling (Stage 1), the proportion of radioactivity (Test and Control) associated with the Ficoll RBC fraction gradient and treatment-emergent AEs (defined as AEs with onset after the initiation of apheresis collection) will be summarized descriptively.</td></tr>
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2.0 LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AABB	Formerly known as the American Association of Blood Banks
ACD-A	Anticoagulant Citrate Dextrose Solution-Formula A, USP (2.13% free citrate ion)
ADE	Adverse Device Effect. There is a causal relationship between the device and an adverse event.
AE	Adverse Event (defined in **Section 9.3.1**)
ALI	Acute Lung Injury
ARDS	Acute Respiratory Distress Syndrome
ATP	Adenosine 5'-Triphosphate
AUC	Area Under the Curve
BEST	Biomedical Excellence for Safer Transfusion
BMI	Body Mass Index
CAD	Compound Adsorption Device
CBC	Complete Blood Count
CCI	Corrected Count Increment
CD62	P-selectin expression
^{51}Chromium	An isotope of chromium with a radioactive half-life of 27.7 days
CI	Count Increment
CRF	Case Report Form
Control	Fresh autologous platelets
D	Days
Device Malfunction	The failure of a medical device to function according to its requirements.
DPI	Days post infusion of autologous radiolabeled platelets
DS	Dual Storage (two storage containers)
DH	Dartmouth-Hitchcock Medical Center
EDTA	Ethylenediaminetetraacetic acid

ESC	Extent of Shape Change
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
H	Hour
HBC	Hoxworth Blood Center
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HPLC	High-Performance Liquid Chromatography
HSR	Hypotonic Shock Response
HTLV	Human T-Lymphotropic Virus
ICF	Informed Consent Form
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
^{111}Indium	An isotope of indium with a radioactive half-life of 2.80 days
INTERCEPT Platelet or Platelet Component	Apheresis platelets suspended in in 100% plasma and treated with the INTERCEPT Blood System for Platelets
INTERCEPT Blood System® for Platelets	A medical device that inactivates infectious pathogens (viruses, bacteria, and protozoa) and leukocytes using amotosalen and UVA light in platelet components.
IRB	Institutional Review Board
LDH	Lactate dehydrogenase
Min	Minute
MPV	Mean Platelet Volume
mrem	Millirem
mSv	Millisievert
NSAID	Non-steroidal anti-inflammatory drug

PAS	Platelet additive solution
PC	Platelet Component
PHI	Protected Health Information
PRP	Platelet-Rich Plasma
QA	Quality Assurance
RBC	Red Blood Cell
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
Sponsor	Cerus Corporation
SV	Small volume
TACO	Transfusion-Associated Circulatory Overload
TAD	Transfusion-Associated Dyspnea
TA-GVHD	Transfusion-Associated Graft Versus Host Disease
Test	Apheresis platelets suspended in in 100% plasma and treated with the INTERCEPT Blood System for Platelets
TRALI	Transfusion-Related Acute Lung Injury
TTI	Transfusion-Transmitted Infection
UADE	Unanticipated Adverse Device Effect
UVA	Ultraviolet A light
WB	Whole blood
WBC	White Blood Cell
WNV	West Nile Virus

3.0 BACKGROUND INFORMATION

The INTERCEPT Blood System for Platelets is intended to be used for ex vivo preparation of pathogen-reduced apheresis platelet components (PCs) in order to reduce the risk of transfusion-transmitted infection (TTI), including sepsis, and as an alternate to gamma irradiation for prevention of transfusion-associated graft-versus-host disease (TA-GVHD).

The INTERCEPT Blood System for Platelets is a Class III medical device consisting of plastic disposable sets and an illumination device (INTERCEPT Illuminator), which provides a controlled dose of ultraviolet A (UVA) light to the PC. The final product is either transfused or stored according to blood center standard operating procedures (SOP), US Food and Drug Administration (FDA), and American Association of Blood Banks (AABB) Guidelines.

The INTERCEPT Blood System for Platelets was approved by the FDA in December 2014 for treatment of platelets collected in a commercially available platelet additive solution. In March 2016, the FDA approved the use of the INTERCEPT System for treatment of platelets suspended in 100% plasma. As part of the approval, Cerus committed to a post-approval study to evaluate the recovery and survival of apheresis platelets suspended in 100% plasma and treated with the INTERCEPT Blood System for Platelets. Cerus also received approval for INTERCEPT Processing Sets manufactured with alternate plastics and design changes and the INT100G2 Illuminator in May 2018.

The current maximal expiration dating period for INTERCEPT platelets in the U.S. is 5 days. Prior limitations to storing platelets beyond 5 days have been due to increased risk of bacterial proliferation associated with initial contamination of the platelet components and subsequent increased risk of transfusion related sepsis (Kleinman, et al. 2013). These limitations may be resolved by the INTERCEPT pathogen reduction technology to reduce the risk of transfusion related sepsis. Extension of apheresis platelet component expiration dating to 6 or 7 days after collection would increase the availability and reduce the wastage of PCs. The feasibility for extension of storage of INTERCEPT platelets for up to 7 days without increasing the risk of bacterial contamination and transfusion-related sepsis is supported by studies demonstrating effective inactivation of bacteria generally >4 $\log_{10}$ with negative cultures at outdate (Schmidt 2015, Wagner 2016), *in vitro* platelet function studies (CLI 00123 and CLI 00124) and an *in vivo* recovery and survival pilot study (CLI 00099) of apheresis-derived PCs suspended in either 100% plasma or platelet additive solution (35% plasma/ 65% PAS-3). The therapeutic efficacy of INTERCEPT PCs stored for 7 days is supported by a Phase 3 study (Lozano, et al. 2011) and post-marketing experience obtained in several European countries where the INTERCEPT Blood System for Platelets is licensed for 7 days of storage. Four years of safety data from Switzerland with INTERCEPT platelets stored up to 7 days have demonstrated no cases of definite transfusion related sepsis (Amsler and Jutzi, Swissmedic Haemovigilance Annual Report 2014). Routine experience in Innsbruck, Austria showed no

increase in platelet usage when implementing INTERCEPT platelets stored for up to 7 days, and no effect in clinical outcomes in massively transfused patients (Amato 2016, Nussbaumer 2017). Cumulatively, this body of evidence warrants the in vivo characterization of recovery and survival of INTERCEPT PCs stored for up to 7 days.

This study will compare the *in vivo* post-infusion radiolabeled recovery and survival of INTERCEPT platelets (Test) suspended in 100% plasma and stored for up to 7 days to fresh autologous platelets (Control) prepared from platelet rich plasma (PRP) isolated from whole blood. The results of this study are intended to support an extended shelf-life claim beyond 5 days of storage for INTERCEPT platelets suspended in either 100% plasma or 35% plasma/ 65% PAS-3 using the currently approved INTERCEPT platelet processing sets (Small Volume, SV, Large Volume, LV or Dual Storage, DS).

Although radiolabeling studies have been performed with stored apheresis platelets in additive solutions (Vassallo 2010 and Dumont 2013) and apheresis platelets in 100% plasma (Dumont 2002 and Vassallo 2004), quality control of the platelet samples processed for radiolabeling has not been performed. Cerus has evaluated variations of the Biomedical Excellence for Safer Transfusion Collaboration (BEST) platelet handling methods prior to radiolabeling to optimize platelet recovery and improve platelet quality (VAL 00376 and REL 00556). These studies have shown that the platelet preparation procedure described in the BEST version 4.2.1 protocol may not be optimal for preparing stored apheresis platelets for radiolabeling (Steps 4.2 and 5.2 of the BEST Platelet Radiolabeling Procedure Version 4.2.1, **Appendix B**). The first two steps in preparing stored platelet samples for radiolabeling involve anticoagulation and acidification of the sample, using ACD-A, and a soft spin to separate and remove contaminating RBCs and WBCs (**Table 1**). These steps are not necessary for stored apheresis platelets since the platelet components are collected in ACD-A and have low RBC and WBC contamination due to the apheresis collection procedures. Furthermore, the first two steps of the platelet preparation steps have been shown to decrease physical platelet recovery and negatively impact platelet quality (described further in **Section 3.2.1.3**). The Variant 1 of BEST method (referred to as Variant 1, **Appendix C**) does not include the first two steps of the platelet preparation steps (**Table 1**).

Table 1 Differences between the BEST 4.2.1 Radiolabeling Method and Variant 1 for Preparation of Stored Apheresis Platelet Sample[$]

	BEST 4.2.1 Method	Variant 1 Method*
Anticoagulant added to the platelet sample	ACD-A (15%, pH 4.5 - 5.5)	None
Soft Spin	200g, 5 minutes, harvest Platelet Rich Plasma	Not done
Hard Spin	Platelet Rich Plasma (PRP) 1500-2000g, 15 minutes	Platelet Sample 1500-2000g, 15 minutes
Resuspension of platelet pellet	ACD-A/Saline (pH 6.5 - 6.8)	ACD-A/Saline (pH 6.5 - 6.8)

*As described by Snyder, et al 1986.
[$] Control autologous whole blood platelets are prepared using the BEST 4.2.1 method in both arms of both study Stages.

An additional objective of this study is to compare the recovery and survival results for INTERCEPT platelets (Test) prepared for radiolabeling using the procedures outlined by BEST or Variant 1. This comparison will test the hypothesis that optimizing platelet recovery and retaining platelet *in vitro* quality while preparing samples from platelet components for radiolabeling will influence the outcome of post-infusion recovery.

3.1 Name and Description of the Investigational Product

The INTERCEPT Blood System for Platelets is a medical device (Table 2, Figure 1) consisting of a single-use processing set (INTERCEPT Processing Set for platelets) and an illumination device (INTERCEPT Illuminator), which provides a controlled dose of ultraviolet A (UVA) light to the platelet component(s). The apheresis-derived platelet component (PC), prepared with the INTERCEPT Blood System for Platelets, is an investigational biological product in this study (INTERCEPT platelet component). The radiolabeled Control platelets are also considered investigational.

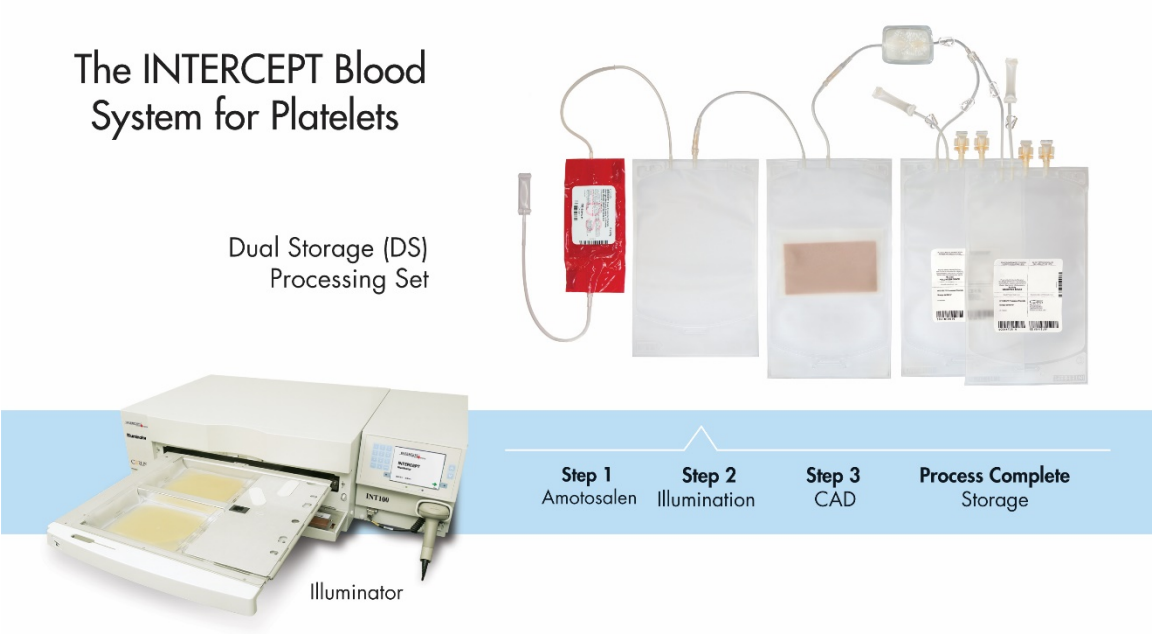
Table 2 Components of the INTERCEPT Platelet Processing Sets

Component	Description
Amotosalen (S-59, psoralen derivative) solution container	**Platelet SV Processing Set (INT2130B):** 15 mL, 3 mM amotosalen in 0.924% NaCl packaged in a 20 mL, flexible, heat-sealed plastic container, with light protective sleeve **Platelet LV and DS Processing Sets (INT2230B and INT 2530B):** 17.5 mL, 3 mM amotosalen in 0.924% NaCl packaged in a 20 mL, flexible, heat-sealed plastic container, with light protective sleeve
Illumination container	Heat-sealed, plastic bag
Compound Adsorption Device (CAD)	Immobilized beads (wafer) in mesh pouch
CAD container	Platelet SV and LV Processing Sets (INT2130B and INT2230B): 1000 mL capacity Platelet DS Processing Set (INT 2530B): 1300 mL capacity
Platelet storage containers	Platelet SV and LV Processing Sets (INT2130B and INT2230B): One platelet storage bag – 1300 mL storage capacity **Platelet DS Processing Set (INT 2530B):** Two platelet storage bags – 1300 mL storage capacity each

The Dual Storage processing set (INT25) that will be used in this study is currently approved and consists of the following sequentially integrated components: 17.5 mL of 3 mM amotosalen solution, a 1 liter Renolit Transfufol 8300 illumination container, a 1.3 liter Renolit Transfufol 8300 container with compound adsorption device (CAD, Renolit Transfufol 8300 binder material), an in-line filter, and two 1.3 liter Transfufol 8300 storage containers.

The INTERCEPT Illuminator is capable of illuminating two platelet illumination containers per processing period. Each PC rests on a UVA-transparent tray and undergoes reciprocal shaking during illumination. The INTERCEPT Illuminator delivers a target dose of 3.9 Joules/cm^2 (J/cm^2) UVA treatment to each PC through a pair of opposing banks of fluorescent lamps. The INT100 G2 INTERCEPT Illuminator will be used for this study.

Figure 1 INTERCEPT Blood System for Platelets



3.2 Summary of Nonclinical and Clinical Studies

The nonclinical and clinical studies are summarized in the Package Insert (SPC 00701-AW v3.0 INTERCEPT Blood System for Platelets – Dual Storage (DS) Processing Set). The following paragraphs provide additional background for the proposed study.

3.2.1 In Vivo Assessments of Stored INTERCEPT Platelets

Extension of platelet storage for up to 7 days for INTERCEPT platelets has been explored in *in vitro* studies (CLI 00094, CLI 00095, CLI 00123 and CLI 00124). The results from these studies show that the *in vitro* quality of INTERCEPT platelets stored in 65% PAS-3/35% plasma or 100% plasma is adequate when compared to untreated platelets and similar to the results published for conventional Amicus platelets stored in InterSol (Vassallo 2010) and Trima platelets in 100% plasma stored for 5 days (Dumont 2013).

In addition, Cerus has conducted an *in vivo* pilot recovery and survival study (CLI 00099) for INTERCEPT PCs suspended in platelet additive solution (35% plasma/65% PAS-3) and 100% plasma stored for 7 days and a study of recovery and survival of INTERCEPT platelets in 35% plasma/65% PAS-3 stored for either 6 or 7 days (CLI 00116).

3.2.1.1 Recovery and Lifespan of Radiolabeled Autologous INTERCEPT 7-day Stored Apheresis Platelet Components (CLI 00099)

A pilot study (CLI 00099) assessed the post-infusion recovery and lifespan (also referred to as survival) of 7-day INTERCEPT PCs stored in either 35% plasma/65% PAS-3 or 100% plasma, as compared to "fresh" radiolabeled platelets. Preliminary results provided evidence of the feasibility for INTERCEPT platelets to be stored for up to 7 days, while maintaining adequate transfusion viability, regardless of the suspension media.

Mean radiolabel 24-h recovery ratios (Test/Control ["fresh" autologous platelets]) of INTERCEPT apheresis-derived PCs suspended in 35% plasma and 65% PAS-3 and stored for 7 days was 65.3% and for INTERCEPT platelets in 100% plasma was 70.9%. Mean lifespan ratio (Test/Control) was 57.5% for INTERCEPT PCs suspended in 35% plasma and 65% PAS-3 and for INTERCEPT PCs in 100% plasma was 61.0% (**Table 3**). Although, the small sample sizes and wide standard deviations limit the conclusions that can be derived from this pilot study, the results suggest 7-day storage of INTERCEPT platelets is feasible.

Table 3 Recovery and Lifespan of Radiolabeled Autologous INTERCEPT Platelets Stored for 7-days Compared to Fresh Controls (Study CLI 00099)

	Apheresis PCs in 35% Plasma and 65% PAS-3*		Apheresis PCs in 100% Plasma*	
	Mean (SD) 24-h Recovery (%)	Mean (SD) Lifespan (h)	Mean (SD) 24-h Recovery (%)	Mean (SD) Lifespan (h)
Control (fresh, n = 6)	53.4 (8.9)	214.2 (32.3)	60.5 (16.7)	198.6 (25.3)
Test (IBS, n = 6)	35.0 (8.3)	119.6 (27.1)	40.6 (11.6)	120.6 (30.9)
Ratio T:C (%)	65.3 (8.8)	57.5 (16.3)	70.9 (26.2)	61.0 (15.7)

* ACD-A/Saline was used in the first step of the BEST sample preparation steps (Steps 4.2 and 5.2, **Appendix B**)

3.2.1.2 Recovery and Lifespan of Radiolabeled Autologous INTERCEPT 6 and 7-day Stored Apheresis Platelet Components (CLI 00116)

CLI 00116, a randomized, cross-over analysis of platelet recovery and survival after autologous transfusion of 6-day and 7-day stored INTERCEPT treated Amicus platelets in PAS-3 (InterSol), was conducted at both HBC and Dartmouth Hitchcock (DH). While conducting CLI 00099 and CLI 00116 studies, modifications to BEST version 4.2.1 were implemented at HBC to improve platelet yield in response to low recovery after processing platelet samples for radiolabeling; whereas, BEST version 4.2.1 was followed at DH for CLI 00116. The modifications implemented at HBC involved combining the pellets from both the soft and hard spins due to observations of platelet loss during the soft spin of the platelet sample in ACD-A.

In study CLI 00116, the DH Investigator noted that 4 of 15 recovery assessments for Control (fresh) WB platelets demonstrated recoveries of >100% (101.5; 106.8%; 113.1% and 193.5%); the results were highly variable and not aligned with historical data (Dumont 2013; Vassallo 2010) (communication from Dr. Dumont dated 4/11/2017). In addition, initial data from HBC in CLI 00116 was reported by the Investigator to show diminished performance with respect to the in vivo recovery/survival outcomes compared to CLI 00099.

At DH it was noted also that radioactivity of the standards was not sufficiently high above background radioactivity to provide reliable measurement of specific activity. This problem may have been responsible for the unusual results showing radioactivity recoveries of > 100% in 4 subjects. A modification in the preparation of the standard samples for gamma counting was instituted in CLI 00116 by using decreased dilution before counting. This resulted in a 50 to 100-fold increase in the corrected radioactivity counts for the standards above background and provided good precision. These DH studies demonstrated the need to implement controls to ensure adequate signal-to-noise ratios when measuring the radioactivity associated with the standards as described in this protocol.

CLI 00116 was terminated for futility caused by poor platelet recovery yields after radiolabeling.

3.2.1.3 Method optimization of preparation of stored platelets for radiolabeling

A small investigational study was performed to assess the radiolabeling methodologies used at HBC during CLI 00116. The results of this study indicated that the addition of acidic (pH 4.5 - 5.5), undiluted ACD-A to stored apheresis platelets before the first soft spin per the BEST protocol (designed to remove residual RBC and WBC from PRP derived from fresh blood) resulted in poor platelet recovery yield and inferior *in vitro* platelet quality of the aliquots compared with platelets resuspended in diluted, pH adjusted (pH 6.5-6.8) ACD-A/saline. Together with the recovery and survival results from CLI 00099, these data indicated that injury may be induced by the addition of a high [H+]-containing solution (ACD-A) when added to stored apheresis PCs.

Based on the investigational study results, a prospective *in vitro* study (VAL 00376) was designed and completed to assess two alternative platelet preparation methods based on the BEST version 4.2.1 protocol for preparation of stored platelet samples for radiolabeling using both untreated and INTERCEPT platelets in 100% plasma, collected on the Trima platform and stored for 5 days. The results of this study showed that platelet aliquots processed with variations of the BEST protocol for preparing platelet samples prior to radiolabeling [Variant 1 (hard spin only) or Variant 2 (addition of buffered ACD-A/saline instead of ACD-A and recombining slow and fast spin pellets)] led to improved *in vitro* platelet pH levels, yield recovery and quality, when compared to the samples processed with the original

BEST version 4.2.1 method. Platelet aliquots prepared with either Variant 1 or Variant 2 modifications exhibited similar *in vitro* characteristics, but slightly differed with respect to platelet yield recovery (proportion), platelet lysis, platelet activation and metabolism. Cumulatively, the study results supported the use of either variant method as an improved platelet sample preparation method for stored apheresis platelets compared to the original BEST version 4.2.1 method. Variant 1 was selected for further study as the use of a soft spin and recombining of pellets in Variant 2 was considered redundant.

Following the completion of VAL 00376, and based on FDA concerns that the Variant 1 procedure would result in higher RBC and WBC contamination and therefore might provide misleading results for platelet recovery and survival, Cerus conducted another study to characterize the RBC and WBC contamination level of samples prepared using either the BEST or Variant 1 methods (REL 00556).

Data from REL 00556, provided in **Table 4**, confirm that when the BEST protocol is performed on INTERCEPT platelets, stored for 7 days, the *in vitro* recovery of platelets is reduced by ~29.4% (BEST 2391 ±381: Variant 1 3389 ±378), raising the possibility that radiolabeling would be biased by the labeling of a selected subset of platelets when using the BEST protocol. Although the manual and flow cytometry-based RBC counts showed that apheresis platelet samples prepared with the Variant 1 procedure have higher RBC and WBC contamination than the BEST protocol, the levels are well below levels considered to be material to the outcome of radiolabeling studies. Both the BEST and Variant 1 protocols resulted in < 1:10,000 contamination of the platelet preparations with either RBC or WBC by cell count and <1:1,000 by cell volume (assuming that the Mean Platelet Volume was 10% of the volume of an RBC or lymphocyte).

