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Protocol Number: CLIN-0001

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Revision History

REVISION	DESCRIPTION
v. 1.0	Final Draft
v. 1.1	Draft with changes in response to FDA conditional IDE approval
v. 1.2	Changes to add FDA requested rejuvination at end of storage and clarify protocol for sites and CRF design.

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2.0 Introduction / Background

2.1 Background

The primary goal of New Health Sciences, Inc. is to improve red cell storage through novel storage methods. Based on our review of the pertinent literature, there is substantial evidence suggesting that prolonged exposure to oxygen during storage results in oxidative damage to the red blood cells leading to decreased therapeutic potential. Therefore, removal of oxygen from red blood cell products prior to storage has potential to preserve the cells in a more physiologically relevant state.

Currently, NHSi has focused on the design and development of a dual compartment bag system designated as the Hemanext Red Blood Cell Processing System. After standard processing of donated whole blood units into leukoreduced packed red blood cells (LR-RBCs) with the appropriate additive solutions, the LR-RBCs would then be placed in the oxygen reduction bag (ORB) which allows for the rapid diffusion of oxygen out of the blood, through a sterile, oxygen-permeable membrane, and into iron-based oxygen sorbents. After processing, the blood is transferred again from the ORB into the Hemanext storage bag (HSB) which will preserve the anaerobic state of the LR-RBC product for the duration of cold storage.

NHSi has conducted preliminary storage tests to ascertain the effects of anaerobic storage on overall blood health in various storage solutions. The research team has focused primarily on percent hemolysis, which is mandated by the FDA to remain below 1% for the duration of storage, as well as ATP and 2,3-DPG levels.

2.2 Product Information

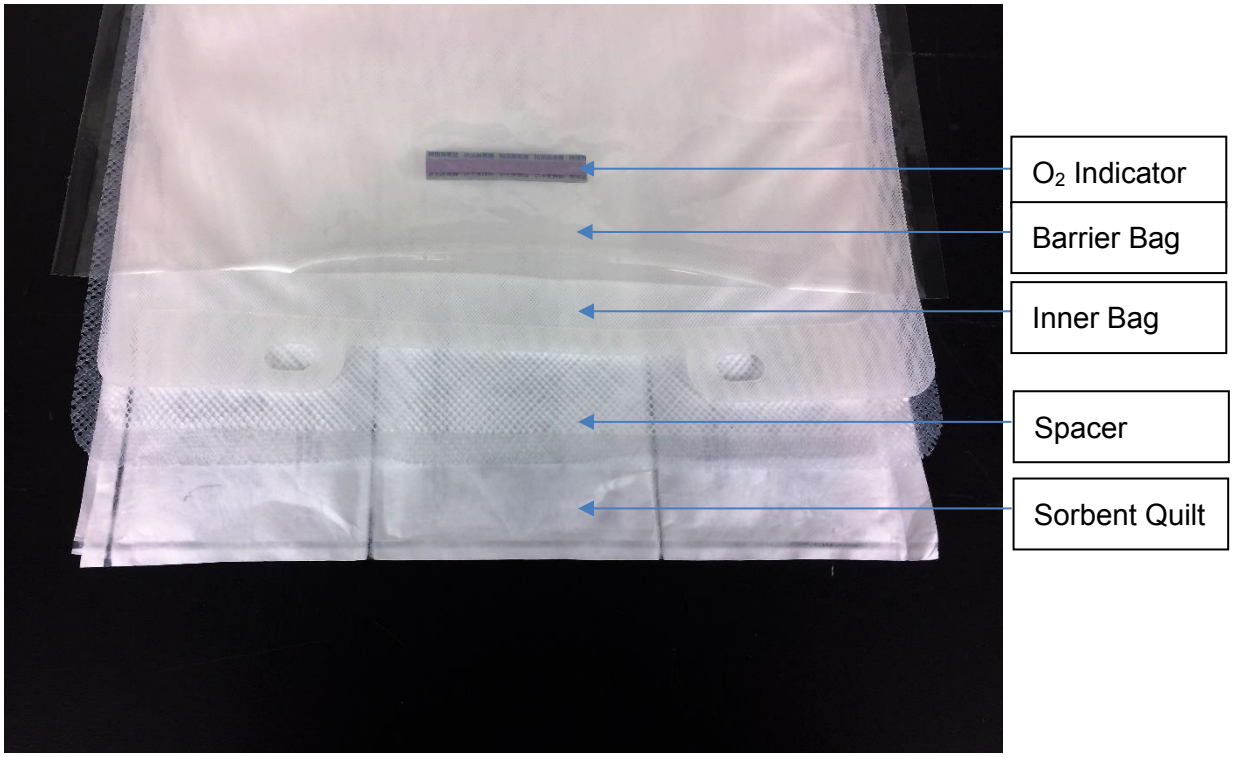
2.2.1 Investigational Product

The Hemanext Red Blood Cell Processing System is a blood storage device that reduces oxygen and carbon dioxide from leukocyte reduced red blood cells in additive solution. The Hemanext System is an assembly of two disposables, the Oxygen Reduction Bag (ORB) and the Hemanext Storage Bag (HSB). See Figure 1 below for the concept configuration.

Figure 1: Investigational Product – Hemanext Processing System Assembly

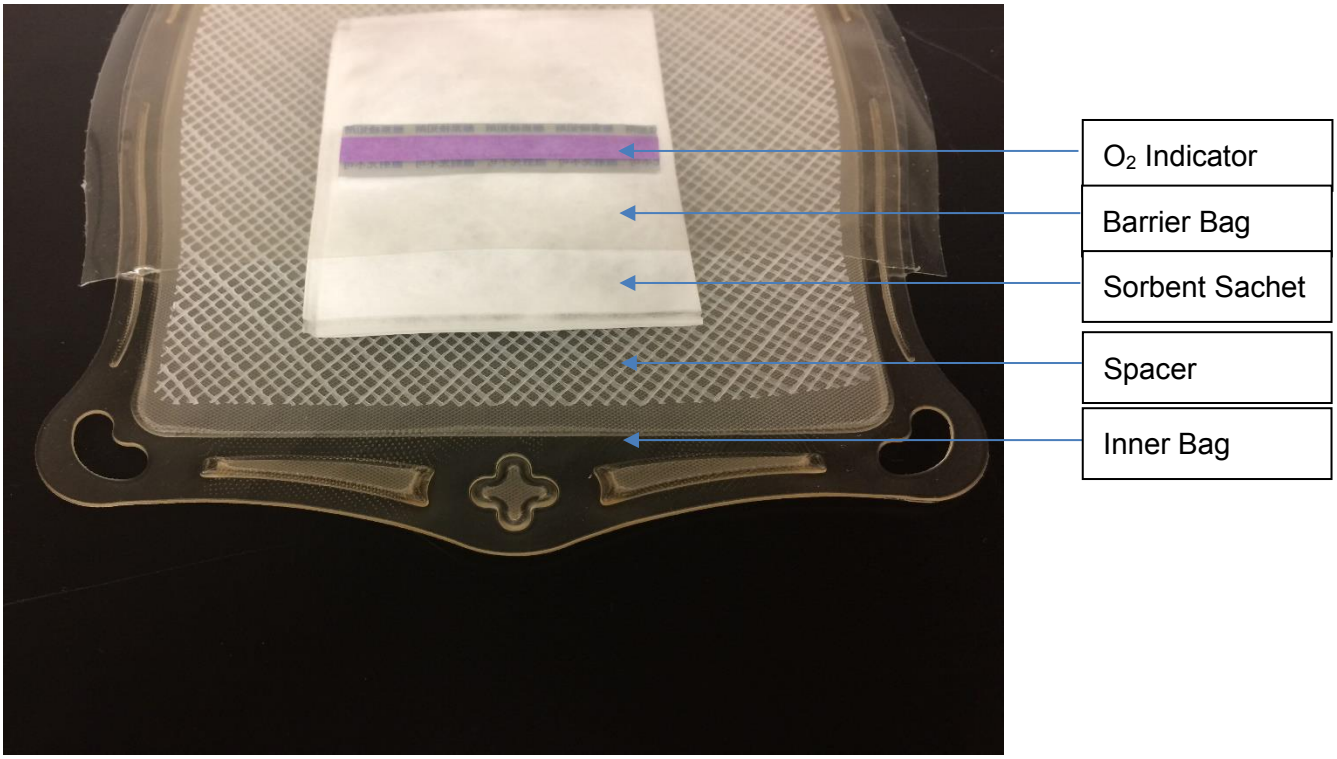
The ORB consists of the following elements: (a) an inner bag; (b) barrier bag; (c) spacer and (d) quilt of 9 sorbent sachets. The inner bag holds the AS-3 LR RBC and is housed in an outer barrier bag. The barrier bag ensures that there is no ingress of oxygen into the ORB during or post O_2/CO_2 reduction. The inner bag is physically separated from the barrier bag by polyester fabric elements embedded into the outer surface of the inner bag. Materials of construction of the inner bag ensure that oxygen released from the LR RBC is optimally transferred to the surrounding space. The sorbent quilt ensures that oxygen released from the LR RBC is absorbed. The spacer (perforated patterned polyethylene stabilizer) is strategically placed in the space between the inner bag and the sorbent quilt to ensure that all of the surface area of the inner bag is clear and able to transfer oxygen. The oxygen indicator is designed to change color if exposed to oxygen. The ORB has tubing to allow sterile docking of the LR RBC bag. The ORB is connected to the ASB bag via integral tubing. The connections facilitate the transfer of the oxygen reduced LR RBC blood. See Figure 2 below for the concept configuration.

Figure 2: Investigational Product, ORB components



The HSB consists of the following elements: (a) inner bag; (b) barrier bag; (c) spacer and (d) single sorbent sachet. The materials of construction of the HSB ensure that it is capable of maintaining the appropriate low oxygen environment for additive stored LR RBC in refrigerated environments for 42 days. The inner bag holds the blood and is housed inside the oxygen impermeable barrier bag. A sorbent is strategically placed in the space, between the inner and outer bags, to ensure that low oxygen levels are maintained post reduction by the HSB. See Figure 3 below for the concept configuration.