Table 4 Comparison of Platelet, RBC and WBC Count in Platelet Samples Prepared for Radiolabeling Using the BEST (WB Derived Fresh Platelets and INTERCEPT Stored Platelets) and Variant 1 Method (INTERCEPT Stored Platelets) (Study REL 00556)

		Fresh Whole Blood (N = 12)		INTERCEPT Apheresis Unit (Day 7) (N = 12)			t-Test (D7 BEST vs D7 Variant 1)
Assay	**Method**	**Input Units**	**BEST**	**Input Units**	**BEST**	**Variant 1**	
Automated Platelet Count, $10^3/\mu L$	Advia 120	214 ±53 (166 - 338)	1376 ±597 (320-2537)	1111 ±148 (906-1481)	2422 ±372 (1961-3338)	3446 ±419 (3027-4431)	<0.001
Automated RBC Count, $10^3/\mu L$	Advia 120	3904 ±286 (3545 - 4320)	169 ±85 (45-315)	100 ±23 (70-150)	165 ±40 (110-255)	248 ±44 (195-350)	<0.001
Manual RBC Count, $10^3/\mu L$	Hemo-cytometer	ND	1.16 ±0.69 (0.23-2.79)	0.05 ±0.08 (0.01-0.33)	0.02 ±0.03 (0.00 - 0.10)	0.09 ±0.14 (0.01 - 0.54)	0.07
Flow Cytometry based RBC count, $10^3/\mu L$	CD235a Antibody	3926 ±463 (3271 - 4816)	0.19 ±0.22 (0.01 - 0.84)	0.01 ±0.02 (0.00 - 0.07)	0.02 ±0.03 (0.00 - 0.07)	0.11 ±0.10 (0.01 - 0.27)	0.003
Flow Cytometry based White Cell Count WBC, per µL	Leucocount	6603±1220 (4953 - 8468)	37±36 (4-135)	0.1 ± 0.1 (0.0 - 0.2)	0.1 ± 0.1 (0.0 - 0.3)	0.2 ± 0.3 (0.0 – 1.0)	0.15

The labeling efficiency using Chromium-51 has been reported to be 10-fold lower for platelets compared to RBCs, 9% versus 90% (Baldini 1960), whereas Indium-111 will preferentially label platelets compared to RBCs or WBCs (Mountford 1985). Even with the differences in labeling efficiency for Chromium-51 (which would represent half of the samples collected for INTERCEPT stored platelets, as the radiolabeling is randomized between Test and Control for Chromium-51 and Indium-111). Cerus data for RBC contamination levels suggests that the contribution of the RBC levels between stored apheresis platelets processed with the BEST or Variant 1 protocols represents a scientifically insignificant difference (<1% of Chromium-51 labeling).

Furthermore, the BEST protocol includes one final sample on Day 10, 11, or 12. The last sample is called the "baseline" sample and is used to correct for inadvertent labeling of other blood components (BEST 2005). The rationale for this sample is that contaminating RBCs have a longer in vivo lifespan than platelets and that any radioactivity remaining in the circulation after Day 10 must be from contaminating RBCs in the sample preparation or from in vivo elution for the radiolabel from platelets and labelling of other blood components.

In summary, RBC contamination for stored, INTERCEPT platelets represents approximately 0.0008% or 0.0035% of the cellular content in the sample, using the BEST or Variant 1 procedures, respectively and therefore is not scientifically relevant as a source of contamination. In comparison, the RBC contamination of the fresh WB derived platelets is approximately 0.0085% of the cellular content and the WBC contamination is 0.0035% of the cellular content for WB derived platelets (versus 0.000000044% for stored, INTERCEPT apheresis platelets).

CLI 00127 will evaluate both Variant 1 and the BEST procedure, which is the current standard for preparing stored platelet samples for recovery and survival studies. RBC contamination will also be assessed using a Ficoll gradient technique as a secondary confirmation of RBC contamination levels.

3.3 Summary of Potential Risks and Benefits

3.3.1 Benefits of Study Participation

There are no direct benefits to the subjects in this study.

3.3.2 Risks of Study Participation

A summary of the safety experience with INTERCEPT platelets is provided in the Package Insert (SPC 00701-AW v3.0 INTERCEPT Blood System for Platelets – Dual Storage (DS) Processing Set). The cumulative experience suggests no excess treatment-related morbidity associated with the transfusion of INTERCEPT PCs compared to conventional PCs. With the limited exposure to autologous platelets in the study proposed herein (approximately 10 to 30 mL infusion), systemic risks are considered unlikely.

3.3.2.1 Risk of Receiving an Incorrectly Labeled Platelet Component

PCs will be prepared, labeled, and tracked using site SOPs to ensure that appropriate identification of study components is maintained at all times. Verification of each subject's platelet component will occur prior to infusion as an additional check on component identity.

3.3.2.2 Risk of Transfusion-Transmitted Infectious Disease

The risk of transfusion-transmitted infections (TTI) is considered minimal for study subjects who will receive approximately 10 to 30 mL of washed radiolabeled autologous Test and Control platelets. All standard viral pathogen testing performed routinely by the Blood Centers will be performed. Since subjects will be receiving autologous platelets that have been screened for TTIs, the risk of infection transmission is minimal. All Test PCs will be treated with the INTERCEPT system which has demonstrated high levels of bacterial inactivation efficacy through 5 days and 7 days of storage (Wagner, et al. 2015) and four years safe experience with 7 day stored INTERCEPT PC in Switzerland. Bacterial contamination is possible during radiolabeling due to required use of an open system. However, only a small aliquot of labeled platelets will be re-infused shortly after labeling, thus the risk of bacterial proliferation due to contamination during the radiolabeling procedure is minimized. Based on laboratory studies demonstrating inactivation of viruses and bacteria, the infectious disease risk to recipients of Test platelets is reduced compared with that of conventional platelet components.

3.3.2.3 Risk of Transfusion Reaction

Transfusion reactions known to occur following transfusion of conventional platelets may occur following infusion of study platelets (e.g., fever, chills, itching, rash, hives, bronchospasm, nausea/vomiting, hypertension/hypotension, TRALI, TACO, and anaphylaxis). The risk of experiencing a transfusion reaction is considered minimal from the small autologous infusions used in this study (approximately 10-30 mL).

3.3.2.4 Risk of Apheresis Collection

Risks associated with the apheresis collection of platelets include: blood pressure changes, a temporary decrease in platelets, a small loss of white blood cells, problems with vein access (including pain, bleeding, and infection at the catheter site), effects of the anticoagulant on the donor's calcium level (including paresthesias from citrate-induced hypocalcemia), and allergic reactions to the anticoagulant or other products used, which may be life-threatening. On very rare occasions, risks associated with the apheresis collection include nerve damage, serious problems and death. Allergic reaction due to iodine allergy is possible if an iodine solution is used to cleanse the phlebotomy site to meet standards in 21 CFR 640.4(f). Blood pressure changes can sometimes cause nausea, fatigue, and dizziness. Platelet loss may increase the risk of bleeding from cuts or wounds. Venous access problems can cause bruising referred to as a hematoma. While donating, a supply of calcium antacid tablets is usually kept close by to replenish the calcium lost. Because the anticoagulant works by binding to the calcium in the blood, a donor's levels of calcium - and especially of active calcium ions - decrease during the donation process. The lips may begin to tingle or there may be a metallic taste; since calcium

enables the function of the nervous system, nerve-ending-dense areas (such as the lips) are susceptible, at least during the donation process. Unusually low calcium can cause more serious problems such as fainting, nerve irritation and short-duration tetany. Such an acute hypocalcemia is usually due to low calcium levels prior to donation, aggravated by the anticoagulant. Hypocalcemia can be curtailed by modestly increasing dietary calcium intake in the days prior to donation. Nerve damage which is more rarely observed may result in numbness, pain or paralysis. Serious problems are extremely rare and may include heart attack and stroke. The apheresis procedure can also result in a need for extended medical treatment and can be fatal. Apheresis donors are routinely monitored during the long donation process.

3.3.2.5 Risk Related to Whole Blood Collection

Use of experienced phlebotomists will reduce the likelihood of these types of injuries. Blood collection may be associated with hematoma formation, local irritation, or infection. Local care can be given to prevent hematoma formation, according to standard medical practice.

3.3.2.6 Risk Related to Infusion Procedure

The infusion procedure may be associated with hematoma formation, local irritation, or infection. Local care can be given to prevent hematoma formation, according to standard medical practice.

3.3.2.7 Risk Related to Radiation Exposure

^{111}Indium Oxine (^{111}In) and $Na_2{}^{51}CrO_4$ (^{51}Cr) are routinely used for radiolabeling and infusion of fresh or stored platelets in humans. The amount of radiation that the subjects will receive in each study infusion is estimated to be up to approximately 60 mrem, below the annual maximum limits typically considered to be safe.

No known risks are associated with ^{51}Cr administration at the level of chromium exposure used in this study. Allergic dermatological reactions have been reported in association with ^{111}In.

4.0 TRIAL OBJECTIVE

The principle objective of this study is to evaluate the hypothesis that INTERCEPT Platelets in 100% plasma stored for 5 or more days (up to 7 days) after apheresis collection retain sufficient viability for therapeutic transfusion efficacy. The post-infusion recovery and survival of radiolabeled 7- day INTERCEPT platelets (Test) stored in 100% plasma will be measured in comparison to their "fresh" radiolabeled platelet (Control) according to FDA guidance for platelet testing (FDA 1999) in Stage 2 of this study protocol.

A secondary objective is to compare the recovery and survival results for Test platelets prepared for radiolabeling using the BEST method or Variant 1 in Stage 1 of this study protocol. This comparison will evaluate the hypothesis that preparation methods prior to radiolabeling may influence *in vitro* quality of the radiolabeled platelets and post-infusion viability outcomes. In addition, *in vitro* function will also be evaluated for the stored Test INTERCEPT platelets.

5.0 TRIAL DESIGN

5.1 Overall Trial Design and Plan

The study population will consist of healthy subjects who meet the FDA, AABB, and site-specific research donor eligibility criteria for an apheresis platelet collection.

In each stage, apheresis platelets (single or double) will be collected in 100% plasma on the Trima Accel® Automated Blood Collection system. Each study apheresis collection will be processed using the INTERCEPT Blood System for Platelets; apheresis platelets containing a platelet dose of 3.0 to 7.9 $x10^{11}$ platelets in 300 to 420 mL of plasma will be processed using the INTERCEPT Dual Storage (DS) processing set. The INTERCEPT process will begin on either the day of collection (Day 0) or the day following collection (Day 1); illumination must occur within 24 hours after the end of collection. Test platelet components will be stored for up to 7 days, after collection, in 100% plasma. *In vitro* platelet function will be evaluated on Days 0/1 (pre-treatment) and at end of storage (Day 7) for both stages (**Table 11**).

At the end of storage, duplicate aliquots of Test platelets will be aseptically removed from each subject's INTERCEPT platelet storage container and prepared, in tandem, for radiolabeling using either BEST version 4.2.1 method (Stage 1) or Variant 1 modification (Stages 1 and 2). One of the Test platelet sample aliquots will be used to assess the *in vitro* quality prior to and following the pre-radiolabeling platelet sample preparations. The indices to be measured in Stage 1 are volume, $pH_{22°C}$, CD62P, platelet count, RBC count, and WBC count (**Table 10**). Assessments of these indices will enable the determination of platelet processing recovery due to each sample preparation method and evaluation of residual RBC and WBC contamination in samples prior to radiolabeling. In Stage 2, only platelet processing recovery (calculated from volume and platelet count) and $pH_{22°C}$ measured following the pre-radiolabeling platelet sample preparations. The second Test platelet sample aliquot will be used for radiolabeling.

The study will be performed in two stages (**Table 5**). Stage 1 is a randomized, 2-period crossover design. Test platelets stored for 7 days will be radiolabeled using either the BEST or Variant 1 methods (depending on the period and randomization scheme for the Test platelets) for 12 healthy subjects (6 subjects from each site). The recovery and survival for Test platelets prepared with the BEST and Variant 1 methods will be compared with each other and against the fresh platelet Control. Stage 2 is a single arm study in which all Test platelets will be prepared for radiolabeling using the Variant 1 methodology. The recovery and survival for Test platelets will be compared against the fresh platelet Control. Recovery and survival of INTERCEPT platelets will be assessed after 7 days of storage for 24 evaluable subjects (target 12 subjects with a minimum of 8 at a single site).

Up to a total of 36 subjects with evaluable (Test and Control) recovery and survival data may be obtained. Randomized subjects who do not have both paired (Test and Control) recovery and paired survival data for a given stage or who are unable to provide a second matched donation for a cross-over stage will be replaced, unless the enrollment goal is met.

Table 5 Description of Study Stages

		Period 1			Period 2		
		Test, INTERCEPT Platelets		Control, Fresh Platelets	Test, INTERCEPT Platelets		Control, Fresh Platelets
Stage	**Arm**	**Storage Duration (post collection)**	**Sample Prep**	**Sample Prep**	**Storage Duration (post collection)**	**Sample Prep**	**Sample Prep**
Stage 1[a]	Arm 1	7 days	Variant 1 (n=6)	BEST (n=6)	7 days	BEST (n=5)	BEST (n=5)
	Arm 2	7 days	BEST (n=7)	BEST (n=7)	7 days	Variant 1 (n=6)	BEST (n=6)
Stage 2	Single Arm (n=24)[b]	7 days	Variant 1	BEST	Not Applicable, Single Arm		

[a] Based on correspondence with FDA (BQ200481, July 8, 2020), Stage 1 was not completed. The n represents the number of subjects which have completed the period.
[b] Stage 1 subjects with evaluable Variant 1 method data will contribute to the requirement of the 24 subjects for Stage 2.

On the day corresponding to the end of storage for the Test component, healthy subjects will return to the site, and 43 (±2) mL of whole blood (WB) will be drawn into a syringe containing 9 mL of Anticoagulant Citrate Dextrose Solution, Formula A (ACD-A). The sample will be used to prepare fresh Control platelets from platelet rich plasma following the BEST methodology. In addition, a blood sample in EDTA will be drawn for use in determination of radiolabel elution.

Test and Control will be randomly radiolabeled with either ^{51}Cr as sodium radiochromate ($Na_2{}^{51}CrO_4$) or ^{111}In as indium oxine, depending upon randomization period and assignment. Subjects will be randomized with equal probability to the radiolabeling sequences ($^{111}In/^{51}Cr$ vs. $^{51}Cr/^{111}In$) for Test/Control. After radiolabeling, the autologous Control and Test platelet samples will be simultaneously infused into the subject.

Blood samples will be drawn immediately before infusion and for radioactivity measurements at 1 hour ± 15 min and 2 hours ± 15 min post-infusion (Day 0), and 6 more samples will be drawn at 1, 2, 3, 4 (or 5 or 6), 7 (or 8), and 11±1 days post-infusion (DPI), at approximately the same time of day as the radiolabeled platelet infusion was administered (±4 hours).

For subjects enrolled in the Stage 1 cross over study, there will be a minimum washout period of four weeks between the two study periods (e.g., four weeks after the last blood sample at 11±1 DPI in period 1). Subjects will be monitored for safety (adverse events) from the first apheresis procedure until 24 hours after the last DPI blood sample is drawn.

Stage 1 is a randomized, 2-period crossover design. Test platelets stored for 7 days will be prepared for radiolabeling using either the BEST or Variant 1 methods (depending on the period and randomization scheme for the stored apheresis platelets) for up to 12 healthy subjects. The post-infusion recovery for Test platelets prepared with the BEST and Variant 1 methods will be compared with each other and against the fresh platelet Control. Stage 1 will have up to 12 completed pairs.

Stage 2 is a single arm study in which all Test platelets will be prepared for radiolabeling using the Variant 1 method. Recovery and survival of Test platelets will be assessed after 7 days of storage for 24 evaluable subjects; Stage 1 Variant 1 data, will be applied to Stage 2 to achieve 24 evaluable pairs based on FDA feedback **(**BQ200481). The goal of this stage is to complete the qualification of Test platelets collected on the Trima separator and stored for 7 days in 100% plasma using the Variant 1 procedure.

The duration of the study for each Stage 1 subject will be 17-19 days following apheresis per period with an initial screening window of up to 15 days, or a total duration of approximately 77 to 81 days. For subjects enrolled in Stage 2 the duration is approximately 32 to 34 days.

5.2 Randomization and Blinding

Cerus will generate the randomization scheme separately for each study stage to be executed by the blood centers. In this study, eligible subjects who are enrolled in Stage 1 will be randomized in 1:1 ratio to one of the two platelet preparation procedure sequences for Test platelets (BEST/Variant 1 or Variant 1 /BEST). Subjects will also be randomized across radiolabeling sequences (^{111}In/^{51}Cr or ^{51}Cr/^{111}In for Test/Control) for both Stage 1 and Stage 2. In addition, an equal number of subjects will be targeted at each study site for both Stage 1 (6 subjects per site) and Stage 2 (12 subjects with a minimum of 8 at a single site). Subjects will receive both Test and Control platelets at the same time. To reduce the potential confounding effect from radiolabel for the BEST vs. Variant 1 comparison in Stage 1, the same randomized radiolabeling sequence (either ^{51}Cr or ^{111}In for Test platelets) will be utilized throughout both donation periods for a given subject as applicable. Randomized subjects who do not have both paired (Test and Control) recovery and paired survival data for a given stage or who are unable to provide a second matched donation for a cross-over stage will be replaced, unless the enrollment goal is met.

In this open-label study, the sites and subjects will not be blinded to the randomization assignment.

5.3 Treatment and Dose

5.3.1 Administration of Study Product

The study platelets (approximately 10 to 30 mL of washed radiolabeled fresh [Control] and 7-day stored autologous radiolabeled platelets [Test]) are administered by intravenous infusion in a peripheral vein using a butterfly infusion needle.

5.3.2 Preparation of the Study Test Platelet Component

5.3.2.1 Apheresis Platelet Collection

Study subjects who qualify to participate in either stage of the study will provide one single or double apheresis platelet collection (Stage 2) or two single or double apheresis platelet collections (Stage 1) as specified in **Section 5.3.6.2**. All study apheresis donations will be collected in 100% plasma on the Trima Accel® automated blood collection system according to blood center SOPs and the manufacturer's instructions, with leukocyte reduction by centrifugation as part of the collection process and with anticoagulant ACD, using FDA approved collection sets (Trima product code: 80400 or 80440). A small sample may be acquired to obtain the subject's platelet count.

The subject's platelet count and body mass prior to the first donation will determine if a single or double-dose collection will be performed. For Stage 1 each subject will provide the same type of apheresis collection for both study apheresis platelet collections. If a subject is unable to provide a second evaluable donation (two attempts, at least two weeks apart, to obtain a second donation can be made if the subject meets the eligibility requirements for donation), the study subject will be replaced.

After collection, the platelet components will be handled following the manufacturer's instructions which include static rest followed by continuous agitation at 20°C to 24°C. Platelets will have an RBC content $<4x10^6$/mL and should be free of visible aggregates prior to INTERCEPT treatment. Platelet components with an RBC content $>4x10^6$/mL will be excluded from the study.

At several different steps in the preparation of the Test component, the component volume will be measured by weight. The tare weight for each of the different platelet containers (empty dry container with standardized tube length) will be used to determine the net product weight. Tare weights will be reported.

Platelet volume (mL) will be determined using the following equation:

- [Gross weight (g) of the component – tare weight of the container (g)] ÷ specific gravity of platelets]
- The specific gravity of platelets used for calculations for this protocol will be:
- 100% plasma: 1.03 g/mL Apheresis Platelets

5.3.2.2 Preparation of Test Platelets

Platelet input components will be sampled and evaluated for parameters described in **Table 11**.

Treatment with INTERCEPT (**Figure 2**) will begin within 24 hours of collection. The processing steps described in SPC 00701-AW v3.0, (INTERCEPT Blood System for Platelets-Dual Storage (DS) Processing Set) will be followed, unless superseded by instructions presented in this protocol (i.e. sampling of components and extended storage durations).

Each Test input component will contain 3.0 to 5.2×10^{11} platelets in 300-390 mL in 100% plasma (single collection) or 5.3 to 7.9×10^{11} platelets in 375-420 mL in 100% plasma (single or double collection). If the input platelet component volume is greater than 420 mL, the volume will be adjusted to 300 to 420 mL by sterile connection to an empty container and removing the excess volume, which will be discarded. The Test input platelet dose and volume should be comparable for both treatment periods of Stage 1.

Each Test input component will be connected to the tubing of the INT25 Dual Processing Set using a sterile connection device (SCD). The final storage containers of the INTERCEPT set will be labeled “Test-A” for single collections and randomly labeled “Test-A” and “Test-B” for collections with input doses $\geq5.3\times10^{11}$ platelets/unit.

Amotosalen will be added to the component by passing the entire contents of the Test input component through the amotosalen container and into the illumination container. After the component is mixed and air has been returned to the input container, the tubing connecting the amotosalen container to the illumination container will be sealed and the amotosalen container and the original platelet container will be detached.

Test in-process platelet components will then be illuminated with a target dose of 3.9 J/cm^2 UVA treatment on the INTERCEPT Illuminator. A treatment report will be printed and attached to the processing batch production record.

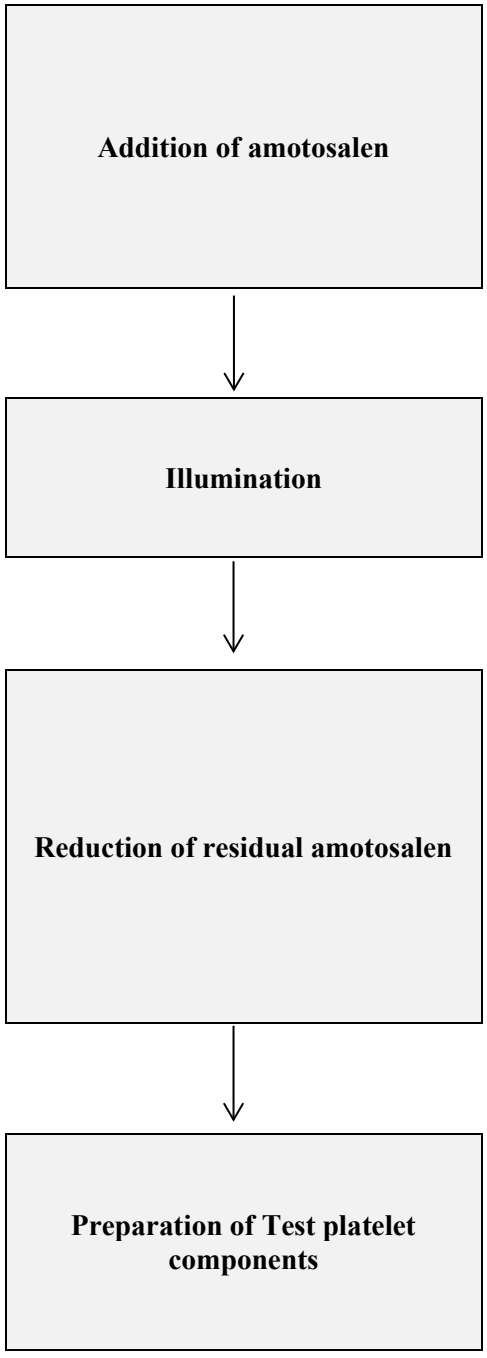
Following illumination, Test in-process platelets will be transferred to the container with the CAD, the illumination container will be detached, and the platelets will be placed in an

incubator at 20°C to 24°C on a flatbed platelet agitator with rotation speed of ≥60 rpm. The time the platelets are transferred to the container with the CAD and the time the CAD is placed into agitated storage will be recorded.

The duration of agitated storage with the CAD will be 12 to 24 hours. The time when the container with the CAD is removed from agitated storage will be recorded. For cross-over stages the CAD duration for the second period should be within 2 hours of the first period. The container with the CAD will be hung and platelets will be transferred by gravity into 1 or 2 storage containers and any air returned to the empty container with the CAD. The INTERCEPT storage containers and connected tubing will be weighed; the net weight will be used to determine the total post INTERCEPT volume. This volume will be used for determination of platelet dose and volume recoveries. For input platelet dose greater than 5.3×10^{11}, the platelet volume will be divided as evenly as possible (by weight) to make 2 equal INTERCEPT platelet (Test) components, randomly designated “Test-A” and “Test-B” that will remain connected during storage or will be stored in two separate containers for input platelet dose greater than 7.1×10^{11} according to the Instructions for Use. The empty container with the CAD will be detached and discarded using a heat seal technique to preserve sterility. The individual INTERCEPT storage containers will be weighed for calculation of post-treatment volume and the post INTERCEPT sample will be removed as described in **Section 5.3.2.1** for assessment of the indices described in **Table 11**.

All INTERCEPT platelets (Test) will be stored under standard conditions of continuous agitation using licensed platelet agitator and temperature control (20°C to 24°C) until the time of radiolabeling on Day 7 post- collection.

Figure 2 Flow Diagram of the INTERCEPT Process for Platelets



- Weld the tubing from the platelet apheresis container to the amotosalen container tubing using a sterile connection device.
- Hang the platelet apheresis container, break cannulas on both ports of the amotosalen container and allow the platelets to flow through the amotosalen container and into the illumination container.

- Illuminate the platelet/amotosalen mixture using an INTERCEPT Illuminator.

- Hang the illumination container.
- Break the cannula on the exit port of the illumination container and allow platelets to flow into the compound adsorption device (CAD) container.
- Disconnect and discard the illumination container and agitate the container with the CAD at 20°C to 24°C on a flatbed platelet agitator with rotation speed of ≥60 rpm for 12-24 hours.

- Break the cannula on the exit port of the container with the CAD and allow platelets to flow into one (5.2×10^{11} platelets) or two ($\geq5.3\times10^{11}$ platelets) storage containers.
- Seal tubing and remove treated platelet components.

5.3.2.3 Labeling

5.3.2.3.1 *Test Platelet Components*

During apheresis collection, processing, and infusion of autologous platelets, the platelet components will be properly labeled to definitively identify the subject and ensure correct infusion of autologous platelets. Minimal information to be provided on the label will include:

- IDE number (BB-IDE 6200)
- Protocol number (CLI 00127)
- Site/Subject ID
- Platelet preparation and radiolabeling method (BEST or VAR 1)
- Date of collection
- Study Stage
- End of Storage Date (based on 7-day Storage Duration)

In addition, for Test platelet components stored in two containers, the label will be identified as “Test-A” or “Test-B.”

All study platelet components will contain the label:

Caution: Investigational Product - Limited by United States Law to Investigational Use

5.3.2.3.2 *Platelet Samples*

The minimal information to be provided on the label of study samples will include:

- Protocol Number (CLI 00127)
- Site/Subject ID
- Platelet preparation and radiolabeling method (BEST or VAR 1)
- Treatment group (Test or Control)
- Timepoint (Input, Post INTERCEPT, Day 7, and DPI)

5.3.2.4 Radiolabeling of Test

On the day of scheduled radiolabeling and infusion (Day 7 post-apheresis), samples will be aseptically removed from the component for radiolabeling and *in vitro* platelet function testing. The Test platelets are prepared for radiolabeling using either the BEST version 4.2.1 method (Stage 1; **Appendix B**) or the Variant 1 method (Stage 1 and Stage 2; **Appendix C**). The Test platelet sample will be evaluated prior to and post preparation for radiolabeling (**Table 10**).

5.3.2.5 Preparation of Control Platelets – Fresh Platelets

On the scheduled day of radiolabeling/infusion approximately 43 (±2) mL of whole blood is collected from the subject in a syringe containing ACD-A to prepare the Control platelets (fresh platelets). The fresh platelets are separated from the whole blood by centrifugation to prepare platelet rich plasma. The Control platelets will be labeled with the alternate radionuclide to the one used for the paired Test platelets. The Control platelets are radiolabeled according to the BEST version 4.2.1 method (**Appendix B**).

5.3.2.6 Platelet Infusion

The study platelets (approximately 10 to 30 mL of washed radiolabeled fresh [Control] and 7-day stored, washed, radiolabeled autologous platelets [Test]) are administered by intravenous infusion in a peripheral vein using a 19-gauge butterfly infusion needle.

5.3.3 Duration of Subject Participation

Within 15 days prior to participating in either study stage, subjects will be screened for eligibility. For each enrolled subject, study participation will include 17-19 days following the apheresis platelet collection, depending on the day of the last blood sample for radioactivity. For Stage 2 the total duration will be approximately 32-34 days; for Stage 1 with two study periods (crossover), total duration will be approximately 77-81 days, including the interim wash out interval.

The study schematic design is displayed in **Table 6**.

Table 6 Study Schema

Period (order randomly assigned, stratified by site)	Screening	Apheresis Collection (TRIMA collector)	INTERCEPT Treatment (Day post collection)	Sampling, Radio-labeling, Infusion, Subject Blood Sampling (Day post collection)	Subject Blood Sampling (Day Post Infusion)					
Stage 1: 7 days storage, BEST and Variant 1										
Period x (Variant 1)	Day -15 to 0, or 4 week washout	Day 0	Day 0-1	Day 7 / DPI 0 [a]	DPI 1[b]	DPI 2	DPI 3	DPI 4 (or 5 or 6)[b]	DPI 7 (or 8)	DPI 11±1 [b]
Period y (BEST)	Day -15 to 0, or 4 week washout	Day 0	Day 0-1	Day 7 / DPI 0 [a]	DPI 1[b]	DPI 2	DPI 3	DPI 4 (or 5 or 6)[b]	DPI 7 (or 8)	DPI 11±1[b]
Stage 2: 7 days storage, Variant 1 only										
NA	Day -15 to 0	Day 0	Day 0-1	Day 7 with Variant 1 / DPI 0 [a]	DPI 1	DPI 2	DPI 3	DPI 4 (or 6)	DPI 7	DPI 11±1 [b]

[a] Samples will be acquired pre infusion and approximately 1 and 2 hours post infusion on DPI 0.
[b] A portion of the subject's radiolabeled whole blood sample will be subjected to a Ficoll gradient to allow characterization of RBC contamination.

5.3.4 Schedule of Assessments and Procedures

The study procedures and assessments are displayed in **Table 7**. The following assessments should be completed at the designated time period(s).

Table 7 Study Assessments

SCREENING AND ENROLLMENT	
Both stages: Day -15 to 0 **Subject Screening**	• Sign informed consent form • Medical history and demographics • Blood donor physical exam (including height, weight, and vital signs: temperature, sitting blood pressure and heart rate) • Concomitant medications (including drugs of abuse) and nicotine and/or cannabis inquiry • Urine drug screening test • Blood sample for hematology and chemistry panels (see list of specific parameters in Table 9) • Blood donor screening panel (including ABO, Rh type, and viral screening panel: HIV, HBV, HCV, HTLV, syphilis, and WNV) • Urine or blood pregnancy test (for females of child-bearing potential) • Review of inclusion/exclusion criteria
PLATELET APHERESIS COLLECTION AND STORAGE (Stage 1 [each period] and Stage 2)	
Both stages: Day 0	**Subject Procedures Prior to Apheresis Collection** • Weight • Vital signs • Blood sample for hematology and chemistry panels [a] (including subject platelet count) (see list of specific parameters in **Table 9**) • Concomitant medications and nicotine and cannabis review • Update baseline medical history **Apheresis collection** • Autologous single or double apheresis platelet collection and concurrent plasma using the Trima cell separator • Collected and suspended in 100% plasma • AEs and SAEs

Both stages: Day 0-1	**Platelet Component Procedures- Prior to INTERCEPT Treatment (In Vitro Platelet Assessments, see Table 11)** • Visual inspection to confirm absence of platelet aggregates • Confirm RBC content $<4\times10^6$ • Component weight pre and post-sampling (for volume calculation) **Platelet Component -Initiate INTERCEPT Treatment**
Both stages: Day 1-2	**Platelet Component Procedures - Post INTERCEPT Treatment** • Component weight • Component count
BACTERIAL TESTING	
Stage 1 (each period): Day 4, 5 or 6 **Stage 2: Day 4, 5 or 6**	A sample for bacterial screening is withdrawn 1-3 days before the scheduled radiolabeling/infusion, although bacterial testing is not routinely required for INTERCEPT PCs stored for 5 days (see Table 11).
RADIOLABELING AND INFUSION OF AUTOLOGOUS PLATELET ALIQUOTS	
Stage 1 (each period): Day 7 **Stage 2: Day 7**	**Subject Procedures On the Day of Infusion (Prior to Infusion)** • Weight • Vital Signs • Urine or blood pregnancy test for females • Blood sample for hematology and chemistry panels (see list of specific parameters in Table 9) • Collect blood sample for preparation of fresh platelets (Control) • Collect blood sample in EDTA for determination of radiolabel elution • Concomitant medications and nicotine and/or cannabis review • Collect AEs and SAEs **Test Platelet Component Procedure Prior to Radiolabeling** • Obtain aliquot for radiolabeling • Platelet component must pass all release criteria (Table 8) in order to be radiolabeled/infused **Platelet Component - In Vitro Platelet Assessments** • Obtain sample for *in vitro* assessments (Table 11) **Radiolabeling of Test Platelet Aliquots** • Prepare sample for radiolabeling following BEST or Variant 1 procedure (Stage 1) or Variant 1 (Stage 2) Assessment of platelet sample prior to preparation with BEST or Variant 1 method and immediately before to radiolabeling (Table 10) • Volume (Stages 1 and 2) • Platelet count (Stages 1 and 2)

	• RBC Count (Stage 1) • WBC count (Stage 1) • pH22°C (Stages 1 and 2) • CD62P (Stage 1) • Radiolabel Test platelets using method and labeling assignments based on randomization **Radiolabeling Control Platelet Aliquots** • Prepare fresh platelet sample (Control) • Radiolabel Control platelets using BEST method and labeling assignments based on randomization **Radioactive Standard** • Reserve samples of radiolabeled combined injectate before infusion to be used as a radioactive standard (pre-infusion)
	Subject Procedures - Infusion of Study Platelets • Vital signs (pre-infusion) • Infuse radiolabeled platelet aliquot from both the autologous apheresis collection treated with INTERCEPT Blood System and stored 7 days (Test) and autologous fresh whole blood derived platelets (Control) • At approximately 0 (pre-infusion sample) and 1 hour ± 15 min and 2 hours± 15 min post-infusion: o Vital Signs o Blood sample to measure radioactivity to calculate recovery and survival o Concomitant medications and nicotine and/or cannabis review o Collect AEs and SAEs o Discharge
Both Stages: 1, 2, 3, 4 (5 or 6), and 7 (or 8) DPI **Within ±4h of the infusion time on** **0 DPI** **(Stage 1-Day 7 and Stage 2-Day 7)**	**Subject Procedures – Post-Infusion** • Blood sample to measure radioactivity to calculate recovery and survival and to characterize RBC contamination • Vital Signs • Concomitant medications and nicotine and/or cannabis review • Collect AEs and SAEs

Both Stages **11± 1 DPI** **Within ±4h of the infusion time on 0 DPI (Stage 1-Day 7 and Stage 2-Day 7)**	**Subject Procedures – Post Infusion Final Visit** • Physical examination • Vital Signs • Weight • Blood sample to measure radioactivity to calculate recovery and survival and to characterize RBC contamination • Blood sample for hematology and chemistry panels (see list of specific parameters in **Table 9**) • Urine or blood pregnancy test for females • Concomitant medications and nicotine and/or cannabis review • Collect AEs and SAEs

[a] Hematology and chemistry panels do not need to be repeated if screening labs are performed within 7 days prior to apheresis.

5.3.5 Subject Screening

5.3.5.1 Before Each Study Stage (Day -15 to 0)

Prior to participating in the study, healthy volunteer subjects will be screened for eligibility. Subject screening procedures will be performed within 15 days preceding the apheresis collection. Screening procedures will include the following:

- Obtain signed informed consent
- Medical history and demographics
- Blood donor physical exam (including height, weight, and vital signs: temperature, sitting blood pressure, and heart rate)
- Concomitant medications (including drugs of abuse), and nicotine and/or cannabis inquiry
- Urine drug screening test
- Blood sample (~13 mL) collection for laboratory tests: hematology and chemistry panels (see list of specific parameters in **Table 9**)
- Blood sample (~24 mL) collection for donor screening panel (including ABO, Rh type, and viral screening panel: HIV, HBV, HCV, HTLV, syphilis, and WNV)
- Urine or blood pregnancy test (for females of child-bearing potential)
- Review of inclusion/exclusion criteria

Subjects who meet all inclusion and none of the exclusion criteria will visit the laboratory as specified in the Schedule of Assessments (see **Table 7**) and Study Schema (**Table 6**) for each stage of the study.

5.3.6 Platelet Apheresis Collection and Storage

5.3.6.1 Day 0: Prior to Apheresis Collection

Prior to apheresis collection in any study stage/period, the donor weight and vital signs will be recorded. A blood sample (approximately 13 mL) for hematology (including platelet count) and chemistry panels (see list of specific parameters in **Table 9**) will be taken; hematology and chemistry panels do not need to be repeated if screening assessments were performed within 7 days prior to apheresis. Subjects will be asked about concomitant medications, nicotine and/or cannabis use, and updates to baseline medical history.

5.3.6.2 Day 0: Apheresis Collection

Study subjects who qualify to participate in any study stage will provide one or two single or double apheresis platelet collections (depending upon whether it is a crossover stage) as described in **Section 5.3.2.1**.

Subjects will be asked about AEs (serious or non-serious) that may have taken place during or following the collection, and these will be recorded.

5.3.6.3 Day 0-1: Post Apheresis Collection Input Sample

A sample (approximately 10-20 mL) will be aseptically removed from the component for *in vitro* platelet assessments (**Table 11**).

5.3.6.4 Day 0-1: Platelet Pathogen Reduction with the INTERCEPT Blood System

INTERCEPT treatment (**Figure 2**) will begin within 24 hours of collection (Day 0) following the steps outlined in **Section 5.3.2.2**. The INTERCEPT platelets will be weighed for calculation of post-treatment volume.

Following the INTERCEPT treatment process, the individual INTERCEPT storage containers will be weighed for calculation of post-treatment volume. A post INTERCEPT sample will be removed using the integrated sample bulb (on the "Test-A" container and approximately 3 mL of platelet component will be withdrawn to measure the post-INTERCEPT platelet count. The volume, dose and platelet yield retention will be calculated (**Table 11**).

5.3.7 Bacterial Testing

A sample (approximately 8 to 20 mL) of the Test platelets will be withdrawn 1, 2 or 3 days prior to the end of storage for bacterial contamination screening. Testing for bacterial contamination will utilize an approved system for aerobic culture of potential viable bacteria. Bacterial detection testing is not considered a licensure requirement for INTERCEPT platelet components treated within the first 24 hours after collection based on studies that have demonstrated effective inactivation of bacteria through 7 days of storage. INTERCEPT technology is approved to replace current bacterial detection methods. However, bacterial detection will be used in this study because the study subjects are healthy volunteers who will derive no benefit from platelet reinfusion. Bacterial detection testing will provide an extra measure of safety for these volunteer subjects. In addition, in the event that any subject experiences a post transfusion febrile reaction, the bacterial detection samples will be useful to evaluate the cause of the febrile reaction.

5.3.8 Platelet Storage: Stage 1 Days 1-7; Stage 2 Days 1-7

All INTERCEPT platelets will be stored under standard conditions of continuous agitation with approved devices and temperature control (20°C to 24°C) until the end of Day 7 post-collection for each stage.

5.3.9 Platelet Radiolabeling and Infusion, Day 7 (0 DPI)

5.3.9.1 Subject Procedures Prior to Infusion

On the scheduled infusion day (0 DPI or Day 7 of component storage), subjects will return to the laboratory. Subject's weight and vital signs, urine or blood pregnancy test (if applicable subject is female), and a blood sample (approximately 13 mL) for hematology and chemistry panels (see list of specific parameters in **Table 9**) will be taken. Subjects will be asked about concomitant medications, nicotine and/or cannabis use and AEs (serious or non-serious) that may have taken place.

5.3.9.2 Collection of Fresh Platelets (Control)

On the day of scheduled infusion, 43 (±2) mL of the subject's blood will be drawn into a syringe containing 9 mL of ACD-A for preparation of the Control PRP platelets. In addition, approximately 10 mL of the subject's blood will be collected in an EDTA tube to determine the elution for each radiolabel using the BEST version 4.2.1 method (**Appendix B**).

Fresh platelets will be prepared from the 43 (±2) mL sample according to the following procedure: Collect 43 (±2) mL of whole blood into a syringe containing 9 mL of ACD-A and

transfer into two 50 mL conical screw-top tubes and rest for 30 minutes (alternatively, 43 mL of whole blood can be collected with 7 mL of ACD-A followed by a 1-2 h holding period).

The tubes will be centrifuged at 200 × g for 15 minutes to prepare platelet-rich plasma (PRP), and the PRP from each tube will be transferred to another conical tube. ACD-A equal to 15% of the PRP volume will be added. Prior to radiolabeling the Control platelet sample will be evaluated as described in **Section 8.2.1**.

5.3.9.3 Release criteria of Test platelets

At the end of storage (Day 7), prior to radiolabeling, Test platelet sample must fulfill the release criteria, as detailed in **Table 8**. Test components that do not fulfill the release criteria, will not be radiolabeled or transfused and hence will not be included in the primary efficacy analysis for the platelet recovery and survival.

Table 8 Release Criteria on Day of Radiolabeling/Infusion

Attribute	Method	Specification
Description	Visual Inspection	Semi-opaque liquid in plastic container
Labeling	Visual Inspection	Affixed and completed
Product integrity	Visual Inspection	No evidence of clots, fibrin strands, aggregates, or compromised container integrity
Bacterial contamination	BacT/ALERT	Negative to date
Records review	Data capture sheets	Complete and reviewed

5.3.9.4 Preparation of Test Platelets for radiolabeling– Test Platelets Stored for 7 Days

On the day of scheduled radiolabeling and infusion (Day 7 post-apheresis), two matched samples of 10-30 mL will be aseptically removed from the "Test-A" container for radiolabeling. For Test platelets stored in one container, the sample for radiolabeling will be removed before the unit is sampled for *in vitro* platelet function testing. The final storage containers will be weighed to determine platelet component volume after sampling.

The Test platelets are prepared for radiolabeling using either the BEST version 4.2.1 method (Stage 1; **Appendix B**) or the Variant 1 method (Stage 1 and Stage 2; **Appendix C**) and protocol specific modifications listed in **Table 15** and **Table 16,** respectively. Prior to processing and immediately before radiolabeling the Test platelet samples will be evaluated as described in **Section 8.2.1**.

Percentage process recovery prior to the radiolabeling procedure will be calculated from the pre and post processing platelet counts and sample volumes for Test platelets.

5.3.9.5 Test and Control Platelet Radiolabeling

Previously stored Test platelets and fresh PRP Control platelets will be radiolabeled according to randomization assignment for that subject with either ^{51}Cr as sodium radiochromate ($Na_2{}^{51}CrO_4$), or ^{111}In as indium oxine, following the labeling and washing procedures outlined in the BEST version 4.2.1 procedure (**Appendix B**) or the Variant 1 procedure (**Appendix C**), dependent upon Stage and where applicable, randomization. The isotope labels will be determined by a random number table such that equal numbers of fresh platelets (Control) and stored Test platelets will be labeled with each isotope.

For both Control and Test platelets, after resuspension in a small volume of ACD-A/saline, either 50–100 µCi ^{51}Cr or 50–60 µCi of ^{111}In is added to the platelets and incubated for 20 +2 minutes at room temperature to achieve radioactive labeling. The platelets are washed to remove excess radioactivity and then resuspended in autologous platelet-poor plasma.

5.3.9.5.1 *Radiolabeling Efficiency*

Efficiency of labeling will be measured for each radioactive isotope according to the BEST version 4.2.1 procedure (**Appendix B**) or Variant 1 procedure (**Appendix C**), as applicable.

5.3.9.5.2 *Elution of Radioactivity from Labeled Platelets*

Radiolabel spontaneously dissociates (elution) from platelets both *in vitro* and *in vivo* (Holme, et al. 1993, Snyder, et al. 2004). To correct for elution before calculation of recovery and survival, the mean elution of radioisotope into the plasma after radiolabeling will be measured *in vitro* and modeled using an assay to estimate *in vivo* elution using the BEST elution assessment method (BEST version 4.2.1 method, **Appendix B**).

5.3.9.6 Stored Test Platelet In Vitro Function Testing

An aliquot (10-20 mL) of the stored Test platelets will be aseptically removed from the subject's test container on the scheduled day of infusion; pH and *in vitro* platelet function assessments will be performed (**Table 11**).

For Test components stored in one container, remove the sample for radiolabeling before the unit is sampled for *in vitro* platelet function testing. For platelets stored in two containers, the samples for pH assessment and for radiolabeling will be removed from "Test-A" container and sample for the *in vitro* platelet function testing will be removed from the "Test-B" container.

5.3.9.7 Retention of Test Platelet Component

Once all of the end of storage sampling is complete, the Test components will be placed in refrigerated storage and reserved for future bacterial culture in the event of a febrile transfusion reaction indicative of sepsis (temperature elevation >2°C from pre-infusion or greater than 1°C with rigors). Three days post infusion, the Test components will be discarded following institutional practices.

5.3.9.8 Infusion of Test and Control Aliquots and Post-Infusion Subject Procedures

After radiolabeling, and a negative pregnancy test (if subject is female), the autologous fresh PRP Control platelets and stored radiolabeled INTERCEPT platelets (Test) will be combined and infused into the subject (approximately 10-30 mL). Subject weight and vital signs will be measured prior to infusion and a sample will be obtained from the combined radiolabeled fresh and stored platelets before infusion and used as a radioactive standard.

Blood samples (approximately 10 mL) will be drawn from the subject pre-infusion and for radioactivity measurements at 1 hour ± 15 min and 2 hours ± 15 min from the end of infusion. For details on radioactivity measurements, see **Section 8.2.2**.

At each blood sampling time, subject vital signs, concomitant medications, AEs and SAEs will be recorded, and when completed, the subject will be discharged.

5.3.10 Subject Procedures: 1 – 7 DPI

On days corresponding to 1, 2, 3, 4 (or 5 or 6), and 7 (or 8) DPI in each stage and study period, subjects will return to the research unit at approximately the same time of the radiolabeled platelet infusion (within 4 hours). Blood samples (10 mL) will be drawn to measure radioactivity. An additional 2-4 mL will be collected on 1 and 4 (5 or 6) DPI to characterize RBC contamination (study Stage 1 only). The exact times of sampling will be recorded. Vital signs will be recorded, and subjects will be questioned about concomitant medications, and nicotine and/or cannabis use and AEs/SAEs.

5.3.11 Subject Procedures – End of Study

On the day corresponding to 11±1 days post-infusion in each stage and each study period, where applicable, subjects will return to the research unit at approximately the same time of the radiolabeled platelet infusion (within 4 hours). Blood samples (25-27 mL) to measure radioactivity and to characterize RBC contamination will be drawn as well as for hematology and chemistry panels (see list of specific parameters in **Table 9**). The exact time of sampling will be recorded. Subjects will undergo a physical examination, vital signs and weight will be recorded, and subjects will be questioned about concomitant medications, and nicotine and/or

cannabis use and AEs/SAEs. A pregnancy test will be done for females. Adverse events occurring within 24 hours after the last blood draw will be recorded.

5.3.12 Study Procedure Definitions

The following list is a description of the procedures to be completed during the study:

5.3.12.1 Informed Consent

An institutional review board (IRB) approved informed consent must be signed and dated by each study subject prior to any study procedures being performed, and a copy must be given to the subject.

5.3.12.2 Inclusion and Exclusion Criteria Assessment

Each study stage will enroll separate cohorts of study subjects. All subjects must qualify for any study stage based on the inclusion and exclusion criteria specified in **Section 6.1** and **Section 6.2**, respectively.

5.3.12.3 Medical History, Demographics, and Prior Medications

A complete medical history will be obtained including a review of all major organ systems. The medical history, demographics, a list of prior medications (including drugs of abuse) and history of nicotine and/or cannabis use will be taken at screening. Demographics will include gender, date of birth or age at time of screening, race, and ethnicity. Prior medications will include all medications taken for 4 weeks prior to screening, including oral contraceptives. In addition, a brief medical history, and a review of concomitant medications including drugs of abuse, and nicotine and/or cannabis use will be performed on Day 0 of both study stages.