Figure 3: Investigational Device, HSB Components



The LR RBC unit bag is connected using an FDA cleared, sterile docking device to the ORB. The sorbent quilt between the inner bag and the barrier bag creates an O_2/CO_2 starved environment. LR RBC are then transferred by gravity to the ORB inner bag. Once the full unit volume is in the ORB inner bag; the Hemanext system is transferred to a tray on an agitator at room temperature (22+2°C). Its contents are evenly distributed, creating a large thin layer of LR RBC contacting the inner surface of the ORB inner bag. The agitator moves in a side to side motion causing the LR RBC to travel within the inner bag in a wave-like motion at the same rate as the shaker, maintaining a constant agitation of RBC and additive solution. Solution in direct contact with the inner bag surface releases oxygen through the material and into the O_2/CO_2-starved environment between the inner bag and barrier bag of the ORB. Maintaining this agitation of red blood cells and residual plasma\additive solution ensures that oxygen saturated RBCs that are furthest from the walls of the bag exchange oxygen molecules with low oxygenated solution closest to the bag surface, which in turn lose the gained oxygen by constant exposure to the oxygen-starved environment on the outer side of the ORB inner bag. The O_2/CO_2 absorption rate is characteristic of the oxygen reduction container material, exchange surface area, volume of RBC and agitation. At the end of the exposure time of 3 hours, the resulting deoxygenated LR RBC is transferred to the HSB in the kit. The HSB utilizes the same principles as the ORB to preserve a low oxygen and CO_2 environment within its contents. It utilizes a sorbent to maintain this environment and preserve the blood at low O_2 and CO_2

levels for its storage duration of up to 42 days. The HSB is also a transfusion ready device.

Figure 4: Diffusion Mechanism for O_2 Removal

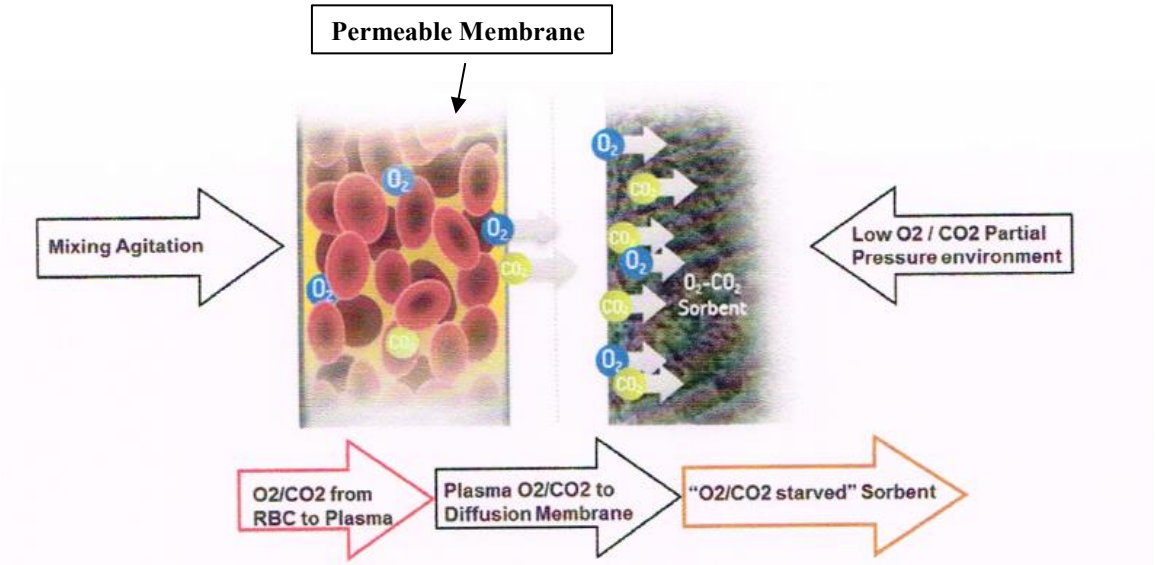
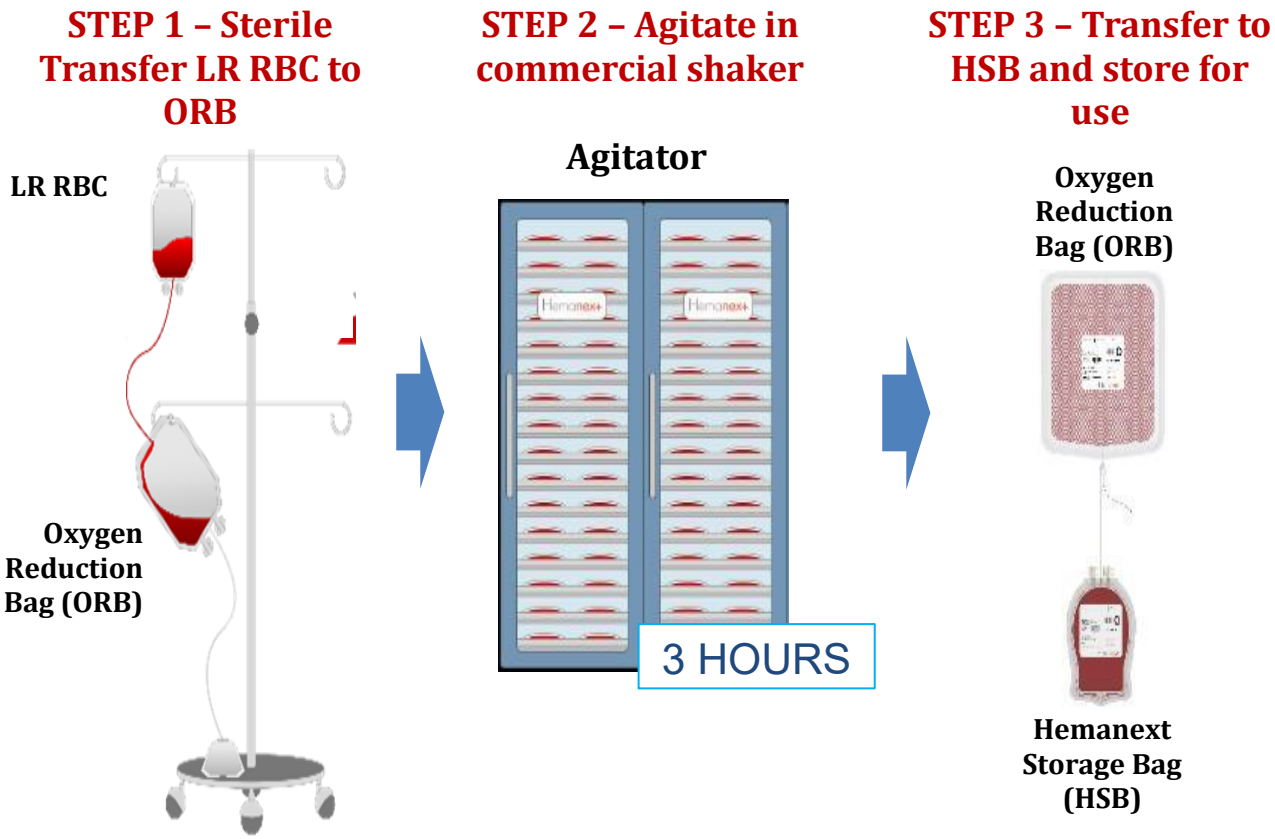