5.3.12.4 Physical Examination

A physical examination will be performed at screening and on the final day of each study stage and study period, where applicable, or upon early termination. The examination will include all major body systems with the exception of genitourinary. Personnel performing the physical exams must be qualified by training and state licensure.

5.3.12.5 Vital signs

Vital signs will be obtained for each study stage at screening, and for each study stage or period, as applicable, on Day 0 (prior to the apheresis collection), on 0 DPI, the day of scheduled infusion, (pre-infusion and at 1 hour ± 15 min and 2 hours ± 15 min post-infusion), and on all follow up days, or upon early withdrawal. Study vital signs will include temperature, sitting blood pressure (to be taken after 5 minutes of sitting), and heart rate.

5.3.12.6 Weight, Height, and Body Mass Index

Subjects will be weighed in street clothes after removing shoes and coat or jacket. Subject height will be measured without shoes. Weight will be measured for each study stage at screening, and for each study stage or period, as applicable, on Day 0 (prior to apheresis collection), prior to infusion of radiolabeled platelets (Day 7), and at the end of study, or upon early termination. Height will be measured at screening only. Subjects will have their body mass index (BMI) calculated at screening using the following formula: BMI (kg/m^2) = weight (kg)/height (m^2). Blood volume will be calculated using the method in the BEST Platelet Radiolabeling Procedure version 4.2.1 (**Appendix B**).

5.3.12.7 Blood Sampling

5.3.12.7.1 *Stage 1*

Subject blood samples will be collected at each time indicated in the Schedule of Assessments (**Table 7**). The date and time of sample collection, as well as the date and time of dose administration, will be recorded. Up to approximately 24 mL of blood will be collected for blood donor screening and viral screening. Up to approximately 13 mL of blood will be collected at each of the protocol-specified time points for hematology and chemistry panels. Approximately 10 mL for radioactivity measurements will be collected at each of the protocol-required time points 0, 1 hour ± 15 min, 2 hours± 15 min and 2, 3, and 7 (or 8) DPI. Up to approximately 12-14 mL for radioactivity measurements and RBC contamination assessment will be collected at each of the protocol-required time points 1, 4 (or 5 or 6), and 11 DPI. In addition, blood samples will be collected to prepare Control platelets (approximately 43 mL) and the radiolabeling elution assessments (up to 10 mL). A total of approximately 231 mL of subject blood will be obtained during each study period for screening, hematology, chemistry, and radioactivity measurements; for Stage 1, the total will be approximately 462 mL

5.3.12.7.2 *Stage 2*

Subject blood samples will be collected at each time indicated in the Schedule of Assessments (**Table 7**). The date and time of sample collection, as well as the date and time of dose administration, will be recorded. Up to approximately 24 mL will be collected for blood donor screening and viral screening. Up to approximately 13 mL will be collected at each of the protocol-specified time points for hematology and chemistry panels. Approximately 10 mL will be collected for the radioactivity measurements at each of the protocol-required time points. An additional approximately 2-4 mL will be collected for the RBC contamination assessment at the protocol required time point 11± 1 DPI. In addition, blood samples will be collected to prepare Control platelets (approximately 43 mL) and the radiolabeling elution

assessments (up to 10 mL). A total of approximately 223 mL of subject blood will be obtained during study Stage 2 for screening, hematology, chemistry, and radioactivity measurements.

5.3.12.8 Laboratory Tests

The local laboratory will perform the subject hematology and chemistry panel tests specified in **Table 9**.

Table 9 Subject Clinical Laboratory Tests

Hematology	Serum Chemistry
Hematocrit Hemoglobin Red Blood Cell Count Platelet Count White Blood Cell Count (*with Differential*)	Blood Urea Nitrogen Calcium Carbon Dioxide Chloride Creatinine Glucose Potassium Sodium

Samples (approximately 13 mL) for clinical laboratory tests will be taken at screening for all stages, Day 0 of each collection (not required if Stage specific screening clinical laboratory tests were performed within 7 days of collection), at the end of the storage/day of scheduled infusion (one or two study periods, depending upon stage), and at the last scheduled blood draw (11± 1 DPI), or upon early termination.

All samples will be collected in accordance with acceptable laboratory procedures and processed according to the local laboratory standard procedures. Copies of the laboratory certificates and procedure, or reference to the procedure, will be filed in the study records. Laboratory values that are out of range will be identified and may be repeated at the Investigator's discretion after notification of the sponsor. The Investigator will determine if any out of range laboratory values are clinically significant and will record these on the AE case report form (CRF). The Investigator may refer the subject for medical treatment, as appropriate, until this occurs.

5.3.12.9 Radioactivity Measurements

See **Section 8.2.2**.

5.3.12.10 In Vitro Platelet Function Testing

See **Section 8.2.3**, **Table 11**.

5.3.12.11 Pregnancy Test

A urine or blood pregnancy test will be performed on all female subjects of childbearing potential at screening for all stages, and for each study period, on all female subjects prior to infusion and on the last day of the study period, or upon early termination.

5.3.12.12 Drug Screening Test

A urine drug screening test will be performed on all subjects during screening in both stages, including both periods 1 and 2 in Stage 1.

5.4 Accountability of Investigational Product

The investigational platelet products will be produced by each participating blood center according to instructions for use or as specified by the protocol. Investigational product accountability of the INTERCEPT for Platelets Dual Storage sets, apheresis collection products and the whole blood collection products will be maintained by the Investigator or designee.

5.4.1 Handling and Disposal

The investigational product produced at the site will be handled and disposed of according to the site's SOP.

6.0 SELECTION AND WITHDRAWAL OF SUBJECTS

The study population consists of healthy volunteer subjects who meet the FDA, AABB and institutional eligibility criteria for autologous apheresis platelet donation and the study inclusion/exclusion criteria detailed below. In each study stage, subjects who do not have both paired (Test and Control) recovery and paired survival data may be replaced, and a sufficient number of subjects will be enrolled to provide up to 12 (Stage 1) and 24 (Stage 2) subjects with evaluable *in vivo* recovery and survival data.

6.1 Subject Inclusion Criteria

- Age ≥18 years, of either gender
- Normal health status (as determined by the Investigator review of medical history and blood donor physical exam)
- Meet FDA, AABB and site specific guidelines for blood donation and apheresis platelet donation; travel, tattoos/piercings and/or male to male sexual contact deferrals do not apply
- Complete blood count (CBC) and serum chemistry values within normal limits or within guidelines, as above
- Pre-donation platelet count of more than 150×10^9 platelets/ L
- Negative blood donor screening test panel for HIV, HBV, HCV, HTLV, syphilis, and WNV (during screening)
- Subjects of childbearing potential must agree to use a medically acceptable method of contraception throughout the study. A barrier method of contraception must be included, regardless of other methods.
- Signed and dated informed consent form

6.2 Subject Exclusion Criteria

- For participation in Stage 2, received any previous infusion in this study.
- Clinically significant acute or chronic disease (as determined by the Investigator).
- Pregnant or nursing females
- Subjects of childbearing potential not using effective contraception
- Disease states or conditions that preclude apheresis platelet donation per AABB reference standards

- Subject received platelet inhibitor within 14 days of donation (e.g., clopidogrel, ticlopidine, amphetamines (e.g., Adderall, Dexedrine)).
- Subjects with positive cocaine and/or amphetamine results from urine drug screen.
- Treatment with aspirin or aspirin-containing medications within 7 days of apheresis or treatment with non-steroidal anti-inflammatory drugs (NSAID), anti-platelet agents or other drugs affecting platelet viability within 3 days of apheresis (e.g. ibuprofen or other NSAIDs)
- Splenectomized subjects
- History of known hypersensitivity to indium or chromium
- Receipt of an investigational product within the past 28 days

6.3 Subject Withdrawal Criteria

Subjects may withdraw from the study at any time. The Investigator may remove a subject for reasons of safety or non-compliance.

The Investigator must determine the primary reason for withdrawal and record it on the CRF. The Investigator or the Medical Monitor may exercise his/her medical judgment to terminate a subject's participation in the study due to clinically significant changes in any clinical or laboratory parameters. The study sponsor also reserves the right to terminate the study at any time. All data normally collected at completion of the study must be collected either at the time of the subject's withdrawal, or before or during the scheduled study close-out. The Investigator will indicate if the withdrawal is associated with indirect or direct COVID-19 related circumstances.

The primary reason for withdrawal should be noted in the CRF using the following categories:

- Adverse Event: The subject has experienced an AE that, in the opinion of the subject or Investigator, requires early termination (withdrawal). The Investigator must complete an AE CRF for each AE. If a subject is withdrawn from the study due to an AE, the Investigator is required to follow the subject until the event is resolved or it becomes stable.
- Significant Protocol Deviation: The subject failed to adhere to protocol requirements.
- Withdrawal of Consent: The subject (or other responsible individual) wishes to withdraw from the study for non-medical reasons, as determined by the Investigator.
- Death

- Investigator Discretion: In the Investigator's judgment, it is in the subject's best interest to be withdrawn from the study.
- Study Termination: The Sponsor, IRB, or FDA terminates the study.
- Lost to Follow-Up

6.4 Stopping Rules

There are no pre-specified stopping rules for this study. The Sponsor may stop the study for any reason.

7.0 TREATMENT OF SUBJECTS

7.1 Treatments

In each study stage, and in each study period of Stage 1, each subject will provide one apheresis platelet component and receive one infusion of autologous radiolabeled fresh Control platelets combined with Test platelets stored for 7 days (approximately 10-30 mL). Platelets are administered by intravenous infusion in a peripheral vein via a 19-gauge butterfly infusion needle.

Procedures will be as follows: On Day 0 of each study period, each healthy volunteer subject provides a single or double apheresis platelet collection.

On Day 7 (0 DPI), subjects will return to the laboratory, and 43(±2) mL of blood will be drawn into a syringe containing 9 mL of ACD-A, USP (2.13% free citrate ion) (alternatively, 43 mL of WB can be collected with 7 mL of ACD-A followed by a 1-2 h holding period) and held for 30 minutes. Fresh platelets will be prepared from this sample for radiolabeling according to the BEST method. Two matched aliquots (10-30 mL) of the stored autologous Test platelets will be aseptically removed from each subject's platelet storage container and prepared for radiolabeling according to either the BEST (Stage 1) or the Variant 1 (Stage 1 and Stage 2) method, depending upon randomization, where applicable, and study stage. Previously stored (Test) and fresh platelets (Control) will be radiolabeled with either ^{51}Cr as sodium radiochromate ($Na_2{}^{51}CrO_4$), or ^{111}In as indium oxine, depending upon randomization order, following the labeling and washing procedures outlined in **Appendix B** (BEST method) or **Appendix C** (Variant 1). The isotope labels will be determined by a random number table such that equal numbers of fresh platelets and stored INTERCEPT platelets will be labeled with each isotope. Aliquots of the fresh Control will be radiolabeled with the BEST method and stored Test platelets will be radiolabeled in tubes with the BEST method (Stage 1) or the Variant 1 (Stage 1 and Stage 2) method, depending upon stage, and where applicable, method randomization. After radiolabeling, the autologous fresh and stored platelets will be simultaneously infused into the subject (approximately 10-30 mL). The pregnancy test for females is required to be negative before infusion in each period.

Subject blood samples for radioactivity measurements will be drawn during each period as follows: pre-infusion,1 ± 15 min and 2 hours post-infusion, and on Days 1, 2, 3, 4 (or 5 or 6), 7 (or 8), and 11 ± 1 DPI (see schedule in **Table 6** and **Section 5.3.12.7 Blood Sampling**).

7.1.1 Subject Confinement and Restrictions

Subjects will visit the study site on Day 0 to have single or double apheresis platelets collected. On day of scheduled infusion, subjects will return to the study site, and blood samples will be drawn to prepare a fresh platelet sample (Control) and to test for radiolabel elution. Fresh and stored INTERCEPT platelets will be radiolabeled and infused to the subjects (autologous infusion). Subjects will have to stay in or return to the study site or facility where blood is being drawn until the 2 hours ± 15 min post-infusion blood sample is drawn. Thereafter, subjects will visit the study site on 1, 2, 3, 4 (or 5 or 6), 7 (or 8), and 11 ±1 DPI (within 4 hours of the initial infusion time).

Subjects will be instructed to abstain from treatment with aspirin or aspirin-containing medications within 7 days of apheresis or treatment with non-steroidal anti-inflammatory drugs (NSAID), anti-platelet agents or other drugs affecting platelet viability within 3 days of apheresis (e.g., ibuprofen or other NSAIDs).

Subjects will not engage in any strenuous activity while confined to the research unit and will follow the rules governing their activities, as set forth by the unit.

7.2 Concomitant and Excluded Therapy

Concomitant medications (including drugs of abuse) will be recorded during study participation. Subjects taking medications that preclude apheresis platelet donation per AABB reference standards and subjects with positive amphetamine and/or cocaine test results are excluded from participating in the study.

Treatment with aspirin or aspirin-containing medications within 7 days of apheresis or treatment with non-steroidal anti-inflammatory drugs (NSAID), anti-platelet agents or other drugs affecting platelet viability within 3 days of apheresis (e.g., ibuprofen or other NSAIDs) will preclude apheresis.

7.3 Subject Compliance

Healthy subjects who understand the study commitments and sign the informed consent will be enrolled in the study.

Treatment compliance will be tracked by the Investigator or designee, recorded on the CRF, and monitored by the Sponsor.

8.0 ASSESSMENT OF EFFICACY

8.1 Efficacy Parameters

8.1.1 Primary Efficacy Endpoints

For Stage 1 (BEST and Variant 1) and Stage 2:

- Post infusion recovery of Test platelets at end of storage (Day 7)
- Post infusion survival of Test platelets at end of storage (Day 7)

Post-infusion platelet recovery and survival will be estimated by using radiolabeled platelets with a computerized multiple hit gamma function analysis with a curve fitting algorithm. Post-infusion blood samples will be corrected for plasma associated radioisotope and the spontaneous *in vitro* and *vivo* dissociation (elution) of radiolabels using the method described in the BEST version 4.2.1 (**Appendix B**).

Recovery and survival of INTERCEPT components will be evaluated separately for platelets prepared for radiolabeling with the BEST and Variant 1 procedures. Results will be compared as described in the statistical plan.

8.1.2 Secondary Efficacy Endpoints

- Product parameters at end of INTERCEPT treatment:
  - Platelet count, volume, and dose ($\geq 3.0\times10^{11}$)
  - Platelet yield retention ($\geq$80%)
- Test Product Parameters at the end of Storage
  - pH $_{22°C}$ ($\geq$6.2)

8.1.3 Additional Endpoints

Additional analyses will include the *in vitro* function parameters of the stored INTERCEPT platelet component and the platelet samples (after radiolabeling preparation process) prior to radiolabeling.

- Product parameters at end of storage (Day 7):
  - Platelet count, platelet dose, and component volume.
  - *Biochemical assessments:* glucose, lactate, pO_2, pCO_2, bicarbonate, LDH, % of baseline adjusted lysis (LDH activity platelet concentrate supernatant at end-of-

storage – Day 0 / Total LDH activity in Triton × 100 lysed platelet concentrate), and ATP. (Normalized O_2 consumption, normalized CO_2 production, normalized glucose consumption, normalized lactate production and normalized ATP production values will be calculated per platelet.)

- *Functional assessments:* HSR, ESC, CD62 (p-selectin expression) MPV, morphology score

- Test platelet sample parameters prior to processing and again before radiolabeling (Table 10).
  - Platelet count, volume (Stages 1 and 2)
  - Calculated % platelet yield (platelet recovery) during BEST or Variant 1 procedures (Stages 1 and 2)
  - WBC count (WBC contamination) (Stage 1)
  - RBC count (RBC contamination) (Stage 1)
  - $pH_{22°C}$ (Stages 1 and 2)
  - CD62P (Stage 1 and 2 assessed prior to processing, and Stage 1 assessed again before radiolabeling)
- RBC contamination in subject's radiolabeled WB sample

8.2 Methods and Timing of Efficacy Parameters

8.2.1 Assessment of Stored Platelet Samples Prior to Radiolabeling

A paired sample of stored (7 day or 0 DPI) Test platelets will be assessed prior to processing and immediately prior to radiolabeling step (resuspended hard spin pellet) to evaluate platelet yield (Stages 1 and 2), RBC (Stage 1) and WBC content (Stage 1) (**Table 10**).

Table 10 Assessment of Test Platelet Sample Prior to Radiolabeling

Assay	Study Stage	Method	Stored Test Platelet Component Sample	Processed Platelet Sample for Radiolabeling Test
Sample volume	1 and 2	NA	X	X
Platelet Count	1 and 2	Automated counter	X[a]	X
RBC Count	1	Hemocytometer or Flow Cytometry	-	X
WBC Count	1	Leucocount	-	X
pH $_{22°C}$	1 and 2	Blood Gas Analyzer or ion selective electrode	X[a]	X
CD62P	1 and 2	Flow Cytometry	X[a]	X[b]

[a] Test platelets only will be analyzed as part of end of storage *in vitro* assessments outlined in Table 11.
[b] The processed platelet sample for radiolabeling test is assessed for CD62P in Stage 1 only.

Characterization of residual RBCs will be performed either by manual counting or using an antibody to CD235a which is expressed on erythrocytes and erythrocyte precursors and is associated with the MNS blood group (Reid 2009; Gahmberg, et al. 1978).

Characterization of residual WBCs will be performed using the Leucocount Kit (Becton Dickenson) which uses propidium iodide that stains the nucleic acids of the DNA contained within the WBCs and is analyzed using flow cytometry.

8.2.2 Radioactivity Measurements and Recovery and Survival Estimation

Samples will be obtained from the radiolabeled Control and stored Test platelets before infusion and used as a radioactive standard. By measuring the volume infused, the total dose of radioactivity infused will be calculated. The radioactivity of the samples will be determined by use of a gamma counter. Sample dilutions for enumeration of radioactivity of the standards will be calibrated to ensure that measured values are at least 10-fold background values. Duplicates of the subject's WB will be measured for radioactivity while singletons of all other samples will be evaluated. Post-infusion blood samples will also be corrected for plasma associated radioisotope and the spontaneous *in vitro* and *vivo* dissociation (elution) of radiolabels using the BEST elution assessment method (BEST version 4.2.1, **Appendix B**).

8.2.3 Red Blood Cell Contamination Assessment

Post infusion blood samples will be also used to determine the level of RBC/WBC contamination in the radioactivity measurements. Ficoll gradient centrifugation of the WB sample will allow for separation of the RBCs and some WBCs from the plasma, WBC and platelets. The radioactivity associated with the RBC and non-RBC associated fractions will be measured. RBC contamination will be determined on 1, 4 (or 5 or 6) and 11±1 DPI in Stage 1 and only on 11±1 DPI in Stage 2.

8.2.4 In Vitro Platelet Assessments

Samples for analysis of pH need to be analyzed shortly after preparation. The remaining indices listed in **Table 11** should be tested or prepared for testing following site SOPs. Samples for assessment of ATP will be prepared by the site and sent to a centralized lab for analysis. Samples for assessment of CD62P, LDH, glucose, morphology (if fixed) and lactate can be prepared and stored for analysis at a later date per site specific SOPs.

Table 11 In Vitro Platelet Function Assays to Evaluate INTERCEPT Platelet Components

Assay	Pre INTERCEPT Day 0-1	Post INTERCEPT	End of Storage
Component weight (g)	X	X	X
Platelet count ($\times 10^3/\mu L$)	X	X	X
Platelet dose ($\times 10^{11}$ cells/unit) [a]	X	X	X
Mean platelet volume (MPV) (fL)	-	-	X
Morphology score (max 400)	-	-	X
$pH_{22°C}$	X	-	X
pO_2 (37°C) (mm Hg)[c]	-	-	X
pCO_2 (37°C) (mm Hg)[c]	-	-	X
HCO_3- (37°C) (mmol/L)[c]	-	-	X
Supernatant glucose (mmol/L)[c]	-	-	X
Supernatant lactate (mmol/L)[c]	-	-	X
Supernatant LDH (U/L)[c]	X	-	X
Total LDH (U/L)	X	-	X
ATP (µmol/dL)[c]	X	-	X
Extent of Shape Change (%) (ESC)	-	-	X
Hypotonic Shock Response (%) (HSR)	-	-	X
CD62P (% P-selectin expression)	X	-	X

Assay	Pre INTERCEPT Day 0-1	Post INTERCEPT	End of Storage
Platelet lysis (%)[b]	X	-	X
Bacterial culture	-	1-3 days before end of storage	-

[a] Platelet dose is calculated from the platelet count and volume.
[b] Calculated from ratio of LDH activity in 1 mL of platelet concentrate supernatant to the total LDH activity in 1 mL of Triton X-100 lysed platelet concentrate.
[c] The values for these parameters will also be normalized for platelet count.

Specific assays and equipment for the assessment of the *in vitro* parameters will be detailed in the study manual and the clinical study report.

9.0 ASSESSMENT OF SAFETY

9.1 Safety Parameters

9.1.1 Safety Endpoints

- Adverse events
- Vital signs
- Hematological profile
- Serum chemistry profile

9.2 Methods and Timing of Safety Parameters

Subjects will be actively monitored for AEs during apheresis collection and during the radiolabeled platelet infusion, until they are discharged from the study site. After discharge from the study site, all AEs reported by the subject to the study staff will be recorded on the CRF. AE and SAE data may be collected by phone or in person by study staff.

Vital signs are collected for all stages at screening, and for each study period prior to apheresis collection (Day 0), on the day of infusion (pre-infusion, and approximately 1 and 2 hours post-infusion), and on 1, 2, 3, 4 (or 5 or 6), 7 (or 8) and 11 ± 1 DPI. Clinically significant changes in vital signs as assessed by the study investigator will be recorded as AEs.

Blood samples for hematology and chemistry panels will be obtained from each subject at screening, and for each study period, prior to apheresis platelet collection (Day 0), prior to platelet infusion, and at the last scheduled blood draw (11±1 DPI). Clinically significant laboratory findings will be recorded as AEs. The subject laboratory assessments are identified in **Table 9**.

9.3 Recording and Reporting Adverse Events

The Investigator records and assesses all AE/SAEs that occur following the start of the apheresis collection through 24 hours after the last DPI blood sample is drawn. All AE/SAEs are recorded on the CRF.

9.3.1 Definition of Adverse Event

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered an investigational product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with

the use of an investigational product whether or not related to the investigational product (ICH E2A II/A/1, 21 CFR 312.32).

9.3.2 Definition of Serious Adverse Event

A SAE is any untoward medical occurrence that at any dose results in any of the following outcomes:

- Death
- Life-threatening event (results in an immediate risk of death from the reaction as it occurred)
- Hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgement, they may jeopardize the subject and/or the subject may require intervention to prevent one of the outcomes listed in this definition.

9.3.3 Definition of Unanticipated Adverse Device Effects

Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

9.3.4 Assessing Relationship of the Adverse Event to the Study Procedure

The Investigator is required to assess whether there is a reasonable possibility that the study procedures (apheresis platelet collection and/or study infusion) caused or contributed to an AE, per **Table 12**. Determination of whether there is a reasonable possibility that the study apheresis platelet collection or the infusion of radiolabeled study platelets caused or contributed to an adverse event includes assessing temporal relationships, biologic plausibility, dechallenge/rechallenge information (if available), association (or lack of association) with underlying disease, and presence (or absence) of a more likely cause.