Figure 5: Hemanext Process



2.3 Previous clinical experience

Two research studies were conducted to confirm the readiness of the Hemanext RBC processing system for clinical trials in the United States (see table 1 below).

An initial pool and split study was performed with the partnership of Rhode Island Blood Center for donor collection. The storage data was measured internally in NHSI's Blood Lab. Key blood quality metrics (such as ATP and 2,3 DPG) compared favorably for the experimental product to the control product.

After a final product design was reached, a study was conducted independently at the American Red Cross Norfolk lab. The study increased the hold time to 12 hours and was designed to be a pilot of the experimental arm of this protocol. All acceptance criteria were met, and results similar to internal testing were collected. In addition, RBC Morphology was equivalent, it was confirmed that Hemanext may be ATP/DPG rejuvenated at 42 days, and oxygenation dissociation curves matched the DPG profile of Hemanext.

3.0 Purpose / Scope

This trial will serve to generate data sufficient to evaluate the in vitro and in vivo performance of the Hemanext product using Leukoreduced Red Blood Cells generated under conditions of 12-hour room temperature hold.

The objective of this clinical study is to evaluate CP2D/AS-3 leukocyte-reduced RBC stored anaerobically for 42 days after deoxygenation with the Hemanext Storage System. In addition to 24-hour recovery and hemolysis, a list of standard blood quality parameters will be measured.

It is expected, based on these data, that Hemanext processing will maintain physiological levels of these blood quality parameters (such as 2,3 DPG).

4.0 Safety / Regulatory Requirements

4.1 Rationale for Human Use

The human use portions of this study are whole blood collection, and the measurement of autologous, in vivo, radiolabeled 24-hour red cell recovery and survival of red blood cells stored in AS-3® additive. All other study procedures involve in vitro bench testing only.

While in vitro testing provides an estimate of the in vivo red blood cell recovery and survival, the FDA requires in vivo studies to establish efficacy of a blood storage system; therefore autologous, radiolabeled, in vivo red cell recoveries will be performed in this study.

See section 7.1 for additional information regarding study-associated risks.

Table 1: Prior Hemanext™ Studies

Study ID (Year Initiated)	Study Name	Purpose	Number of Subjects	Outcome
RESPR27 (2016)	Hemanext Processing System with 42-day Storage and in-vitro Characterization of Resultant Oxygen Reduced, Leukoreduced, Red Blood Cells in AS-3 vs. Control.	The purpose of this pilot study is to generate data of the current design of the Hemanext Processing System (consisting of an oxygen reduction bag "ORB" and an anaerobic storage bag "HSB", 60" tubing, y-connector and 2 ratchet clamps) and Hemanext process.	20 donors. 10 pools of two donors.	Hemanext O_2/CO_2 reduced RBC meet all in vitro acceptance criteria.
RESPR34 (2017)	12-hour Processing with The Hemanext Red Blood Cell Processing System with 42-day Storage and in-vitro Characterization of Resultant Oxygen Reduced, Leukoreduced, Red Blood Cells in CP2D/AS-3	Confirm the feasibility of the current design of the HEMANEXT RBC PROCESSING SYSTEM and Hemanext process for preparing RCC in 12 hours from blood donation. Obtain P50 values that correspond with DPG levels and rejuvenation data on the post Day-42 stored products to show that Hemanext process does not compromise the ability of Rejuvesol to increase ATP and DPG levels in anticipation of FDA pivotal study.	10 donors	Hemanext met acceptance criteria, P50 tracked 2,3 DPG, and Hemanext demonstrated ability to be ATP/DPG rejuvenated.

4.2 Regulatory Requirements

The clinical investigation shall be conducted according to FDA regulations (21 CFR), as applicable.

This study will be conducted under IRB (Institutional Review Board) approvals. All potentially eligible donors will read, have the opportunity to ask questions on, and sign Informed Consent Forms approved by the respective IRB prior to any study related procedures.

5.0 Study Design / Analysis

5.1 Study Design

In vitro and in vivo performance of O_2/CO_2 reduced red blood cells produced with the Hemanext System will be used to demonstrate the acceptability the final product for clearance. To accomplish this, the study will require a total of 100 study donors. The study entails a randomized, paired, 2-x-2 crossover design where every study donor (n = 100) who completes the study will donate a total of two whole blood units with individual units being donated at least 56 days (8 weeks) apart. One unit will be used as the test and the other unit will be used for the control. The order in which the IP and the CP will be used to collect, filter and store the whole blood and appropriate blood products (within the context of the crossover design) will be randomized.

The full clinical investigation is summarized in Table 2. See the study's flow diagram in **Section** 13.1 for an additional reference.

Table 2: Hemanext™ Clinical Trial Design Summary

Hemanext Pivotal Clinical Trial Summary		
'n' (evaluable) *		93
Pre-filtration storage & filtration conditions		20-24°C
Randomized, Paired, Crossover	Investigational unit filtration	<8 hours + up to 4 hours processing
	Control unit filtration	< 8 hours
In vivo subgroup study ('n')		√ (24-28)
Post-filtration assessments		√
Baseline assessments		√
Post-storage assessments		√

5.2 Study Primary Endpoints and Acceptance Criteria

The primary endpoints that will be evaluated in this clinical trial are RBC mass recovery, hemolysis and 24-hour in vivo red cell recovery. The endpoint-associated study acceptance criteria are listed below:

- A one-sided 95% lower confidence limit for the true proportion of packed RBC units with hemolysis at day 42 of < 1% is > 95%.
- The mean 24-hour, post-transfusion, in vivo red cell recovery of at least 75% with a standard deviation of ≤ 9%, and the lower limit of a one-sided 95% confidence interval for the population proportion of successes is ≥ 70%.
- Red Blood cell recovery ≥ 85%; with 95% confidence that at least 95% of units are ≥ 85%.

5.3 Donor/Data Criteria

5.3.1 Sample Size

The sample sizes described within the protocol were selected to demonstrate meeting study primary endpoints that conform to the FDA's 95-95 rule (95% confidence and 95% reliability). For successful endpoint outcomes, the study requires meeting the acceptance criteria in 92 of 93 test units per test condition. Therefore, 93 evaluable study donors will complete the study. In the event of two (2) donors/units with unmet acceptance criterion occurring out of ninety-three (93) evaluable procedures (i.e., within one or more study arm(s) for one or more acceptance criteria/primary endpoints), the study will be discontinued.

5.3.2 Intended Study Enrollment

Definitions:

- **Study Donor:** an individual who has signed the informed consent form and is enrolled in the study.
- **Evaluable Study Donor:** an individual who is enrolled in the study, meets the inclusion criteria, and completes two Day 42 test periods.

This clinical trial requires enrolling a total of 93 evaluable study donors. However, due to the study design which incorporates a 2x2 crossover and the in vivo RBC recovery and survival substudy, the study will purposely enroll up to 100 study donors to account for study withdrawal, loss to follow-up and/or study donors who meet study criteria for the first donation of the crossover, but fail to meet study criteria for the second donation.

The study sites will make efforts to balance gender during screening.

Once enrolled, if a study donor's status should change from evaluable to non-evaluable per protocol definitions (see section 5.3.5), or if the study donor should discontinue follow-up (e.g., either voluntarily withdraw from the trial or become

lost to follow-up), then that study donor may be replaced in order to maintain intended study enrollment goals. Such replacement would increase the total number of enrolled study donors, but maintain the final number of evaluable study donors.

In instances where up to 100 study donors have been enrolled and all have undergone their first donation, consecutive donors will return for their second donation until a complete set of 93 matched data pairs have been obtained for both in vitro and, when applicable, in vivo parameters. As a result, it is possible that a study donor who donates an evaluable unit of whole blood will not be asked to return to provide a second unit of whole blood in instances where a complete data set has already been ascertained within the relative study arm.

Site A is not participating in enrolling donors for the in vivo cohort of the study. Site A will target enrollment of 40 evaluable donors for in vitro testing only. Site H will target enrollment 46 evaluable donors for in vitro testing of which 14 will also be enrolled for the cohort of in vivo testing. Site B target enrollment 14 evaluable donors for both in vivo and in vitro testing.

Table 3: Target Enrollment by site

Site	In vitro	In vivo
Site A: American Red Cross, Norfolk	40	0
Site B: Blood Center of Wisconsin	14	14
Site H: Hoxworth Blood Center	46	14
Total	**100**	**28**

5.3.3 Inclusion/Exclusion Criteria

The study donor must conform to the criteria listed in Table at the time of study donor selection and must meet study eligibility in order to participate in the study. Any deferral criteria will be based on local procedures and SOPs. Note, further, that later evaluability determinations (see section 5.3.5) may impact study-eligible individuals' eligibility/evaluability and/or full participation within the study.