Table 12 Causality Assessment Scale

Imputability Level		Explanation
0	Excluded	When there is conclusive evidence beyond reasonable doubt for attributing the adverse reaction to alternative causes.
	Unlikely	When the evidence is clearly in favor of attributing the adverse reaction to causes other than apheresis platelet collection or the infusion of radiolabeled study platelets.
1	Possible	When the evidence is indeterminate for attributing adverse reaction either to apheresis platelet collection or the infusion of radiolabeled study platelets to alternative causes.
2	Likely/ Probable	When the evidence is clearly in favor of attributing the adverse reaction to apheresis platelet collection or the infusion of radiolabeled study platelets.
3	Certain	When there is conclusive evidence beyond reasonable doubt for attributing the adverse reaction to apheresis platelet collection or the infusion of radiolabeled study platelets.

9.3.5 Assessing Severity of Adverse Event

The severity (intensity) of each AE is assessed using the standard severity grading scale provided in **Table 13**.

Table 13 Severity Grading Scale

Grade	Definition
Grade 1	Mild: the recipient required no medical intervention.
Grade 2	Moderate: the recipient may have required medical intervention (e.g. symptomatic treatment) but lack of such would not result in permanent damage or impairment of a bodily function
Grade 3	Severe: signs and symptoms requiring inpatient hospitalization or prolongation of hospitalization directly attributable to the event; and/or the event results in persistent or significant disability or incapacity; or the event necessitated medical or surgical intervention to preclude permanent damage or impairment of a bodily function.
Grade 4	Imminently life-threatening: unstable vital signs with major treatment intervention required to prevent death (e.g. vasopressors, intubation, transfer to intensive care).
Grade 5	Death: adverse event resulting in death following the implicated apheresis platelet collection or transfusion.

9.3.6 Assessing Seriousness of an Adverse Event

The Investigator is required to assess if an AE resulted in any of the following outcomes that result in classification of the event as SAE: death, a life-threatening event (results in an immediate risk of death from the reaction as it occurred), inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, a congenital anomaly/birth defect, or another important medical event (listed in **Section 9.3.2**).

9.3.7 Reporting Adverse Events

All AEs that occur during or after the start of any study apheresis platelet collection or are reported by the subject through the end of the study are recorded on the CRF. In addition, SAEs are recorded on the CRF and reported to the Sponsor. The Sponsor contact information is provided in **Table 14**.

Table 14 Sponsor Contact Information

Name Title	Phone	E-mail
Cerus Product Safety Team	Office: +1 925-288-6014	ProductSafetyOps@cerus.com
Tanya O'Neal, MD Sr. Director, Clinical Development & Product Safety Medical Monitor	Office: +1 925-288-6198	toneal@cerus.com
Richard Benjamin, MD, PhD, FRCPath Chief Medical Officer (Backup Medical Monitor)	Office: +1 925 288-6020	rbenjamin@cerus.com

9.3.7.1 Reporting Serious Adverse Events

When an SAE occurs, the Investigator records the data in the CRF (within 24 hours) and notifies the Cerus Product Safety Manager immediately (within 24 hours). Sponsor contact information is provided in **Table 14**.

9.4 Adverse Event Follow up

The Sponsor reviews each SAE. The Sponsor may request additional appropriate follow-up medical information in order to write a comprehensive narrative.

The Investigators agree to promptly provide follow-up information requested by the Sponsor. All SAEs are followed until resolution or the Investigator judges the event to be chronic or stable.

10.0 STATISTICS

10.1 Analysis Sets

The analysis sets will be defined as follows:

Safety Analysis Set:

All randomized subjects who successfully initiate an apheresis collection.

Evaluable Analysis Set (EAS):

All randomized and infused subjects who have both paired (Test and Control) recovery and paired survival data for the relevant study stage and/or radiolabeling method, and otherwise complied with the protocol without any other major protocol deviation.

Summaries for the efficacy endpoints will be presented using EAS. All other summaries will be presented using the safety analysis set unless stated otherwise.

10.2 Statistical Methods

This study utilizes a paired design to characterize the recovery and survival of autologous apheresis INTERCEPT Test platelet components stored for 7 days compared to their untreated "fresh" Control platelet components.

In addition, the study is designed to evaluate two different approaches (BEST and Variant 1 for Stage 1, and Variant 1 for Stage 2) to radiolabeling stored INTERCEPT platelets for evaluation of recovery and survival. Recovery and survival of INTERCEPT Test platelets will be evaluated separately for each radiolabeling method.

Data will be summarized descriptively by mean, standard deviation, median, and range (minimum, maximum) for continuous parameters and by frequencies and percentages for categorical data variables. Summaries will be presented by radiolabeling method across all study sites and within each study site.

Data will be compared between the two radiolabeling methods in Stage 1. Correlations between the primary and secondary efficacy endpoints may also be explored using graphs and/or regression analysis.

10.2.1 Efficacy Analyses

10.2.1.1 Primary Endpoints

Primary efficacy endpoints are the post infusion recovery and survival of Test platelets at end of storage for both Stage 1 and Stage 2. Post-infusion recovery and survival of platelets will be calculated based on the corrected radioactive counts observed from the six post-infusion sampling time points at 1, 2, 3, 4 (or 5 or 6), 7 (or 8), and 11 ± 1 days post infusion. The following acceptance criteria for recovery and survival will be used to demonstrate non-inferiority of stored Test platelets against fresh Control platelets at the end of Stage 2:

1. Post-infusion recovery:
   H_0: Test - 0.66×Control <0 vs. H_1: Test - 0.66×Control ≥0

The null hypothesis of inferiority will be rejected in favor of the alternative hypothesis of non-inferiority at the two-sided 0.05 significance level if the lower bound of a two-sided 95% CI for the mean treatment difference (Test - 0.66×Control) is greater than or equal to zero. For any platelet recovery values that are more than 100%, they will be capped at 100% for the data analysis.

2. For survival:
   H_0: Test - 0.58×Control <0 vs. H_1: Test - 0.58×Control ≥0

The null hypothesis of inferiority will be rejected in favor of the alternative hypothesis of non-inferiority at the two-sided 0.05 significance level if the lower bound a two-sided 95% CI for the mean treatment difference (Test - 0.58×Control) is greater than or equal to zero.

The two-sided 95% CI will be constructed using the variance estimated from a paired t test. The above acceptance criteria will also be explored for Stage 1 separately for each radiolabeling method that is evaluated (BEST or Variant 1).

The primary analysis population for the primary efficacy endpoints will only include subjects who have both paired (Test and Control) recovery and paired survival data for the relevant radiolabeling method. Subjects who do not have both paired recovery and paired survival data may be replaced to ensure sufficient number of subjects is reached for the primary analysis. Reasons for absence of primary endpoint measurement(s) for any subject will be listed in the study report.

In the event that non-inferiority cannot be declared for the primary endpoints with a minimum of 24 evaluable subjects, additional exploratory analyses may be conducted to quantify the proportions of subjects whose recovery and survival data meet the 0.66 and 0.58 cutoff,

respectively (e.g., platelet recovery from a Test PC greater than or equal to 66% of platelet recover from the paired fresh control).

10.2.1.2 Secondary Endpoints

Data will be summarized descriptively for each stage. The proportions of Test platelet components with platelet dose $\geq 3.0 \times 10^{11}$ (end of INTERCEPT treatment), physical platelet recovery ≥80% (end of INTERCEPT treatment), and $pH_{22°C} \geq 6.2$ (end of storage) will be summarized, with the corresponding lower bound of a one-sided 95% CI for the proportion provided.

10.2.2 Additional Endpoints

10.2.2.1 In Vitro Evaluation of Stored INTERCEPT Platelet Components

In vitro parameters of stored Test platelet components at the end of storage will be summarized descriptively for each study stage.

10.2.2.2 In Vitro Evaluation of Processed Platelet Samples for Radiolabeling

In vitro assessment of processed platelet samples will be summarized descriptively by treatment group (Test vs. Control), sample preparation method (BEST vs. Variant 1), and radiolabeling (^{51}Cr vs. ^{111}In) as applicable. Additionally, the proportions of Test platelet samples prior to radiolabeling with $pH_{22°C} \geq 6.2$ and physical platelet recovery ≥80% will be summarized separately for the BEST and Variant 1 procedures as applicable.

10.2.2.3 Evaluation of Red Blood Cell Contamination

Assessment of the RBC/WBC contamination as by Ficoll gradient centrifugation will be summarized descriptively by day post infusion, study arm (Test and Control), sample preparation method (BEST vs. Variant 1), and radiolabeling (^{51}Cr vs. ^{111}In).

10.2.3 Safety Analyses

Treatment-emergent AEs (defined as AEs with onset on or after the initiation of apheresis platelet collection) will be summarized descriptively. Other safety data (e.g., vital signs, lab data) will be provided in the data listings.

10.3 Determination of Sample Size

A sufficient number of subjects will be enrolled to provide 24 evaluable subjects with both paired (Test and Control) recovery and paired survival data for Stage 2. The sample size of 24 for Stage 2 is chosen to provide reasonable estimates of the primary efficacy endpoints.

10.4 Level of Significance

Unless stated otherwise, statistical significance will be set at the two-sided 0.05 significance level. Note that given the nature of this study, adjustments for multiple hypothesis testing will not be made.

10.5 Criteria for Termination of the Study

There are no pre-specified stopping rules for this study. The Sponsor may stop the study for any reason.

10.6 Procedure for Missing, Unused, and Spurious Data

Missing data will be noted as such. Procedures for imputing data will not be undertaken.

10.7 Deviations from the Statistical Plan

Any changes in the planned analyses instituted after commencement of the study will be documented. The reasons for the modifications and when the changes were made will also be documented.

10.8 Selection of Subjects to be Analyzed

The primary analysis population for the primary efficacy endpoints will only include subjects who have both paired (Test and Control) recovery and paired survival data for the relevant study stage.

11.0 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

Prior to participating in the trial, the Investigator(s)/institution(s) agrees to provide direct access to and copies of source data/documents for collection of baseline data, study data, monitoring, audits, IRB review, and regulatory inspection. Protected health information on source documents will be redacted prior to providing copies of the document to the sponsor. The quality representative(s) of the Sponsor may visit a clinical facility to ensure proper conduct of the protocol, recording of data, and maintenance of records.

All study-related documents and records are subject to inspection and audit by the Sponsor (or designee), and by the FDA, IRB or other relevant regulatory bodies. The Investigator/institution guarantees access to source documents by the study Sponsor or its designee, the FDA, other regulatory bodies and the IRB. If the FDA or local health authority should schedule an inspection, Sponsor should be advised prior to the time this inspection is to occur.

The subjects' apheresis collection records and medical records are considered source data. Data worksheets used during the preparation of study platelet components will be considered source data for processing study platelet components. Analyzer reports or assay results from the laboratory will also be considered source data. The study data will be recorded on the CRF.

12.0 QUALITY CONTROL AND QUALITY ASSURANCE

Collection of accurate, consistent, and reliable data will be ensured through the use of written standard practices and procedures. Clinical study monitors will assure that CRFs are completed correctly and that complete information has been provided. The Cerus Corporation Department of Quality Assurance (QA) will review data collected for this trial. At the discretion of the Sponsor, in addition to the study monitor, a QA representative(s) of the Sponsor may visit a site to ensure proper conduct of the trial in terms of collection and recording of data and maintenance of records as appropriate for a study performed under an Investigational Device Exemption.

The clinical site will be monitored routinely to ensure Good Clinical Practice (GCP) compliance and data quality. The CRF will be verified against the source document and any queries concerning the CRF will be generated and resolved/reconciled to assure data integrity. The clinical site may be audited by the Sponsor or Sponsor designee for quality control and quality assurance purposes, as specified in the Sponsor's SOPs for GCP compliance. The clinical site may also be audited by a regulatory agency.

The study will be monitored by the Sponsor's representatives at all stages of study conduct from inception to completion in accordance with current GCPs. This monitoring will include both centralized and on-site review and source document verification of the data. Centralized data will include remote review of the data (data capture sheets and electronic CRFs) that are provided to Cerus on a regular basis. On-site monitoring involves the visit of a monitor to the investigational site. The Sponsor's monitor or representative will notify the Investigator prior to conducting any investigational site visit. It is important that the Investigator and other study personnel are available during the monitoring visits, and that sufficient time is devoted to the process. The frequency of these visits will depend upon the progress of the study and will encompass all aspects of the study conduct (i.e. review of all study related procedures, tour of the facilities, inspection of equipment and appropriate records, completion of electronic CRFs, recruiting methods, record-keeping, protocol adherence, data collection, AE reporting, etc.). Frequency of on-site monitoring may also be decreased due to institutional restrictions on site access or research activities due to COVID-19 (FDA 2020).

13.0 ETHICS

13.1 Ethical Conduct of the Study

This study will be conducted in compliance with International Council on Harmonization (ICH) E6(R2), Good Clinical Practices (GCP), and US Regulations governing the protection of human subjects (21 CFR Part 50); Institutional Review Boards (21 CFR Part 56), Financial Disclosure by Clinical Investigators (21 CFR Part 54); FDA Guidance for Industry: Collection of Platelets by Automated Methods (2007); and local IRB requirements. Investigators are encouraged to discuss any ethical issues that arise prior to or during the conduct of the study with the Sponsor.

The Protocol and Informed Consent Form will be approved by the Investigator and the Investigator's IRB before the study is initiated.

13.2 Informed Consent

The Informed Consent process must comply with all applicable US Regulations (21 CFR Part 50; 21 CFR Part 640), FDA Guidance for Industry: Informed Consent Recommendations for Source Plasma Donors Participating in Plasmapheresis and Immunization Programs (2007), and the Health Insurance Portability and Accountability Act (HIPAA). A statement that subject medical records must be available for investigations into SAEs must be included in the Informed Consent Form. It should also include any additional information required by local laws relating to institutional review.

The risks and hazards of study participation will be explained to the potential study subjects, under the supervision of a qualified, licensed physician. Written informed consent and HIPAA authorization will be obtained from all study subjects prior to any tests or evaluations. A copy of the signed informed consent will be provided to each subject and will also be maintained in the subject’s medical record.

13.3 Institutional Review Board (IRB)

This protocol will be submitted to an appropriate central or local IRB and its written unconditional approval obtained and submitted to Cerus or its designee before enrollment of the first subject.

Cerus will supply relevant data for Investigators to submit to the IRB for the protocol’s review and approval. Written verification of IRB unconditional approval of the protocol and the subject Informed Consent Form (ICF) will be transmitted to Cerus or its designee prior to granting access to the CRF to study sites. This approval must refer to the study by exact

protocol title and number (including version), identify documents reviewed, and state the date of approval.

The Investigator must promptly report to the IRB all changes in the research activity and all unanticipated problems involving risk to human subjects or others. This includes all SAEs that have resulted in an expedited safety report to the FDA (serious, unexpected AEs possibly related to investigational product). Concurrently, the Investigator must send the study Sponsor documentation of such IRB notification as presented in **Section 9.4**. The Investigator must not make any changes in the research without IRB approval, except where necessary to eliminate apparent immediate hazards to human subjects.

13.4 Participant Recruitment

If an Investigator chooses to advertise for subjects, whether in professional or consumer publications, radio, or television, all advertising must be approved by Cerus and the IRB prior to initiation.

13.5 Disclosure of data

Individual subject medical information obtained as a result of this study is considered confidential, and disclosure to third parties other than those noted below is prohibited. Subject confidentiality will be further assured by utilizing subject identification code numbers to correspond to treatment data in the computer files.

However, such medical information may be given to the subject's personal physician, or to other appropriate medical personnel responsible for the subject's welfare.

In addition, data generated as a result of this study are to be available for inspection upon request by FDA or local health authority auditors, the Sponsor's monitors, or by the IRB. Therefore, absolute confidentiality cannot be guaranteed.

14.0 DATA HANDLING AND RECORD KEEPING

The Investigator will maintain appropriate subject records filed by each participating subject. Clinical information gathered during this study will be recorded on CRFs. As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this trial.

A CRF is required and should be completed for each included subject. The completed original CRFs are the sole property of Cerus and should not be made available in any form to third parties, except for authorized representatives of Cerus or appropriate regulatory authorities, without written permission from Cerus.

It is the investigator's responsibility to ensure completion and to review and approve all CRFs. CRFs must be signed by the investigator or by an authorized staff member. These signatures serve to attest that the information contained on the CRFs is true. At all times, the investigator has final personal responsibility for the accuracy and authenticity of all clinical and laboratory data entered on the CRFs. Subject source documents are maintained at the trial site. In cases where the source documents are the blood donation record, the information collected on the CRFs must match those charts.

In some cases, the CRF may also serve as the source document. In these cases, Cerus and the investigator must prospectively document which items will be recorded in the source documents and for which items the CRF will stand as the source document.

All raw data generated at the blood centers will be retained at the blood center. Raw data generated at any external testing facility will be retained by that facility. Cerus will receive a copy of all primary data. Primary data provided to Cerus and generated by Cerus will be retained in the Cerus Corporation archives, managed from 1220 Concord Avenue, Suite 600, Concord, CA 94520.

Investigator/institution and the Sponsor shall maintain all study records required (by subpart 21CFR 812.140) during the investigation and for a period of two years after the latter of the following the two dates: 1) the date on which the investigation is terminated or completed, or 2) the date that the records are no longer required for purposes of supporting a premarket approval application or a notice of completion of a product development protocol. Investigator/institution shall not destroy any such records prior to obtaining written permission from Cerus.

15.0 PUBLICATION POLICY

The publication policy is part of the financial contract with each Investigator.

16.0 APPENDICES

List of Appendices

Appendix A Literature References

1. Afssaps France Annual Hemovigilance Report 2009.
2. Afssaps 2010 Annual Hemovigilance Report.
3. Amato M, Schennach H, Astl M, Chen CY, Lin JS, Benjamin RJ, Nussbaumer W. Impact of platelet pathogen inactivation on blood component utilization and patient safety in a large Austrian Regional Medical Centre. Vox Sang 2017;112: 47-55.
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5. ANSM 2011 Hemovigilance Activity Report.
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7. The Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Platelet radiolabeling procedure. Transfusion 2006;46 Suppl:59S-66S.
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9. Code of Federal Regulations (CFR). 1998. Title 21, Part 54, Financial Disclosure By Clinical Investigators.
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11. Code of Federal Regulations (CFR). 1973. Title 21, Part 640, Additional Standards for Human Blood and Blood Products.
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2. CLI 00095, A Prospective, Randomized, In Vitro Study of Apheresis Platelet Components in 100% Plasma Prepared with Photochemical Treatment Compared to Untreated Apheresis Platelets in 100% Plasma. Clinical Study Report v 1.0, March 27, 2015.

3. CLI 00099, A Controlled, In Vivo, Pilot Study to Assess the Recovery and Survival of Radiolabeled Autologous INTERCEPT Treated 7-day Stored Apheresis Platelet Components in 35% Plasma and 65% InterSol and INTERCEPT Treated 7-day Stored Apheresis Platelet Components in 100% Plasma. Clinical Study Report v 1.0, September 8, 2015.

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Appendix B BEST version 4.2.1 Radiolabeling Procedure

Platelet Radiolabeling Procedure

The Biomedical Excellence for Safer Transfusion (BEST) Collaborative

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Introduction

This file contains a procedure for the radiolabeling of both stored and fresh platelets and their autologous reinfusion for the determination of recovery and survival as published in the November, 2006, supplement to *Transfusion*. It is being made available on the BEST website in a downloadable format to facilitate implementation as the routine procedure in laboratories performing this kind of work. When referencing this procedure, please cite the *Transfusion* Supplement.

As detailed in other manuscripts in the Supplement, this procedure was derived from the validated, seminal work of Andrew Heaton, Stein Holme and colleagues in Norfolk, Virginia, and adapted for application to Scott Murphy's insightful approach of comparing a new platelet preparation to fresh platelets from the same subject. The effort of adapting the procedure (kindly made available by the American Red Cross) for this purpose and thoroughly discussing and debating its content were undertaken by the members of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative through multiple meetings over a two-year period. The validation of the adaptation and its application can be found reviewed in other papers in this Supplement. Deviations from this procedure should be accompanied by appropriate validation and documentation.

Many individuals contributed substantial time and effort in developing, reviewing, and validating this procedure. In particular, the leadership of the authors of the other articles in this Supplement should be noted as well as the meticulous reviewing undertaken by Lisa Cooke, Louise Herschel, Jill Roger, Sherrill Slichter, and Ralph Vassallo.

BEST welcomes receipt of comments regarding the procedure and suggestions for further development.

Procedure

1.0 Purpose: To describe a technique for radioisotopic labeling and infusion of fresh or stored platelets with ^{111} In Oxine (^{111}Indium) and $Na_2{}^{51}CrO_4$ (^{51}Chromium).

1.1 While the measurement of platelet recovery and survival can be used for a variety of purposes, this procedure is intended for assessment of the *in vivo* viability of platelets that have been stored and/or treated in comparison with fresh platelets from the same subject.

1.2 This procedure includes the basic calculations for determining platelet recovery and survival. The recommended manner of comparing the results between control and test infusions is through comparison of the paired differences and calculation of upper confidence intervals as detailed by Dumont in this supplement (See 2.6.).

2.0 Applicable documents:

2.1 Snyder EL, Moroff G, Simon T, Heaton A, and Members of the Ad Hoc Platelet Radiolabeling Study Group. Recommended methods for conducting radiolabeled platelet survival studies. Transfusion 1986; 26:37-42.

2.2 Panel Report, International Committee for Standardization in Hematology. Recommended methods for radioisotopic platelet survival studies. Blood 1977; 50:1137-1144.

2.3 Holme S., Heaton A., Roodt J. Concurrent label method with ^{111}In and ^{51}Cr allows accurate evaluation of platelet viability of stored platelet concentrates. Brit J Haematol 1993;84:717-723.

2.4 Nadler SB, Hidalgo JU, Bloch T. Prediction of blood volume in normal human adults. Surgery 1962;51:224-232.

2.5 Lötter MG, Rabe WL, Van Zyl JM, et al. A computer program in compiled BASIC for the IBM personal computer to calculate the mean platelet survival time with the multiple-hit and weighted mean methods. Comput Biol Med 1988;18:305-15.

2.6 Dumont LJ. Analysis and reporting of platelet kinetic studies. Transfusion 2005;45 (Suppl) 67S-73S.

3.0 Equipment/Supplies/Reagents:

3.1 Calibrated, temperature-controlled, variable speed, swinging bucket centrifuge

3.2 Calibrated electronic balance, accurate to ± 0.0002 g.

3.3 Gamma counter with 3 inch sodium chloride crystal

3.4 Laminar flow hood

3.5 19-21 gauge butterfly or straight needles

3.6 Sterile transfer pipettes (3.2 mL draw)

3.7 15 mL and 50 mL sterile conical polypropylene plastic tubes (Note: Although some procedural steps specify exact sizes, an alternative appropriate size may be substituted.)

3.8 Evacuated sample tubes with EDTA (powdered or dry "lavender tops")

3.9 ACD-A: Anticoagulant Citrate Dextrose Solution - Formula A

3.10 ACD-A/ Saline Solution: mix 1 part ACD-A to 7 parts sterile (0.9%) Normal Saline, adjusted to pH 6.5 to 6.8 with 1 N NaOH; sterilize by filtration. (Stable for one week when stored in refrigerator; warm to room temperature (20-24°C) before use.)

3.11 0.22µm Sterilizing Filter Unit.

3.12 ^{111}Indium Oxine in ethanol solution containing approximately 1 mCi per vial at the time of manufacturer's calibration. Use within 7 d for platelet labeling. (Changes in ethanol/oxine ratio over time may result in changes in platelet function.)

3.13 ^{111}Indium Oxine Labeling solution: If not premixed, aseptically add 4.0 mL sterile ACD- A/saline solution to the vial of ^{111}Indium Oxine to make 5.0 mL of sterile ^{111}In-Oxine solution for labeling. Mix well. (Stable for one week or until expiration of stock ^{111}In oxine solution.)