Table 4: Inclusion/Exclusion criteria

Inclusion Criteria	Criteria to qualify for Inclusion	Reference
Age	Study donor must be ≥ 18 years of age.	Age of legal consent used for inclusion criteria.
Weight	Study donor must be ≥ 110 pounds.	AABB Standards for Blood Banks and Transfusion Services 28th Edition
Temperature	Study donor's body temperature must be ≤ 37.5°C / 99.5°F (oral).	
Hemoglobin	Study donor's hemoglobin must be ≥12.5 g/dL if female and ≥13.0 g/dL if male.	FDA Standard 21CFR 640.3(b)(3). Study participants must meet **EITHER** hemoglobin or hematocrit criteria.
Hematocrit	Study donor's hematocrit must be ≥ 38% if female and ≥39% if male.	
Donor Eligibility	Study donor must meet all criteria per respective site's Research Blood Donation Record (BDR).	Site documentation
Prior Donation	Study donor's most recent single RBC unit donation must have been ≥56 days prior to study donation. Study donor's most recent double RBC unit donation must have been ≥ 112 days prior to study donation. **Only donors who participate in the in vivo portion of the study:** An additional 2-4 weeks between donations will be required to ensure washout from prior in vivo survival procedures.	AABB Standards for Blood Banks and Transfusion Services 28th Edition
Informed Consent	Study donor must have consented to study participation by reviewing and having expressed understanding the site-respective IRB–approved informed consent form prior to undergoing any study related procedures.	ISO 14155:2011 21 CFR 50
Blood-borne Pathogens	Study donor's testing results from collected blood does not indicate a risk of transfusion-transmitted disease (TTD)*.	N/A
Adverse Events	Study donors must agree to report adverse events from the time of signing the informed consent to twenty-four hours following the end of their active study involvement.	N/A
Pregnancy	Female study donors must not be pregnant or expected to be pregnant. For donors participating in the in vitro portion, this is an interview question only.	N/A

	Only female donors who participate in the in vivo portion of the study: Women of child-bearing age must not be pregnant, as determined by a negative pregnancy test prior to each re-infusion, or be breastfeeding. If acceptable by local procedures, post-menopausal or surgically sterile women may be exempt from the pregnancy testing requirement.	
**Study donors' TTD test results may not be available until several days following enrollment and donation of a whole blood unit. See section 5.3.5 for additional details.*		

For the purpose of this trial, not meeting any of the inclusion criteria constitutes exclusion from the trial (i.e., meeting exclusion criteria).

5.3.4 Donor Withdrawal Criteria

Any study donor that enrolled in the study can withdraw from the study, effectively withdrawing consent, at any time, for any reason, without prejudice or consequence. Study donors are to be informed that they are free to leave the study at any time for any reason without prejudice or consequence in the informed consent document. A study donor may withdraw from the study at any time following blood donation and thus withdraw permission to evaluate their blood product.

If a study donor withdraws from the study, whether prior to completion of the whole blood donation or after completion of the whole blood donation, the data collected up through the time of withdrawal (including testing results) will be included within the study database. Withdrawn study donors will be replaced in all instances where incomplete data required to calculate acceptance criteria result from their withdrawal. If a study donor withdraws at a point when both the whole blood collection and all study analyses are complete, then their withdrawal does not impact the data's entry into the database, their data will be analyzed within the full data set and the study donor will not be replaced.

5.3.5 Evaluable and Non-Evaluable Whole Blood Collections

Collected whole blood's evaluability status per protocol definition (see section 5.3.5.1) will determine whether these data are included within the data set that will be analyzed towards study endpoints. Blood donations deemed unevaluable per protocol definition will necessitate whole blood sample replacement obtained from alternate evaluable study donors such that intended population targets are maintained.

5.3.5.1 Definition of Non-Evaluable Whole Blood Collection

Each of the following issues or occurrences defines a non-evaluable whole blood collection. Partial data sets from donors who provide data and/or blood donations will be entered into the database and reported within descriptive statistics as outlined within the study's statistical analysis plan (SAP).