3.14 $Na_2\ ^{51}CrO_4$, Chromium-51

3.15 18 gauge spinal needles sterile

3.16 Assorted sterile syringes and needles (Note: Although some procedural steps specify exact sizes, any appropriate size may be substituted.)

3.17 Positive displacement pipettes for whole blood aliquots.

3.18 Assorted size pipettes and tips

3.19 SDS (Sodium Dodecyl Sulfate) 20%; Dissolve 20g sodium dodecyl sulfate in 100 mL of distilled water. (Store at room temperature for up to one year.)

NOTE: All vessels holding platelets or plasma that will be reinfused must be properly labeled to definitively identify the subject.

4.0 Preparation of Platelets for Labeling with ^{111}Indium Oxine

4.1 Preparation of freshly collected platelets

4.1.1 Using a 19-gauge needle, collect 43 mL (± 2 mL) of venous blood into a 60 mL sterile plastic syringe containing 9 mL of ACD-A. Avoid a traumatic venipuncture at the start of blood drawing. Care should be taken to avoid excessive negative pressure in the syringe during draw to prevent activation of the platelets. (Alternatively, 43 mL of whole blood can be collected with 7 mL of ACD-A followed by a 1-2 h holding period in Step 4.1.3.)

4.1.2 At the same time as 4.1.1, collect one 5 or 7 mL blood sample from the subject into an EDTA tube. Mix well. Reserve for determination of radiolabel elution (see Step 9.0).

4.1.3 Using sterile technique, in a laminar flow hood, remove the needle and express the contents of the syringe into two sterile 50 mL screw-cap conical centrifuge tubes. A hold period of 30 minutes for the ACD-A/blood mixture before further processing should be utilized.

4.1.4 Centrifuge (soft spin) the conical tubes at 180 to 200 x g for 15 minutes at 20 to 24°C with the brake off to produce red cell-poor platelet–rich plasma (PRP).

4.1.5 Remove the PRP from both tubes with a sterile transfer pipette or spinal needle and syringe and place into a single 50 mL sterile conical plastic centrifuge tube. Avoid aspirating any contaminating red cells.

4.1.6 Add a volume of sterile ACD-A equal to 15% of the volume of PRP to the PRP. Cap the tube and mix gently by inversion. Continue with Step 4.3.

4.2 Preparation of aliquot from stored platelet unit

4.2.1 Ensure unit is thoroughly mixed and platelets are completely suspended.

4.2.2 Using sterile technique, in a laminar flow hood, gently remove 10 mL from the stored platelet unit using a 20 mL syringe and 16 ga needle.

4.2.3 Remove the needle and express the contents of the syringe into a 15 mL screw-cap conical labeled centrifuge tubes containing 1.5 mL sterile ACD-A . Cap and mix gently by inversion.

4.2.4 Centrifuge (soft spin) the conical tubes at 180 to 200 x g for 5 minutes at 20 to 24°C with the brake off to produce red cell-poor platelet-rich plasma (PRP).

4.2.5 Remove the PRP with a sterile transfer pipette or spinal needle and syringe and place into a new 15 mL sterile conical plastic centrifuge tube. Avoid aspirating any contaminating red cells. Continue with Step 4.3.

4.3 Centrifuge (hard spin) the ACD-PRP at 1500-2000 x g for 15 minutes at 20 to 24ºC with brake off.

4.4 Remove the platelet-poor plasma (PPP) produced, as completely as possible and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 x g for 15 minutes with brake off), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 4.6.2 and 4.6.5).

4.5 Using a sterile pipet, resuspend the harvested platelet pellet with 2 mL of ACD-A/saline solution. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting radiolabeling. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure).

4.6 Platelet Labeling with 111 Indium-Oxine

4.6.1 Add 50 to 60 µCi of ^{111} Indium-Oxine labeling solution (Step 3.13) to the washed platelet suspension. Incubate at 20 to 24°C for 20 minutes to achieve labeling. (Note: 1 Ci = $3.7x10^{10}$ Bq).

4.6.2 At the end of incubation, add 3.5 mL of ACD-A/saline and 0.5 mL of the reserved PPP (from Step 4.4) directly to the incubation tube containing the platelets in ACD-A/saline and ^{111}In.

4.6.3 Centrifuge the tube at 1250 x g at 20 to 24°C for 10 minutes with brake off.

4.6.4 Remove the supernatant and retain in a separate test tube. Determine the activity of this supernatant and the labeled pellet in a dose calibrator. Calculate and note the efficiency of labeling by dividing the activity of the pellet by the combined activities of the pellet and supernate.

4.6.5 Gently resuspend the platelet-pellet in 2 mL of PPP reserved previously (from Step 4.4). (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) After complete resuspension, add an additional 4 mL of PPP.

5.0 Preparation of Platelets for Labeling with $Na_2{}^{51}CrO_4$

5.1 Labeling of fresh platelets

5.1.1 Using a 19-gauge needle, collect 43 mL (± 2 mL) of venous blood into a 60 mL sterile plastic syringe containing 9 mL of ACD-A. Avoid a traumatic venipuncture at the start of blood drawing. Care should be taken to avoid excessive negative pressure in the syringe during draw to prevent activation of the platelets. (Alternatively, 43 mL of whole blood can be collected with 7 mL of ACD-A followed by a 1-2 h holding period in Step 5.1.3.).

5.1.2 At the same time as 5.1.1, collect one 5 or 7 mL blood sample from the subject into an EDTA tube. Mix well. Reserve for determination of radiolabel elution (see Step 9.0).

5.1.3 Using sterile technique, in a laminar flow hood, remove the needle and express the contents of the syringe into two sterile 50 mL screw-cap conical centrifuge tubes. A hold period of 30 minutes for the ACD-A/blood mixture before further processing should be utilized.

5.1.4 Centrifuge (soft spin) the conical tubes at 180 to 200 x g for 15 minutes at 20 to 24°C with the brake off to produce red cell-poor PRP.

5.1.5 Remove the PRP from both tubes with a sterile transfer pipette or spinal needle and syringe and place into a single 50 mL sterile conical plastic centrifuge tube. Avoid aspirating any contaminating red cells.

5.1.6 Add a volume of sterile ACD-A equal to 15% of the volume of PRP to the PRP. Cap the tube and mix gently by inversion. Continue with Step 5.3.

5.2 Preparation of aliquot from stored platelet unit

5.2.1 Ensure unit is thoroughly mixed and platelets are completely suspended.

5.2.2 Using sterile technique, in a laminar flow hood, gently remove 20 (± 2 mL) mL from the stored platelet unit using a 30 mL syringe and 16 ga needle.

5.2.3 Remove the needle and express 10 mL of the contents of the syringe into each of two 15 mL screw-cap conical labeled centrifuge tubes each containing 1.5 mL sterile ACD-A . Cap and mix gently by inversion.

5.2.4 Centrifuge (soft spin) the conical tubes at 180 to 200 x g for 5 minutes at 20 to 24°C with the brake off to produce red cell-poor platelet-rich plasma (PRP).

5.2.5 Remove the PRP with a sterile transfer pipette or spinal needle and transfer to two new sterile 15 mL conical plastic centrifuge tubes. Avoid aspirating any contaminating red cells. Continue with Step 5.3.

5.3 Centrifuge (hard spin) the conical tubes containing the ACD-PRP at 1500-2000 x g for 15 minutes at 20 to 24ºC with brake off.

5.4 Remove the platelet-poor plasma (PPP) produced, as completely as possible from both tubes and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 x g for 15 minutes at 20 to 24ºC with brake off), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 5.6.2 and 5.6.5).

5.5 Resuspend the harvested platelet pellet in one tube with 3 mL of ACD-A/saline solution. Using a sterile transfer pipette, transfer the resuspended platelets to the other tube containing the platelet pellet. Resuspend the second platelet button. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting the next step.

5.6 Platelet Labeling with Na251CrO4

5.6.1 Add 50 to 100 µCi $Na_2{}^{51}CrO_4$ to the washed platelet suspension. Incubate at 20 to 24°C for 20 minutes to achieve labeling.

5.6.2 At the end of incubation, add 2.5 mL of ACD-A/saline and 0.5 mL of the reserved PPP (from Step 5.4) directly to the incubation tube containing the platelets in ACD-A/saline and $Na_2{}^{51}CrO_4$.

5.6.3 Centrifuge the tube at 1250 x g at 20 to 24°C for 10 minutes with brake off.

5.6.4 Remove the supernatant and retain in a separate test tube. Determine the activity of this supernatant and the labeled pellet in a dose calibrator. Calculate and note the efficiency of labeling by dividing the activity of the pellet by the combined activities of the pellet and supernatant.

5.6.5 Gently resuspend the platelet-pellet in 2 mL of PPP reserved previously (from Step 5.4). (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) After complete resuspension, add an additional 4 mL of PPP.

6.0 Injectate and Standard Preparation

6.1 Label five 100 mL volumetric flasks and five 12 x 75 mm polystyrene tubes with the isotope used, subject name and/or unit identification, date prepared and In STD, Cr STD, and STD I, II or III.

NOTE: The In STD and the Cr STD are necessary only if calculation of “cross-up” and “cross-down” corrections requires them.

Add 1.8 mL 0.9% saline to each 12 x 75 mm standard capped tube. Weigh each tube and record the weights. Take care that the saline does not touch the cap.Add 10 mL of 0.2% SDS-saline solution (1 part saturated (20%) SDS + 99 parts 0.9% saline) to each flask.

6.2 Mix each single labeled platelet aliquots (from Steps 4.6.5 and 5.6.5). Draw 0.3mL of each suspension into its own 1cc syringe through a 16-gauge needle.

6.3 Weigh the filled syringe, needle and needle cover accurately. Record the weight.

6.4 Carefully add 0.2 mL of each injectate directly into each flask, “Cr STD” or “In STD”, as appropriate. Add 0.9% saline to the flask to bring to volume. Mix carefully. Weigh the syringe, needle and needle cover accurately and determine the mass of labeled injectate placed in each standards flask.

6.5 Draw the appropriate radioactive dose of ^{51}Cr-labeled platelets into a labeled syringe. Weigh the syringe and record the radioactive dose.

NOTE: The activity needed to be injected is dependent on a number of variables, such as the gamma counter’s crystal size. In general, 10-20 μCI are required to be injected for accurate determinations. The injectates of the fresh and stored platelets should have approximately the same activities.

6.6 Remove ^{111}In-labeled platelets from the centrifuge tube until the dose intended to be injected is remaining. Measure and record the radioactive dose. Record the weight of the tube.

6.7 Carefully verify subject identification on the chromium syringe and indium tube. Inject the ^{51}Cr-labeled platelets into the centrifuge tube containing the ^{111}In-labeled platelets.

6.8 Mix the labeled injectate in the tube. Prepare final mixed injectate for infusion by using an 18 gauge spinal needle and a syringe labeled with subject information to aspirate all but at least 1 mL of labeled solution in conical tube. (This remaining amount will be used for preparation of standards and determination of cell bound activity.)

6.9 Remove the spinal needle and replace with a sterile syringe cap. Record the weight of the final injectate syringe and cap.

6.10 Draw approximately 0.8 mL of the remaining labeled solution from the tube containing the ^{51}Cr- and ^{111}In-labeled platelets into a 1cc syringe through a 16-gauge needle to be used for standard preparation.

6.11 Record the weight of the filled syringe, needle, and needle cover.

6.12 Carefully add 0.2 mL of the injectate directly into the flask labeled "STD I”. Add 0.9% saline to the flask to bring to volume. Mix carefully.

6.13 Reweigh the 1 cc syringe, needle and needle cover and record this weight and the difference between the two weights (this being the net weight of the mixed injectate added to the standards flask).

6.14 Repeat Steps 6.14 and 6.15 for the remaining two flasks.

6.15 Pipet 0.2 mL of each standard into the appropriate pre-weighed capped tube containing 1.8 mL saline, one sample tube per standard.

6.16 Weigh the capped tubes and record the weight. Take care that the sample does not touch the cap.

7.0 Infusion Procedure

7.1 Allow recipient to sit in the donor bed to stabilize. Record pre-infusion vital signs.

7.2 Verify that recipient's identifiers match the information on the infusion syringe.

7.3 Perform venipuncture with a 19-21 gauge butterfly infusion needle. Draw one 10 mL EDTA (lavender-top) tube for the pre-infusion sample.

7.4 Flush the line with saline to ensure patency of the vein. Inject labeled platelet suspension slowly. Record exact time (starting from time 1/2 of injectate has been infused).

7.5 Flush butterfly line and needle with 10 mL of sterile 0.9% sodium chloride.

7.6 Remove butterfly needle and apply pressure to injection site. Apply bandage when bleeding has stopped.

7.7 Weigh empty infusion syringe, needle and needle cover. Record weight on the data sheet. Record the net weight of injectate.

7.8 Dispose of used supplies in the receptacle labeled for radioactive waste.

7.9 Measure and record post-infusion vital signs.

8.0 Sampling Procedure and Sample Preparation

8.1 One 5-10 mL EDTA (lavender-top) tube sample is to be drawn by direct venipuncture at the post-infusion times:

8.1.1 Pre-infusion (from Step 7.3).

8.1.2 Optional: 2 hours after infusion. (Collect from the contralateral arm to that used for infusion.)

8.1.3 One sample daily between days 1 through day 7 (except weekends for samples <u>after</u> Day 1) and one final sample on day 10, 11 or 12. (The last sample is called the “baseline” sample and is used to correct for inadvertent labeling of other blood components.)

NOTE: As the points at the beginning of the curve are most important in determining the parameters of the curve, daily sampling on Days 1, 2, and 3 afford more accurate projections of recovery and survival parameters and should be taken if at all possible. A total of at least 5 data points from samplings on Days 1-7 should be obtained.

Delaying the final sample from Day 10 to Day 12 results in lower counts and, potentially, counts that are inadequate to achieve desired accuracy. Obtaining the final sample on Day 10, if possible, is desirable.

8.2 Sample Processing:

NOTE: Processing should be completed as soon as possible after sampling to prevent post-sampling elution from skewing the proportion of activity found in the cellular fraction.

8.2.1 Prior to the infusion, label and weigh capped 12 x 75 mm polystyrene tubes: one for each sampling time (including pre-sample) for whole blood, packed cells and plasma. Tubes for daily samples may be labeled and weighed on the day of use or beforehand.

8.2.2 Just prior to aliquoting sample, thoroughly mix each timed sample from the subject manually or for a maximum of two minutes on a rocker.

8.2.3 Pipet approximately 2.0 mL of the mixed whole blood into the appropriate pre-weighed tube using a positive displacement pipettor and replace cap.

8.2.4 Weigh the capped tube and record the weight. Take care that the sample does not touch the cap.

8.2.5 Pipet approximately 2.0 mL of blood into the appropriate tube labeled for packed cells.

8.2.6 Centrifuge this second set of tubes at 2000 x g for 15 minutes at room temperature.

8.2.7 Pipet the plasma from the packed cell tubes to the appropriate tube labeled for plasma.

8.2.8 Count the "splits" (packed cell and plasma tubes, including the injectate). (When possible, count the “splits” on the day of sampling.)

NOTE: The size of sample volumes may be increased if desired.

9.0 Elution of Radioactivity from Labeled Platelets in Whole Blood

9.1 Add 10 µL of the mixed injectate into a 5 or 7 mL EDTA tube of blood collected from the subject as soon as possible after the infusion of the labeled cells procedure. (See Section 4.1.2 or 5.1.2.).

Note: This timing is anticipating infusion of the radiolabeled platelets at the earliest possible time. If delay in infusion is anticipated, the elution steps described in this section should be begun so that the separation of the supernatant after the next step occurs approximately coincidently with the infusion.

9.2 Mix and incubate the tube for two hours at 37ºC in a wet incubator (water bath) or a dry incubator in a cylinder of water.

9.3 Pipet approximately 2 mL into a 12 x 75 mm capped tube.

9.4 Spin at 2000g for 15 minutes.

9.5 Transfer the supernatant plasma to a capped 12 x 75 mm capped tube.

9.6 Count the splits.

9.7 The activity found in the cells and plasma will be used to evaluate the in vivo cell-bound activity of the labeled platelets for each of the two radiolabels.

Elution = $(CPM_{supernate})/[(CPM_{cells}) + (CPM_{supernate})]$

10.0 Counting the Samples

10.1 Ensure required quality control and preventive maintenance procedures are performed according to the operating instructions. Load racks into counter and start counting. Count whole blood samples once all samples from the subject have been collected and processed (including Day 10, 11 or 12 sample). (One can also count standards and splits, daily.)

10.2 Load the gamma counter in a standard order, such as: blank/background, standards, elutions, pre-infusion, and post-infusion timed specimens in order collected.

10.3 Select appropriate counting program. Set count time to achieve a 2% accuracy, including background.

10.4 When the tubes have finished counting, label the instrument printout. Verify that the background has not drifted significantly. Verify that all samples counted for expected times. Place the gamma counter printouts in the appropriate file.

10.5 Transfer all data to appropriate spreadsheets for calculations. Verify accurate transcription.

11.0 Calculations

11.1 Determine corrected CPM /g for each sample.

Most gamma counters correct automatically for background activity and decay during counting as well as for “cross-up” and “cross-down”, that is, counting of one isotope’s activity in the other’s channels. Samples and standards must all be corrected for these parameters as well as corrected to the same time for calculations to be valid. Using these corrected CPM values reported by the gamma counter ($CPM_{corrected}$), determine CPM/g for standards and timed subject samples as follows:

11.1.1 Standards: STD = $CPM_{corrected}$ * dilution factor/mass counted, where dilution factor = mass of standard/mass of injectate added

NOTE: Since the diluent for the standard is water, the specific gravity of the standard may be assumed to be 1.00, and the final volume of the standard in mL may be taken as its mass in g.

11.1.2 Timed sample activity: SAMPLE = $CPM_{corrected}$ /mass counted

11.2 Determine cell-bound proportion for timed count adjustment

Using timed split samples (Section 8.2.8), determine the proportion of the activity in each timed sample that can be associated with the cellular fraction:

Cell Bound Fraction ($CBF_{Time\ t}$) = (Activity of cells)/(Activity of cells + Activity of plasma) = ($CPM_{cell\ bound}$)/[($CPM_{cell\ bound}$) + (CPM_{plasma})]

11.3 Standard adjustment for injectate elution:

11.3.1 Determine the mean CPM of the three mixed standards (Steps 6.1 and 11.1.1).

11.3.2 Multiply the mean CPM of the standard samples by the elution correction (Step 9.7).

$STD_{corrected}$ = STD * (1-Elution)

11.4 Adjustment for cell-bound proportion and baseline

The following two adjustments should be made to the timed sample CPM/g counts. These adjustments result in "fully adjusted counts" used for curve fitting.

11.4.1 Adjustment of timed sample for cell-bound proportion (Steps 11.1.2, 11.2):

SAMPLE $_{Time\ t}$ = SAMPLE (CPM/g) x ($CBF_{Time\ t}$)

Adjust for non-platelet residual activity baseline, according to the day on which the "baseline" sample was taken. (This results in fully adjusted timed sample counts.).

11.4.1.1 Day 10

Fully adjusted count = SAMPLE $_{Time\ t}$ – [SAMPLE $_{Day\ 10}$ x (1.20-0.20 (Time t/Time of Day 10 sample)]

11.4.1.2 Or, Day 11

Fully adjusted count = SAMPLE $_{\text{Time t}}$ – [SAMPLE $_{\text{Day 11}}$ x (1.22-0.22 (Time t/Time of Day 11 sample)]

11.4.1.3 Or, Day 12

Fully adjusted count = SAMPLE $_{\text{Time t}}$ – [SAMPLE $_{\text{Day 12}}$ x (1.24-0.24 (Time t/Time of Day 12 sample)]

Note: "Time t" is the elapsed time in hours from the time of infusion (Step 7.4) to the time of sampling. "Time of Day 10 sample" is the elapsed time in hours from time of infusion (Step 7.4) to the time the sample was obtained on Day 10 following infusion.

These calculations are based on the published experience of reference 2.3 and the expectation that ^{51}Cr persistence is the primary source of persistent activity. Persistent activity attributable to ^{111}In is less of a concern because of its shorter half-life, its higher elution from any contaminating red cells that were labeled and the lack of oxine to facilitate labeling after elution from platelets.

11.5 Determination of expected time$_0$ count following infusion

11.5.1 Calculate blood volume:

Male: BV (mL) = ([0.3669*{height (m)} ^{3}] + 0.03219*weight (kg) + 0.6041)*1000

11.5.2 Female: BV (mL) = ([0.3561*{height (m)}^{3}] + [0.03308*weight (kg)] + 0.1833)*1000Convert the blood volume calculated in 11.5.1 to blood mass by multiplying the volume by the specific gravity of whole blood. The specific gravity may be taken as 1.05 g/mL, or, if the subject's hematocrit is known, it may be estimated as (1541 + Hct)/1500 (Subject hematocrit is expressed as a percentage rather than a decimal; expected SG usually between 1.053 and 1.060 g/mL.).

11.5.3 Determine expected time$_0$ (t=0) count (Steps 11.3.2, 7.0, 11.5.2)

Time$_0$ Count = STD$_{\text{corrected}}$ * Infusate mass (g) / Blood mass

11.6 Determine recovery for each timed sample (optional)

Take each fully adjusted sample count (11.4.2) and divide by the expected time0 count (11.5.3) to calculate the recovery at each sampling time:

Recovery $_{\text{Time t}}$ = Fully Adjusted Count $_{\text{Time t}}$ / Time$_0$ Count

NOTE: The recovery result that is reported from the study is determined using the time0 extrapolation of the curve fitting program.

11.7 Kinetic curve fitting

The fully adjusted timed-sample counts (Step 11.4.2) for times greater than 20 hours are used for kinetic curve fitting and estimation of recovery and survival. That is, the 2 hour time sample is not used for kinetic curve fitting. These parameters may be estimated using a validated non-linear curve fitting routine found in various statistical and pharmacokinetic computer program packages. The use of COST software, one of these software packages, is described below. Optionally, the recovery of timed-samples calculated in Step 11.6 may be used.

Recovery is determined as the extrapolated y-axis intercept (t=0) of the survival curve, expressed as the blood cell-bound activity in proportion to the expected activity as projected from the blood volume-based dilution.

The fully adjusted count data (CPM/g) for each timed sample point are entered into the COST program as y values, the time after infusion (in hours) as x values, and the expected $time_0$ count. (Optionally, the recovery of each timed sample from 11.6 may be entered as y values.)

11.7.1 Activate the COST program.

11.7.2 Select the appropriate group in COST into which the subject's data will be entered.

11.7.3 Enter the number of data points to be entered.

11.7.4 Enter the x- (time in hours) and y-values (fully adjusted timed sample count, Step 11.4.2) for each timed sample. Double check entry accuracy. (Optionally, enter the y value as recoveries from 11.6).

11.7.5 Enter the $time_0$ count from 11.5.3 into counts/milliliter field. (If the individual timed recoveries from 11.6 are used as y values, enter 100 into the counts/milliliter field in COST to set the 100% recovery point).

11.7.6 Perform data analyses using the multiple hit model.

11.7.7 If outliers are found, determine potential source of error and flag for review by medical director. Remove point from curve only if clearly a spurious result.

11.7.8 Save and print results. Append to unit records.

12.0 Completion of Records

12.1 Complete, review and file all forms, data and other paperwork.

13.0 BEST Protocol Modifications

Since the inception of the BEST protocol, sites have modified some steps of the protocol to BEST 4.2.1 Method. Some of these modifications include improvements to staff safety, adaptations to suit site specific equipment and simplification of the workflow to avoid errors. The protocol specific modifications for the BEST 4.2.1 Method are listed in Table 15.

Table 15 BEST Version 4.2.1 Modifications

Section	BEST Version 4.2.1 Method	Modified BEST Version 4.2.1 Method
3.12	^{111}Indium Oxine in ethanol solution containing approximately 1 mCi per vial at the time of manufacturer's calibration.	^{111}Indium Oxine in ethanol solution containing approximately 1-2 mCi per vial at the time of manufacturer's calibration.
3.13	^{111}Indium Oxine Labeling solution: If not premixed, aseptically add 4.0 mL sterile ACD- A/saline solution to the vial of ^{111}Indium Oxine to make 5.0 mL of sterile ^{111}In-Oxine solution for labeling. Mix well. (Stable for one week or until expiration of stock ^{111}In oxine solution.).	1 mCi ^{111}Indium Oxine Labeling solution: If not premixed, aseptically add 4.0 mL sterile ACD- A/saline solution to the vial of 1mCi ^{111}Indium Oxine to make 5.0 mL of sterile ^{111}In-Oxine solution for labeling. 2mCi ^{111}Indium Oxine Labeling solution: aseptically add 2mL Oxine Suspension buffer to the vial of 2mCi ^{111}Indium Oxine to make 3mL ^{111}Indium Oxine suspension. Aseptically mix approximately 83µL of the ^{111}Indium Oxine suspension with 1mL of ACD-A/Saline to create 50-60 µCi labeling solution. Mix well (Stable for one week or until expiration of stock ^{111}In Oxine solution).
4.1.2	At the same time as 4.1.1, collect one 5 or 7 mL blood sample from the subject into an EDTA tube. Mix well. Reserve for determination of radiolabel elution (see Step 9.0.).	This tube can be taken here or during draw listed in Section 7.3.
4.2.2	Using sterile technique, in a laminar flow hood, gently remove 10 mL from the stored platelet unit using a 20 mL syringe and 16 ga needle.	Using sterile technique, in a laminar flow hood, gently remove two - 10mL from the stored platelet unit using sterilely docked transfer packs.
4.2.3	Remove the needle and express the contents of the syringe into a 15 mL screw-cap conical labeled centrifuge tubes containing 1.5 mL sterile ACD-A. Cap and mix gently by inversion.	Express the contents of the syringe into two – 15 or 50 mL screw-cap conical labeled centrifuge tubes each containing 1.5 mL sterile ACD-A (15% by volume, pH 4.5 – 5.5). Cap and mix gently by inversion or swirling.

Section	BEST Version 4.2.1 Method	Modified BEST Version 4.2.1 Method
4.2.5	Remove the PRP with a sterile transfer pipette or spinal needle and syringe and place into a new 15 mL sterile conical plastic centrifuge tube.	Remove PRP with a sterile transfer pipette or spinal needle and syringe and place into a new 15 or 50 mL sterile conical plastic centrifuge tube.
4.4	Remove the platelet-poor plasma (PPP) produced, as completely as possible and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 x g for 15 minutes with brake off), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 4.6.2 and 4.6.5).	Remove the platelet-poor plasma (PPP) produced, as completely as possible and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 x g for 15 minutes with brake off or brake minimum to allow for addition of PPP to platelet sample within 20 minutes of platelet labeling (Section 4.6.1)), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 4.6.2 and 4.6.5).
4.5	Using a sterile pipet, resuspend the harvested platelet pellet with 2 mL of ACD-A/saline solution. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting radiolabeling. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure).	Using a sterile pipet, resuspend the harvested platelet pellet with 2 mL of ACD-A/saline solution. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting radiolabeling. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure). Before proceeding to radiolabeling the platelets, one aliquot of Test platelets will be stopped and used only for in vitro analysis.
4.6.3	Centrifuge the tube at 1250 x g at 20 to 24°C for 10 minutes with brake off.	Centrifuge the tube at 1200 to 1250 x g at 20 to 24°C for 10 minutes with brake off.
5.2.2	Using sterile technique, in a laminar flow hood, gently remove 20 (± 2 mL) mL from the stored platelet unit using a 30 mL syringe and 16 ga needle.	Using sterile technique, in a laminar flow hood, gently remove two – 20mL from the stored platelet unit using sterilely docked transfer packs.
5.2.3	Remove the needle and express 10 mL of the contents of the syringe into each of two 15 mL screw-cap conical labeled centrifuge tubes containing 1.5 mL sterile ACD-A . Cap and mix gently by inversion.	Express 10 mL of the contents of the syringes into four - 50 mL screw-cap conical labeled centrifuge tubes each containing 1.5 mL sterile ACD-A (15% by volume). Cap and mix gently by inversion or swirling.
5.2.5	Remove the PRP with a sterile transfer pipette or spinal needle and syringe and place into a new 15 mL sterile conical plastic centrifuge tube.	Remove PRP with a sterile transfer pipette or spinal needle and syringe and place into a new 50 mL sterile conical plastic centrifuge tubes.
5.4	Remove the platelet-poor plasma (PPP) produced, as completely as possible from both tubes and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 x g for 15 minutes at 20 to 24ºC with brake off), discard pellet and reserve supernatant PPP in	Remove the platelet-poor plasma (PPP) produced, as completely as possible from both tubes and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 x g for 15 minutes at 20 to 24ºC with brake off or brake minimum to allow for addition of PPP

Section	BEST Version 4.2.1 Method	Modified BEST Version 4.2.1 Method
	a sterile tube for later use (Steps 5.6.2 and 5.6.5).	to platelet sample within 20 minutes of platelet labeling (Section 5.6.1), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 5.6.2 and 5.6.5).
5.5	Resuspend the harvested platelet pellet in one tube with 3 mL of ACD-A/saline solution. Using a sterile transfer pipette, transfer the resuspended platelets to the other tube containing the platelet pellet. Resuspend the second platelet button. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting the next step.	Resuspend the harvested platelet pellet in one tube with 3 mL of ACD-A/saline solution. For Fresh platelets using a sterile transfer pipette, transfer the resuspended platelets to the other tube containing the platelet pellet and resuspend the second platelet button. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting the next step. Before proceeding to radiolabeling the platelets, one aliquot of Test platelets will be stopped and used only for in vitro analysis.
5.6.3	Centrifuge the tube at 1250 x g at 20 to 24°C for 10 minutes with brake off.	Centrifuge the tube at 1200 to 1250 x g at 20 to 24°C for 10 minutes with brake off.
7.3	Perform venipuncture with a 19-21 gauge butterfly infusion needle. Draw one 10 mL EDTA (lavender-top) tube for the pre-infusion sample.	Perform venipuncture with a 19 gauge butterfly infusion needle. Draw one (or 2 if tube for elution was not drawn earlier) 10 mL EDTA (lavender-top) tube for the pre-infusion sample.
7.7	Weigh empty infusion syringe, needle and needle cover. Record weight on the data sheet. Record the net weight of injectate.	Remove needle, cap syringe with syringe cap, and weigh syringe with syringe cap. Record weight on the data sheet. Record the net weight of injectate.
8.1.2	Optional: 2 hours after infusion. (Collect from the contralateral arm to that used for infusion.).	Collect blood draw at 1 and 2 hours after infusion. (Collect from the contralateral arm to that used for infusion.).
8.1.3	One sample daily between days 1 through day 7 (except weekends for samples after Day 1) and one final sample on day 10, 11 or 12. (The last sample is called the “baseline” sample and is used to correct for inadvertent labeling of other blood components.).	Sample on days 1, 2, 3, 4 (or 5 or 6), 7 (or 8) and one final sample on day 10, 11 or 12. (The last sample is called the “baseline” sample and is used to correct for inadvertent labeling of other blood components.).
8.2.3	Pipet approximately 2.0 mL of the mixed whole blood into the appropriate pre-weighed tube using a positive displacement pipettor and replace cap.	This sample is prepared and counted duplicate.
9.3 – 9.6	Pipet approximately 2 mL into a 12 x 75 mm capped tube.	This sample is prepared and counted duplicate.

Appendix C Radiolabeling Procedure for Platelets (Variant 1)

Platelet Radiolabeling Procedure 4.2.1 with Variant 1 Modifications (shown as strikethrough text indicating deletions or capitalized text indicating additions)

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INTRODUCTION

This file contains a procedure for the radiolabeling of both stored and fresh platelets and their autologous reinfusion for the determination of recovery and survival as published in the November, 2006 supplement to *Transfusion*. It is being made available on the BEST website in a downloadable format to facilitate implementation as the routine procedure in laboratories performing this kind of work. When referencing this procedure, please cite the *Transfusion* Supplement.

As detailed in other manuscripts in the Supplement, this procedure was derived from the validated, seminal work of Andrew Heaton, Stein Holme and colleagues in Norfolk, Virginia, and adapted for application to Scott Murphy's insightful approach of comparing a new platelet preparation to fresh platelets from the same subject. The effort of adapting the procedure (kindly made available by the American Red Cross) for this purpose and thoroughly discussing and debating its content were undertaken by the members of the Biomedical Excellence for Safer Transfusion (BEST) Collaborative through multiple meetings over a two-year period. The validation of the adaptation and its application can be found reviewed in other papers in this Supplement. Deviations from this procedure should be accompanied by appropriate validation and documentation.

Many individuals contributed substantial time and effort in developing, reviewing and validating this procedure. In particular, the leadership of the authors of the other articles in this Supplement should be noted as well as the meticulous reviewing undertaken by Lisa Cooke, Louise Herschel, Jill Roger, Sherrill Slichter, and Ralph Vassallo.

BEST welcomes receipt of comments regarding the procedure and suggestions for further development.

PROCEDURE

1.0 Purpose: To describe a technique for radioisotopic labeling and infusion of fresh or stored platelets with ^{111} In Oxine (^{111}Indium) and $Na_2{}^{51}CrO_4$ (^{51}Chromium).

1.1 While the measurement of platelet recovery and survival can be used for a variety of purposes, this procedure is intended for assessment of the in vivo viability of platelets that have been stored and/or treated in comparison with fresh platelets from the same subject

1.2 This procedure includes the basic calculations for determining platelet recovery and survival. The recommended manner of comparing the results between control and test infusions is through comparison of the paired differences and calculation of upper confidence intervals as detailed by Dumont in this supplement (See 2.6.)

2.0 Applicable documents:

2.1 Snyder EL, Moroff G, Simon T, Heaton A, and Members of the Ad Hoc Platelet Radiolabeling Study Group. Recommended methods for conducting radiolabeled platelet survival studies. Transfusion 1986; 26:37-42

2.2 Panel Report, International Committee for Standardization in Hematology. Recommended methods for radioisotopic platelet survival studies. Blood 1977; 50:1137-1144

2.3 Holme S., Heaton A., Roodt J. Concurrent label method with ^{111}In and ^{51}Cr allows accurate evaluation of platelet viability of stored platelet concentrates. Brit J Haematol 1993;84:717-723.

2.4 Nadler SB, Hidalgo JU, Bloch T. Prediction of blood volume in normal human adults. Surgery 1962;51:224-232

2.5 Lötter MG, Rabe WL, Van Zyl JM, et al. A computer program in compiled BASIC for the IBM personal computer to calculate the mean platelet survival time with the multiple-hit and weighted mean methods. Comput Biol Med 1988;18:305-15

2.6 Dumont LJ. Analysis and reporting of platelet kinetic studies. Transfusion 2006;46 (Suppl) 67S-73S

3.0 Equipment/Supplies/Reagents

3.1 Calibrated, temperature-controlled, variable speed, swinging bucket centrifuge.

3.2 Calibrated electronic balance, accurate to ± 0.0002 g.

3.3 Gamma counter with 3 inch sodium chloride crysta

3.4 Laminar flow hood.

3.5 9-21 gauge butterfly or straight needle

3.6 Sterile transfer pipettes (3.2 mL draw)

3.7 15 mL and 50 mL sterile conical polypropylene plastic tubes (Note: Although some procedural steps specify exact sizes, an alternative appropriate size may be substituted.)

3.8 Evacuated sample tubes with EDTA (powdered or dry "lavender tops").

3.9 ACD-A: Anticoagulant Citrate Dextrose Solution - Formula A

3.10 ACD-A/ Saline Solution: mix 1 part ACD-A to 7 parts sterile (0.9%) Normal Saline, adjusted to pH 6.5 to 6.8 with 1 N NaOH; sterilize by filtration. [Stable for one week when stored in refrigerator; warm to room temperature (20-24°C) before use.]

3.11 0.22µm Sterilizing Filter Unit

3.12 ^{111}Indium Oxine in ethanol solution containing approximately 1 mCi per vial at the time of manufacturer's calibration. Use within 7 d for platelet labeling. (Changes in ethanol/oxine ratio over time may result in changes in platelet function.)

3.13 ^{111}Indium Oxine Labeling solution: If not premixed, aseptically add 4.0 mL sterile ACD- A/saline solution to the vial of ^{111}Indium Oxine to make 5.0 mL of sterile ^{111}In-Oxine solution for labeling. Mix well. (Stable for one week or until expiration of stock ^{111}In oxine. solution.)

3.14 $Na_2\ ^{51}CrO_4$, Chromium-51

3.15 18 gauge spinal needles sterile

3.16 Assorted sterile syringes and needles (Note: Although some procedural steps specify exact sizes, any appropriate size may be substituted.)

3.17 Positive displacement pipettes for whole blood aliquots.

3.18 Assorted size pipettes and tips

3.19 SDS (Sodium Dodecyl Sulfate) 20%; Dissolve 20g sodium dodecyl sulfate in 100 mL of distilled water. (Store at room temperature for up to one year.).

NOTE: All vessels holding platelets or plasma that will be reinfused must be properly labeled to definitively identify the subject

4.0 Preparation of Platelets for Labeling with ^{111}Indium Oxine

4.1 Preparation of freshly collected platelets

4.1.1 Using a 19-gauge needle, collect 43 mL (± 2 mL) of venous blood into a 60 mL sterile plastic syringe containing 9 mL of ACD-A. Avoid a traumatic venipuncture at the start of blood drawing. Care should be taken to avoid excessive negative pressure in the syringe during draw to prevent activation of the platelets. (Alternatively, 43 mL of whole blood

can be collected with 7 mL of ACD-A followed by a 1-2 h holding period in Step 4.1.3.)

4.1.2 At the same time as 4.1.1, collect one 5 or 7 mL blood sample from the subject into an EDTA tube. Mix well. Reserve for determination of radiolabel elution (see Step 9.0)

4.1.3 Using sterile technique, in a laminar flow hood, remove the needle and express the contents of the syringe into two sterile 50 mL screw-cap conical centrifuge tubes. A hold period of 30 minutes for the ACD-A/blood mixture before further processing should be utilized.

4.1.4 Centrifuge (soft spin) the conical tubes at 180 to 200 x g for 15 minutes at 20 to 24°C with the brake off to produce red cell-poor platelet–rich plasma (PRP)

4.1.5 Remove the PRP from both tubes with a sterile transfer pipette or spinal needle and syringe and place into a single 50 mL sterile conical plastic centrifuge tube. Avoid aspirating any contaminating red cells.

4.1.6 Add a volume of sterile ACD-A equal to 15% of the volume of PRP to the PRP. Cap the tube and mix gently by inversion. Continue with Step 4.3

4.2 Preparation of aliquot from stored platelet unit

4.2.1 Ensure unit is thoroughly mixed and platelets are completely suspended.

4.2.2 Using sterile technique, in a laminar flow hood, gently remove 10 mL from the stored platelet unit using a 20 mL syringe and 16 ga needle.

4.2.3 Remove the needle and express the contents of the syringe into a 15 mL screw-cap conical labeled centrifuge tubes ~~containing 1.5 mL sterile ACD-A~~. Cap and mix gently by inversion

~~4.2.4 Centrifuge (soft spin) the conical tubes at 180 to 200 x g for 5 minutes at 20 to 24ºC with the brake off to produce red cell poor platelet rich plasma (PRP).~~

~~4.2.5 Remove the PRP with a sterile transfer pipette or spinal needle and syringe and place into a new 15 mL sterile conical plastic centrifuge tube. Avoid aspirating any contaminating red cells. Continue with Step 4.3.~~

4.3 Centrifuge (hard spin) the ACD-PRP **(FRESH SAMPLE) OR STORED PLATELET SAMPLE** at 1500-2000 x g for 15 minutes at 20 to 24ºC with brake off

4.4 Remove the platelet-poor plasma (PPP) produced, as completely as possible and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 x g for 15 minutes with brake off), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 4.6.2 and 4.6.5)

4.5 Using a sterile pipet, resuspend the harvested platelet pellet with 2 mL of ACD-A/saline solution. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting radiolabeling. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.)

4.6 Platelet Labeling with ^{111} Indium-Oxine

4.6.1 Add 50 to 60 µCi of ^{111} Indium-Oxine labeling solution (Step 3.13) to the washed platelet suspension. Incubate at 20 to 24°C for 20 minutes to achieve labeling. (Note: 1 Ci = $3.7x10^{10}$ Bq)

4.6.2 At the end of incubation, add 3.5 mL of ACD-A/saline and 0.5 mL of **STORED AUTOLOGOUS PLASMA** ~~the reserved PPP (from Step 4.4)~~ directly to the incubation tube containing the platelets in ACD-A/saline and ^{111}In.

4.6.3 Centrifuge the tube at 1250 x g at 20 to 24°C for 10 minutes with brake off.

4.6.4 Remove the supernatant and retain in a separate test tube. Determine the activity of this supernatant and the labeled pellet in a dose calibrator. Calculate and note the efficiency of labeling by dividing the activity of the pellet by the combined activities of the pellet and supernatant.

4.6.5 Gently resuspend the platelet-pellet in 2 mL of **STORED AUTOLOGOUS PLASMA** ~~PPP reserved previously (from Step 4.4)~~. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) After complete resuspension, add an additional 4 mL of **STORED AUTOLOGOUS PLASMA** ~~PPP~~.

5.0 Preparation of Platelets for Labeling with $Na_2{}^{51}CrO_4$

5.1 Labeling of fresh platelets

5.1.1 Using a 19-gauge needle, collect 43 mL (± 2 mL) of venous blood into a 60 mL sterile plastic syringe containing 9 mL of ACD-A. Avoid a traumatic venipuncture at the start of blood drawing. Care should be taken to avoid excessive negative pressure in the syringe during draw to prevent activation of the platelets. (Alternatively, 43 mL of whole blood can be collected with 7 mL of ACD-A followed by a 1-2 h holding period in Step 5.1.3.)

5.1.2 At the same time as 5.1.1, collect one 5 or 7 mL blood sample from the subject into an EDTA tube. Mix well. Reserve for determination of radiolabel elution (see Step 9.0)

5.1.3 Using sterile technique, in a laminar flow hood, remove the needle and express the contents of the syringe into two sterile 50 mL screw-cap conical centrifuge tubes. A hold period of 30 minutes for the ACD-A/blood mixture before further processing should be utilized

5.1.4 Centrifuge (soft spin) the conical tubes at 180 to 200 x g for 15 minutes at 20 to 24°C with the brake off to produce red cell-poor PRP.

5.1.5 Remove the PRP from both tubes with a sterile transfer pipette or spinal needle and syringe and place into a single 50 mL sterile conical plastic centrifuge tube. Avoid aspirating any contaminating red cells.

5.1.6 Add a volume of sterile ACD-A equal to 15% of the volume of PRP to the PRP. Cap the tube and mix gently by inversion. Continue with Step 5.3

5.2 Preparation of aliquot from stored platelet unit

5.2.1 Ensure unit is thoroughly mixed and platelets are completely suspended.

5.2.2 Using sterile technique, in a laminar flow hood, gently remove 20 (± 2 mL) mL from the stored platelet unit using a 30 mL syringe and 16 ga needle

~~5.2.3~~ Remove the needle and express 10 mL of the contents of the syringe into each of two 15 mL screw-cap conical labeled centrifuge tubes ~~each containing 1.5 mL sterile ACD-A~~ . Cap and mix gently by inversion

~~5.2.4 Centrifuge (soft spin) the conical tubes at 180 to 200 x g for 5 minutes at 20 to 24ºC with the brake off to produce red cell poor platelet rich plasma (PRP).~~

~~5.2.5 Remove the PRP with a sterile transfer pipette or spinal needle and transfer to two new sterile 15 mL conical plastic centrifuge tubes. Avoid aspirating any contaminating red cells. Continue with Step 5.3.~~

5.3 Centrifuge (hard spin) the conical tubes containing the ACD-PRP **(FRESH SAMPLE) OR STORED PLATELET SAMPLE** at 1500-2000 x g for 15 minutes at 20 to 24ºC with brake off

5.4 Remove the platelet-poor plasma (PPP) produced, as completely as possible from both tubes and transfer to a new sterile conical tube. ~~Spin PPP a second time (1500–2000 x g for 15 minutes at 20 to 24ºC with brake off), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 5.6.2 and 5.6.5).~~

5.5 Resuspend the harvested platelet pellet in one tube with 3 mL of ACD-A/saline solution. Using a sterile transfer pipette, transfer the resuspended platelets to the other tube containing the platelet pellet. Resuspend the second platelet button. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting the next step

5.6 Platelet Labeling with Na251CrO4

5.6.1 Add 50 to 100 µCi $Na_2{}^{51}CrO_4$ to the washed platelet suspension. Incubate at 20 to 24°C for 20 minutes to achieve labeling

5.6.2 At the end of incubation, add 2.5 mL of ACD-A/saline and 0.5 mL **STORED AUTOLOGOUS PLASMA** ~~of the reserved PPP (from Step 5.4)~~ directly to the incubation tube containing the platelets in ACD-A/saline and $Na_2{}^{51}CrO_4$

5.6.3 Centrifuge the tube at 1250 x g at 20 to 24°C for 10 minutes with brake off

5.6.4 Remove the supernatant and retain in a separate test tube. Determine the activity of this supernatant and the labeled pellet in a dose calibrator. Calculate and note the efficiency of labeling by dividing the activity of the pellet by the combined activities of the pellet and supernatant

5.6.5 Gently resuspend the platelet-pellet in 2 mL of **STORED AUTOLOGOUS PLASMA** ~~PPP reserved previously (from Step 5.4)~~. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) After complete resuspension, add an additional 4 mL of **STORED AUTOLOGOUS PLASMA** ~~PPP~~.

6.0 Injectate and Standard Preparation

6.1 Label five 100 mL volumetric flasks and five 12 x 75 mm polystyrene tubes with the isotope used, subject name and/or unit identification, date prepared and In STD, Cr STD, and STD I, II or III

NOTE: The In STD and the Cr STD are necessary only if calculation of "cross--p" and "cross-down" corrections requires them

6.2 Add 1.8 mL 0.9% saline to each 12 x 75 mm standard capped tube. Weigh each tube and record the weights. Take care that the saline does not touch the cap.

6.3 Add 10 mL of 0.2% SDS-saline solution (1 part saturated (20%) SDS + 99 parts 0.9% saline) to each flask.

6.4 Mix each single labeled platelet aliquots (from Steps 4.6.5 and 5.6.5). Draw 0.3mL of each suspension into its own 1cc syringe through a 16-gauge needle.

6.5 Weigh the filled syringe, needle and needle cover accurately. Record the weight

6.6 Carefully add 0.2 mL of each injectate directly into each flask, "Cr STD" or "In STD", as appropriate. Add 0.9% saline to the flask to bring to volume. Mix carefully. Weigh the syringe, needle and needle cover accurately and determine the mass of labeled injectate placed in each standards flask

6.7 Draw the appropriate radioactive dose of ^{51}Cr-labeled platelets into a labeled syringe. Weigh the syringe, and record the radioactive dose

NOTE: The activity needed to be injected is dependent on a number of variables, such as the gamma counter's crystal size. In general, 10-20 μCI are

required to be injected for accurate determinations. The injectates of the fresh and stored platelets should have approximately the same activities

6.8 Remove ^{111}In-labeled platelets from the centrifuge tube until the dose intended to be injected is remaining. Measure and record the radioactive dose. Record the weight of the tube

6.9 Carefully verify subject identification on the chromium syringe and indium tube. Inject the ^{51}Cr-labeled platelets into the centrifuge tube containing the ^{111}In-labeled platelets

6.10 Mix the labeled injectate in the tube. Prepare final mixed injectate for infusion by using an 18 gauge spinal needle and a syringe labeled with subject information to aspirate all but at least 1 mL of labeled solution in conical tube. (This remaining amount will be used for preparation of standards and determination of cell bound activity.)