- **TTD:** Data/analyses resulting from blood acquired from donors who are subsequently determined to test positive for TTD will result in a determination of non-evaluability and will not be included within study endpoint analyses. Upon TTD positive determination, further analyses of the blood will cease, the study donor will be notified via site-respective procedures, and any collected data will be reported as descriptive statistics.
- **Slow Collection:** Any whole blood collection taking longer than 15 minutes to complete will be considered a slow collection and will be defined by the point that the cannula on the respective system's Donor Line is broken to initiate blood flow into the Donor Bag until the Donor Line is clamped to cease blood flow to the Donor Bag at the end of collection. All whole blood units resulting from a slow collection will be non-evaluable. Information/data from study donors from whom the collection is slow per this definition will only be included within the demographics section of the descriptive statistics.
- **Incomplete Collection:** Upon collection of the full unit and prior to any sampling, filtration or processing, any collection that is stopped prior to reaching the 500 ± 50 mL acceptable volume range will be considered unevaluable. With the exception of descriptive statistics, data resulting from incomplete collection donations will not be included in study-related analyses.
- **Protocol Deviation:** Any procedural protocol deviation that the PI and/or sponsor determine(s) has the potential to impact (both positively and negatively) any acceptance criteria, study endpoint and/or analytical study-critical outcomes will result in a determination of non-evaluability. Procedural protocol deviations must be documented within the eCRFs and all deviations that compromise data validity (i.e., one that impacts evaluability) must be reported to the sponsor within 24 hours (see section 9.3). Examples of protocol deviation that have the potential to impact study outcomes include but are not limited to the following: (1) storage deviation (e.g., electrical outage and unknown storage conditions, unit left out of refrigeration for >30 minutes, sample frozen vs. refrigerated, etc.), (2) incorrect mixing/agitation (e.g., unit dropped from height, unit inappropriately mixed prior to sampling, excess sample drawn into syringe and quickly re-injected back into unit, etc.), (3) other deviation (e.g., incorrect

centrifuge speed/duration, other)). Deviations must further be communicated to the respective IRB per local regulations.

- **Sickle Cell Trait:** Any whole blood unit in the study from a sickle cell trait (SCT) positive study donor will be excluded.
- **Grossly Lipemic Specimens:** Any whole blood unit grossly evaluated (per visual inspection) as lipemic will be non-evaluable relative to only the RBC hemolysis endpoint at Day 42. Any units in question will be reviewed by the PI or designee and if considered non-evaluable, the unit will be removed from the study, and the study donor may be replaced.
- **Positive Bacterial Culture:** This evaluability determination pertains only to study donors who participate in the in vivo portion of the study. Should the outcome of the pre-infusion bacterial culture (see section 6.2.16.11) indicate that the stored LRRBCs are '+' for bacteria, then the unit will be discarded. As a result, the study donor and his/her unit of blood will be non-evaluable and the study donor may be replaced.

5.4 Statistical Analysis Plan

The data from this study will be analyzed to determine whether the primary endpoints and study acceptance criteria (as recommended by FDA and as listed in section 5.2) were met.

In order to assure that 95% confidence in 95% reliability of the of the lower confidence level of the units meet the acceptance criteria for the primary endpoints, the sample size shall be:

- Ninety-three (93) evaluable procedures in which no more than one (1) failure occurs against the acceptance criteria for the primary endpoints.

Secondary analyses, such as differences in populations by site and comparisons between arms, will be described in more detail within the statistical analysis plan. This formal SAP will be developed in conjunction but separately from this protocol, and filed within the trial master file.

The following, at a minimum, will be presented using descriptive statistics:

- **Donor Disposition:** All data on all study donors enrolled in trial including data identified as evaluable and non-evaluable donations will be presented.
- **Demographics:** Age, gender, weight, height, ethnicity and race will be provided for all study donors enrolled in the trial for both evaluable and non-evaluable donations. A comparison to census data will be performed in the final report.
- **Collection procedure-related data:** Collection duration and ambient temperatures will be provided for both evaluable and non-evaluable donations.

- **Filtration & processing procedure-related data:** Temperature, head height, filtration time, filter time, filter weight, and final collection volumes will be provided for evaluable and non-evaluable donations.
- **Laboratory data:** Laboratory data will be provided for evaluable and non-evaluable donations (if applicable). Other than the endpoints detailed in the acceptance criteria, the laboratory data will be presented for information only.
- **Adverse events:** Expected, unexpected and serious adverse events will be reported for all enrolled study donors.

6.0 Test Material and Study Methodology

6.1 Test Material

Disposables directly related to the IP will be labeled as an investigational product.

6.1.1 Product Storage, Accountability and Disposition

Study product will be supplied by NHSi after the study contract has been executed. Study product may not be used in any capacity until proof of IRB approval and IRB-approved informed consent(s) for the study have been received by the Sponsor. Site documentation of 100% accountability of the IP is required beginning with receipt of the product until all product has been dispensed, destroyed and/or returned to the study sponsor. A "Study Product Receipt and Disposition Log" will be supplied by NHSi in the Clinical Trial Binder for documenting each receipt and disposition (i.e., used, discarded or returned) of the study product. The IP are to be stored at room temperature in a secure location upon receipt. Unused product will be returned to the sponsor at the end of the trial unless other documented instructions are provided in advance to the respective clinical site.

6.2 Methodology

6.2.1 Tare Weight Measurements

During the study initiation visit and prior to enrolling the first study donor, tare weight measurements must be determined for the IP. Tare weight instructions and source documents will be supplied within the study's Regulatory Binder, and tare weights will be obtained and confirmed at the individual study sites. Tare weight data must be entered onto the sponsor-provided worksheet(s) and into the appropriate eCRF within the study's EDC system.

All blood and blood component weight measurements will be gross weight measurements. Each site participating in the clinical trial will either be provided with a prepared Tare Kit or will develop a Tare Kit during the SIV. The Tare Kits are to be retained until the completion of the study.

NOTE: The ORB component of the Hemanext RBC Processing system must be weighed individually and cannot be a part of a standard Tare Kit.