6.11 Remove the spinal needle and replace with a sterile syringe cap. Record the weight of the final injectate syringe and cap

6.12 Draw approximately 0.8 mL of the remaining labeled solution from the tube containing the ^{51}Cr- and ^{111}In-labeled platelets into a 1cc syringe through a 16-gauge needle to be used for standard preparation

6.13 Record the weight of the filled syringe, needle, and needle cover

6.14 Carefully add 0.2 mL of the injectate directly into the flask labeled "STD I". Add 0.9% saline to the flask to bring to volume. Mix carefully

6.15 Reweigh the 1 cc syringe, needle and needle cover and record this weight and the difference between the two weights (this being the net weight of the mixed injectate added to the standards flask)

6.16 Repeat Steps 6.14 and 6.15 for the remaining two flasks

6.17 Pipet 0.2 mL of each standard into the appropriate pre-weighed capped tube containing 1.8 mL saline, one sample tube per standard. ALTERNATIVELY, IF THIS PROCESS RESULTS IN STANDARD COUNTS LESS THAN 10-FOLD HIGHER THAN BACKGROUND, PIPET 2.0 ML OF THE STANDARD FROM THE 100ML DILUTED VOLUMETRIC FLASK INTO AN APPROPRIATE PRE-WEIGHED CAPPED TUBE FOR DIRECT COUNTING

6.18 Weigh the capped tubes and record the weight. Take care that the sample does not touch the cap

7.0 Infusion Procedure

7.1 Allow recipient to sit in the donor bed to stabilize. Record pre-infusion vital signs

7.2 Verify that recipient's identifiers match the information on the infusion syringe

7.3 Perform venipuncture with a 19-21 gauge butterfly infusion needle. Draw one 10 mL EDTA (lavender-top) tube for the pre-infusion sample

7.4 Flush the line with saline to ensure patency of the vein. Inject labeled platelet suspension slowly. Record exact time (starting from time 1/2 of injectate has been infused)

7.5 Flush butterfly line and needle with 10 mL of sterile 0.9% sodium chloride

7.6 Remove butterfly needle and apply pressure to injection site. Apply bandage when bleeding has stopped

7.7 Weigh empty infusion syringe, needle and needle cover. Record weight on the data sheet. Record the net weight of injectate

7.8 Dispose of used supplies in the receptacle labeled for radioactive waste

7.9 Measure and record post-infusion vital signs

8.0 Sampling Procedure and Sample Preparation

8.1 One 5-10 mL EDTA (lavender-top) tube sample is to be drawn by direct venipuncture at the post-infusion times:

8.1.1 Pre-infusion (from Step 7.3)

8.1.2 Optional: 2 hours after infusion. (Collect from the contralateral arm to that used for infusion.)

8.1.3 One sample daily between days 1 through day 7 (except weekends for samples after Day 1) and one final sample on day 10, 11 or 12. (The last sample is called the "baseline" sample and is used to correct for inadvertent labeling of other blood components.)

NOTE: As the points at the beginning of the curve are most important in determining the parameters of the curve, daily sampling on Days 1, 2, and 3 afford more accurate projections of recovery and survival parameters and should be taken if at all possible. A total of at least 5 data points from samplings on Days 1-7 should be obtained

Delaying the final sample from Day 10 to Day 12 results in lower counts and, potentially, counts that are inadequate to achieve desired accuracy. Obtaining the final sample on Day 10, if possible, is desirable.

8.2 Sample Processing:

NOTE: Processing should be completed as soon as possible after sampling to prevent post-sampling elution from skewing the proportion of activity found in the cellular fraction.

8.2.1 Prior to the infusion, label and weigh capped 12 x 75 mm polystyrene tubes: one for each sampling time (including pre-sample) for whole

blood, packed cells and plasma. Tubes for daily samples may be labeled and weighed on the day of use or beforehand

8.2.2 Just prior to aliquoting sample, thoroughly mix each timed sample from the subject manually or for a maximum of two minutes on a rocker

8.2.3 Pipet approximately 2.0 mL of the mixed whole blood into the appropriate pre-weighed tube using a positive displacement pipettor and replace cap

8.2.4 Weigh the capped tube and record the weight. Take care that the sample does not touch the cap

8.2.5 Pipet approximately 2.0 mL of blood into the appropriate tube labeled for packed cells

8.2.6 Centrifuge this second set of tubes at 2000 x g for 15 minutes at room temperature

8.2.7 Pipet the plasma from the packed cell tubes to the appropriate tube labeled for plasma

8.2.8 Count the "splits" (packed cell and plasma tubes, including the injectate). (When possible, count the “splits” on the day of sampling.)

NOTE: The size of sample volumes may be increased if desired

9.0 Elution of Radioactivity from Labeled Platelets in Whole Blood

9.1 Add 10 µL of the mixed injectate into a 5 or 7 mL EDTA tube of blood collected from the subject as soon as possible after the infusion of the labeled cells procedure. (See Section 4.1.2 or 5.1.2.)

Note: This timing is anticipating infusion of the radiolabeled platelets at the earliest possible time. If delay in infusion is anticipated, the elution steps described in this section should be begun so that the separation of the supernatant after the next step occurs approximately coincidently with the infusion

9.2 Mix and incubate the tube for two hours at 37ºC in a wet incubator (water bath) or a dry incubator in a cylinder of water

9.3 Pipet approximately 2 mL into a 12 x 75 mm capped tube.

9.4 Spin at 2000g for 15 minutes

9.5 Transfer the supernatant plasma to a capped 12 x 75 mm capped tube

9.6 Count the splits

9.7 The activity found in the cells and plasma will be used to evaluate the in vivo cell-bound activity of the labeled platelets for each of the two radiolabels

$$\text{Elution} = (CPM_{supernate})/[(CPM_{cells}) + (CPM_{supernate})]$$

10.0 Counting the Samples

10.1 Ensure required quality control and preventive maintenance procedures are performed according to the operating instructions. Load racks into counter and start counting. Count whole blood samples once all samples from the subject have been collected and processed (including Day 10, 11 or 12 sample). (One can also count standards and splits, daily.)

10.2 Load the gamma counter in a standard order, such as: blank/background, standards, elutions, pre-infusion, and post-infusion timed specimens in order collected

10.3 Select appropriate counting program. Set count time to achieve a 2% accuracy, including background.

10.4 When the tubes have finished counting, label the instrument printout. Verify that the background has not drifted significantly. Verify that all samples counted for expected times. Place the gamma counter printouts in the appropriate file

10.5 Transfer all data to appropriate spreadsheets for calculations. Verify accurate transcription

11.0 Calculations

11.1 Determine corrected CPM /g for each sample

Most gamma counters correct automatically for background activity and decay during counting as well as for "cross-up" and "cross-down", that is, counting of one isotope's activity in the other's channels. Samples and standards must all be corrected for these parameters as well as corrected to the same time for calculations to be valid. Using these corrected CPM values reported by the gamma counter ($CPM_{corrected}$), determine CPM/g for standards and timed subject samples as follows:

11.1.1 Standards: STD = $CPM_{corrected}$ * dilution factor/mass counted, where dilution factor = mass of standard/mass of injectate added

NOTE: Since the diluent for the standard is water, the specific gravity of the standard may be assumed to be 1.00, and the final volume of the standard in mL may be taken as its mass in g.

Dilution of standards must be calibrated to ensure that measurements at baseline are > 10 fold higher than background

11.1.2 Timed sample activity: SAMPLE = $CPM_{corrected}$ /mass counted

11.2 Determine cell-bound proportion for timed count adjustment

Using timed split samples (Section 8.2.8), determine the proportion of the activity in each timed sample that can be associated with the cellular fraction:

Cell Bound Fraction ($CBF_{Time\ t}$) = (Activity of cells)/(Activity of cells + Activity of plasma) = ($CPM_{cell\ bound}$)/[($CPM_{cell\ bound}$) + (CPM_{plasma})]

11.3 Standard adjustment for injectate elution:

11.3.1 Determine the mean CPM of the three mixed standards (Steps 6.1 and 11.1.1)

11.3.2 Multiply the mean CPM of the standard samples by the elution correction (Step 9.7)

$STD_{corrected}$ = STD * (1-Elution)

11.4 Adjustment for cell-bound proportion and baseline

The following two adjustments should be made to the timed sample CPM/g counts. These adjustments result in "fully adjusted counts" used for curve fitting

11.4.1 Adjustment of timed sample for cell-bound proportion (Steps 11.1.2, 11.2):

$SAMPLE_{Time\ t}$ = SAMPLE (CPM/g) x ($CBF_{Time\ t}$)

11.4.2 Adjust for non-platelet residual activity baseline, according to the day on which the "baseline" sample was taken. (This results in fully adjusted timed sample counts.)

11.4.2.1 Day 10

Fully adjusted count = $SAMPLE_{Time\ t}$ – [$SAMPLE_{Day\ 10}$ x (1.20-0.20 (Time t/Time of Day 10 sample)]

11.4.2.2 Or, Day 11

Fully adjusted count = $SAMPLE_{Time\ t}$ – [$SAMPLE_{Day\ 11}$ x (1.22-0.22 (Time t/Time of Day 11 sample)]

11.4.2.3 Or, Day 12

Fully adjusted count = $SAMPLE_{Time\ t}$ – [$SAMPLE_{Day\ 12}$ x (1.24-0.24 (Time t/Time of Day 12 sample)]

Note: "Time t" is the elapsed time in hours from the time of infusion (Step 7.4) to the time of sampling. "Time of Day 10 sample" is the

elapsed time in hours from time of infusion (Step 7.4) to the time the sample was obtained on Day 10 following infusion

These calculations are based on the published experience of reference 2.3 and the expectation that 51Cr persistence is the primary source of persistent activity. Persistent activity attributable to ^{111}In is less of a concern because of its shorter half-life, its higher elution from any contaminating red cells that were labeled and the lack of oxine to facilitate labeling after elution from platelets

11.5 Determination of expected $time_0$ count following infusion

11.5.1 Calculate blood volume:

Male: BV (mL) = ([0.3669*{height (m)}^{3}] + 0.03219*weight (kg) + 0.6041)*1000

Female: BV (mL) = ([0.3561*{height (m)}^{3}] + [0.03308*weight (kg)] + 0.1833)*1000

11.5.2 Convert the blood volume calculated in 11.5.1 to blood mass by multiplying the volume by the specific gravity of whole blood. The specific gravity may be taken as 1.05 g/mL, or, if the subject's hematocrit is known, it may be estimated as (1541 + Hct)/1500 (Subject hematocrit is expressed as a percentage rather than a decimal; expected SG usually between 1.053 and 1.060 g/mL.).

11.5.3 Determine expected $time_0$ (t=0) count (Steps 11.3.2, 7.0, 11.5.2)

$Time_0$ Count = $STD_{corrected}$ * Infusate mass (g) / Blood mass

11.6 Determine recovery for each timed sample (optional)

Take each fully adjusted sample count (11.4.2) and divide by the expected $time_0$ count (11.5.3) to calculate the recovery at each sampling time:

Recovery $_{Time\ t}$ = Fully Adjusted Count $_{Time\ t}$ / $Time_0$ Count

NOTE: The recovery result that is reported from the study is determined using the time0 extrapolation of the curve fitting program

11.7 Kinetic curve fitting

The fully adjusted timed-sample counts (Step 11.4.2) for times greater than 20 hours are used for kinetic curve fitting and estimation of recovery and survival. That is, the 2 hour time sample is not used for kinetic curve fitting. These parameters may be estimated using a validated non-linear curve fitting routine

found in various statistical and pharmacokinetic computer program packages. The use of COST software, one of these software packages, is described below. Optionally, the recovery of timed-samples calculated in Step 11.6 may be used

Recovery is determined as the extrapolated y-axis intercept (t=0) of the survival curve, expressed as the blood cell-bound activity in proportion to the expected activity as projected from the blood volume-based dilution

The fully adjusted count data (CPM/g) for each timed sample point are entered into the COST program as y values, the time after infusion (in hours) as x values, and the expected $time_0$ count. (Optionally, the recovery of each timed sample from 11.6 may be entered as y values.)

11.7.1 Activate the COST program

11.7.2 Select the appropriate group in COST into which the subject's data will be entered

11.7.3 Enter the number of data points to be entered

11.7.4 Enter the x- (time in hours) and y-values (fully adjusted timed sample count, Step 11.4.2) for each timed sample. Double check entry accuracy. (Optionally, enter the y value as recoveries from 11.6)

11.7.5 Enter the $time_0$ count from 11.5.3 into counts/milliliter field. (If the individual timed recoveries from 11.6 are used as y values, enter 100 into the counts/milliliter field in COST to set the 100% recovery point)

11.7.6 Perform data analyses using the multiple hit model

11.7.7 If outliers are found, determine potential source of error and flag for review by medical director. Remove point from curve only if clearly a spurious result

11.7.8 Save and print results. Append to unit records

12.0 Completion of Records

12.1 Complete, review and file all forms, data and other paperwork.

13.0 Protocol Modifications for Variant 1 of BEST Version 4.2.1

13.1 The protocol specific modifications for Variant 1 of the BEST 4.2.1 Method are listed in **Table 16**. These modifications are similar to those described about in **Table 15**

Table 16 Variant 1 of BEST Version 4.2.1 Method Modifications

Section	Variant 1 of BEST Version 4.2.1 Method	Variant 1 of BEST Version 4.2.1 Method Modifications
3.12	^{111}Indium Oxine in ethanol solution containing approximately 1 mCi per vial at the time of manufacturer's calibration	^{111}Indium Oxine in ethanol solution containing approximately **1-2 mCi** per vial at the time of manufacturer's calibration
3.13	^{111}Indium Oxine Labeling solution: If not premixed, aseptically add 4.0 mL sterile ACD- A/saline solution to the vial of ^{111}Indium Oxine to make 5.0 mL of sterile ^{111}In-Oxine solution for labeling. Mix well. (Stable for one week or until expiration of stock ^{111}In oxine solution.)	1 mCi ^{111}Indium Oxine Labeling solution: If not premixed, aseptically add 4.0 mL sterile ACD- A/saline solution to the vial of 1mCi ^{111}Indium Oxine to make 5.0 mL of sterile ^{111}In-Oxine solution for labeling 2mCi ^{111}Indium Oxine Labeling solution: aseptically add 2mL Oxine Suspension buffer to the vial of 2mCi ^{111}Indium Oxine to make 3mL ^{111}Indium Oxine suspension. Aseptically mix approximately 83µL of the ^{111}Indium Oxine suspension with 1mL of ACD-A/Saline to create 50-60 µCi labeling solution. Mix well (Stable for one week or until expiration of stock ^{111}In Oxine solution).
4.1.2	At the same time as 4.1.1, collect one 5 or 7 mL blood sample from the subject into an EDTA tube. Mix well. Reserve for determination of radiolabel elution (see Step 9.0)	This tube can be taken here or during draw listed in Section 7.3
4.2.2	Using sterile technique, in a laminar flow hood, gently remove 10 mL from the stored platelet unit using a 20 mL syringe and 16 ga needle	Using sterile technique, in a laminar flow hood, gently remove **two – 10mL** from the stored platelet unit using **sterilely docked transfer packs**
4.2.3	Remove the needle and express the contents of the syringe into a 15 mL screw-cap conical labeled centrifuge ~~tubes containing 1.5 mL sterile ACD-A .~~ Cap and mix gently by inversion	Express the contents of the syringe into **two – 15 or 50 mL** screw-cap conical labeled centrifuge tubes ~~each containing **1.5 mL sterile ACD-A** (15% by volume, pH 4.5 – 5.5).~~ Cap and mix gently by inversion **or swirling**
4.3	Centrifuge (hard spin) the ACD-PRP **(FRESH SAMPLE) OR STORED PLATELET SAMPLE** at 1500-2000 x g for 15 minutes at 20 to 24ºC with brake off	Centrifuge (hard spin) the ACD-PRP **(FRESH SAMPLE) OR STORED PLATELET SAMPLE** at 1500-2000 x g for 15 minutes at 20 to 24ºC with brake off
4.4	Remove the platelet-poor plasma (PPP) produced, as completely as possible and transfer to a new sterile conical tube. Spin PPP a second time (1500-2000 x g for 15	Remove **AND SAVE** the supernatant, platelet-poor plasma (PPP) produced, as completely as possible and transfer to a new sterile conical tube. **Spin PPP a second time**

Section	Variant 1 of BEST Version 4.2.1 Method	Variant 1 of BEST Version 4.2.1 Method Modifications
	minutes with brake off), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 4.6.2 and 4.6.5)	**(1500-2000 x g for 15 min with brake off** or brake minimum to allow for addition of PPP to platelet sample within 20 minutes of platelet labeling (Section 4.6.1)**), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 4.6.2 and 4.6.5) (this spin may be done during incubation mock radiolabel)**
4.6.3	Centrifuge the tube at 1250 x g at 20 to 24°C for 10 minutes with brake off.	Centrifuge the tube at 1200 to 1250 x g at 20 to 24°C for 10 minutes with brake off.
4.5	Using a sterile pipet, resuspend the harvested platelet pellet with 2 mL of ACD-A/saline solution. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting radiolabeling. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.)	Using a sterile pipet, resuspend the harvested platelet pellet with 2 mL of ACD-A/saline solution. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting radiolabeling. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) **Before proceeding to radiolabeling the platelets, one aliquot of Test platelets will be stopped and used only for *in vitro* analysis**
4.6.5	Gently resuspend the platelet-pellet in 2 mL of **STORED AUTOLOGOUS PLASMA** ~~PPP reserved previously (from Step 4.4)~~. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) After complete resuspension, add an additional 4 mL of **STORED AUTOLOGOUS PLASMA** ~~PPP~~.	Gently resuspend the platelet-pellet **in 2 mL of PPP reserved previously (from Step 4.4).** (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) **After complete resuspension, add an additional 4 mL of PPP**
5.2.2	Using sterile technique, in a laminar flow hood, gently remove 20 (± 2 mL) mL from the stored platelet unit using a 30 mL syringe and 16 ga needle	Using sterile technique, in a laminar flow hood, gently remove **two – 20mL**from the stored platelet unit using **sterilely docked transfer packs**
5.2.3	Remove the needle and express 10 mL of the contents of the syringe into each of two 15 mL screw-cap conical labeled centrifuge tubes ~~each containing 1.5 mL sterile ACD-A~~ . Cap and mix gently by inversion	Express 10 mL of the contents of the syringes into **four - 50 mL** screw-cap conical labeled centrifuge tubes ~~each containing **1.5 mL sterile ACD-A** (15% by volume, pH 4.5 – 5.5).~~ Cap and mix gently by inversion **or swirling**
5.4	Remove the platelet-poor plasma (PPP) produced, as completely as possible from both tubes and transfer to a new sterile conical tube. ~~Spin PPP a second time~~	Remove the platelet-poor plasma (PPP) produced, as completely as possible and transfer to a new sterile conical tube. **Spin PPP a second time (1500–2000 x g for 15**

Section	Variant 1 of BEST Version 4.2.1 Method	Variant 1 of BEST Version 4.2.1 Method Modifications
	~~(1500–2000 x g for 15 minutes at 20 to 24ºC with brake off), discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 5.6.2 and 5.6.5).~~	**minutes at 20 to 24ºC with brake off)** or brake minimum to allow for addition of PPP to platelet sample within 20 minutes of platelet labeling (Section 5.6.1)**, discard pellet and reserve supernatant PPP in a sterile tube for later use (Steps 5.6.2 and 5.6.5) (this spin may be done during incubation mock radiolabel)**
5.5	Resuspend the harvested platelet pellet in one tube with 3 mL of ACD-A/saline solution. Using a sterile transfer pipette, transfer the resuspended platelets to the other tube containing the platelet pellet. Resuspend the second platelet button. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting the next step	Resuspend the harvested platelet pellet in one tube with 3 mL of ACD-A/saline solution. For Fresh platelets using a sterile transfer pipette, transfer the resuspended platelets to the other tube containing the platelet pellet and resuspend the second platelet button. Make sure a complete resuspension is achieved with no visible aggregates or clumps before starting the next step. **Before proceeding to radiolabeling the platelets, one aliquot of Test platelets will be stopped and used only for *in vitro* analysis.**
5.6.2	At the end of incubation, add 2.5 mL of ACD-A/saline and 0.5 mL **STORED AUTOLOGOUS PLASMA** ~~of the reserved PPP (from Step 5.4)~~ directly to the incubation tube containing the platelets in ACD-A/saline and $Na_2{}^{51}CrO_4$.	At the end of incubation, add 2.5 mL of ACD-A/saline and **0.5 mL of the reserved PPP (from Step 5.4)** directly to the incubation tube containing the platelets in ACD-A/saline and $Na_2{}^{51}CrO_4$
5.6.3	Centrifuge the tube at 1250 x g at 20 to 24°C for 10 minutes with brake off.	Centrifuge the tube at 1200 to 1250 x g at 20 to 24°C for 10 minutes with brake off.
5.6.5	Gently resuspend the platelet-pellet in 2 mL of **STORED AUTOLOGOUS PLASMA** ~~PPP reserved previously (from Step 5.4)~~. (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) After complete resuspension, add an additional 4 mL of **STORED AUTOLOGOUS PLASMA** ~~PPP~~.	Gently resuspend the platelet-pellet in **2 mL of PPP reserved previously (from Step 4.4).** (Failure to obtain complete resuspension of platelets is a cause for discontinuation of the procedure.) **After complete resuspension, add an additional 4 mL of PPP.**
7.3	Perform venipuncture with a 19-21 gauge butterfly infusion needle. Draw one 10 mL EDTA (lavender-top) tube for the pre-infusion sample	Perform venipuncture with a **19 gauge** butterfly infusion needle. Draw **one (or 2 if tube for elution was not drawn earlier)** 10 mL EDTA (lavender-top) tube for the pre-infusion sample

Section	Variant 1 of BEST Version 4.2.1 Method	Variant 1 of BEST Version 4.2.1 Method Modifications
7.7	Weigh empty infusion syringe, needle and needle cover. Record weight on the data sheet. Record the net weight of injectate	Remove needle, cap syringe with syringe cap, and weigh syringe with syringe cap. Record weight on the data sheet. Record the net weight of injectate
8.1.2	Optional: 2 hours after infusion. (Collect from the contralateral arm to that used for infusion.)	**Collect blood draw at 1and** 2 hours after infusion. (Collect from the contralateral arm to that used for infusion.)
8.1.3	One sample daily between days 1 through day 7 (except weekends for samples after Day 1) and one final sample on day 10, 11 or 12. (The last sample is called the "baseline" sample and is used to correct for inadvertent labeling of other blood components.)	**Sample on days 1, 2, 3, 4 (or 5 or 6), 7 (or 8) and one final sample on day 10, 11 or 12.** (The last sample is called the "baseline" sample and is used to correct for inadvertent labeling of other blood components.)
8.2.3	Pipet approximately 2.0 mL of the mixed whole blood into the appropriate pre-weighed tube using a positive displacement pipettor and replace cap	This sample is prepared and counted duplicate
9.3 – 9.6	Pipet approximately 2 mL into a 12 x 75 mm capped tube	This sample is prepared and counted duplicate