6.2.2 Donor Selection, Consent and Enrollment Rate

This study will enroll up to 93 evaluable study donors (see sections 5.3.2, 5.3.3 and 5.3.5 for details regarding intended enrollment, inclusion/exclusion criteria and donor evaluability determinations). Study donors will be selected from site-managed databases of known donors as well as new donors who meet study eligibility criteria. Clinical sites may choose to employ IRB-approved advertisements as a means to assist recruitment.

Individuals who express interest in study participation must undergo site-specific informed consent processes per local requirements. Study donors will be consented by the respective sites' PI or appropriate PI delegate(s) prior to undergoing any study-related activities or assessments using the respective sites' IRB-approved Informed Consent Form, and will follow consenting procedures per local SOP and 21CFR 50.27.

For the purpose of this study, any study donor who has signed the study's informed consent documentation is considered to be enrolled in the study regardless of whether the enrolled individual engages in any study activity beyond ICF signing, donates blood, or is evaluable.

Each study sites' rate of enrollment will be dependent on limitations resulting from both individual study donor's evaluability and sites' ability to process the required number of evaluable study donors per study arm. As a result, the distribution of evaluable study donors may vary per study arm and per site.

6.2.3 Study Donors: Assigning to Study Arm and Randomization to Study Product

Study donors must be randomized to either IP or CP prior to their first donation. A donor randomized to IP will donate their first whole blood unit to be stored with the Investigational Product, and will donate their second whole blood unit to be stored with the Control Product. A study donor randomized to CP will donate their first whole blood unit with the Control Product, and will donate their second whole blood unit with the Investigational Product.

The randomization process will be a manual process that will be recorded within the Subject Enrollment and Screening Log. The randomization procedure will be based on a form generated wholly with SAS that will provide sequentially listed randomized product. Further details regarding the randomization process and associated documentation will be captured within the study's Operation Manual. Documentation relative to randomization will be stored within the study's Regulatory Binder.

6.2.4 Whole Blood Collections

After study donors are consented, initial screen procedures have been completed, and individual donors have been assigned to their study arm and randomized, study donors will donate 500mL ± 10% of whole blood with each whole blood donation. Approximately 20 - 40mL of blood (included within the 500mL ± 10%) will be collected during phlebotomy for CBC, ABO typing, TTD and SCT testing. The total volume of blood removed from any donor at collection will not exceed 550mL.

Study donors who also participate in the in vivo cohort will have whole blood samples of various volumes drawn as part of the cohort. The in vivo study does not require an additional whole blood unit donation. However, as part of the in vivo study, whole blood samples will be drawn on Day 42 (50-65 mL total) and additional whole blood samples will be drawn on Day 43 (10 mL) and 5ml for each subsequent time point for in vivo survival. See section 6.2.11 for details.

6.2.5 Leukoreduction of Whole Blood Units

Each collected whole blood unit will be leukoreduced warm using the leukoreduction filter per the IFU for the Haemonetics WB Leukotrap System (Appendix 13.2). The collection set with leukoreduction filter will be used according to the instructions for use provided at study initiation with the head height specifications included.

6.2.6 Slow Filtrations

Any filtration with a Bag Empty Time (emptying of the Donor Bag) that is greater than one hour will be considered a slow filtration. Slow filtrations will be documented, the Sponsor will be notified immediately and testing will continue to completion if the product is stored in < 12 hours for the IP or < 8 hours for the CP groups.

6.2.7 Whole Blood Processing and Preparation of PRBC

As dictated, each leukoreduced whole blood unit will be processed into PRBCs and platelet-poor plasma (PPP) using a representative hard-spin centrifugation and subsequent aspiration (PRBC/PPP separation).

The PRBC unit will be mixed with the AS-3 additive per the product's instructions for use. PPP will not be analyzed, and this plasma will be discarded per Section 6.2.12.

6.2.8 Blood Product Sampling

Blood product containers will be thoroughly mixed prior to sampling to ensure a representative sample is obtained from a homogeneous blood product.

Thorough mixing of each whole blood unit prior to taking pre-filtration and post-filtration samples is of critical importance for the calculation of filtration red cell recovery.

All sample aliquots will be taken without damaging the integrity of the test system or blood unit. Evaluation samples from whole blood and RBC units will be taken via a sterile connection between a sterile transfer bag to the blood product container using an FDA approved sterile connection device and then transferring an aliquot to the transfer bag.

When sampling, care must be taken to ensure that air is not transferred to the blood component container from the sample transfer bag. If a red cell unit is removed from cold storage for sampling purposes it must be returned to cold storage (1-6°C) within 30 minutes of the time of removal.

6.2.9 Microvascular Analysis

This study incorporates an evaluation of deformability using an experimental assay to characterize red cell deformability called the Microvascular Analyzer (MVA). The MVA measured perfusion of an artificial microvascular network under physiological conditions. Additional details on the procedure for this assay are documented in the study operations manual. This testing will only be performed on donors participating in the in vivo portion of the study.

For the purposes of this study, the assay will be considered optional and inability to complete it will not be documented as a protocol deviation. The Sponsor and sites may mutually agree to discontinue the testing at any time should difficulties with the equipment be encountered.

6.2.10 Laboratory Storage and Testing Methods

All study blood products will be stored under standard blood banking conditions. All study blood products will be segregated from normal blood product inventory. PRBC units are stored for 42 days at 1-6°C.

All testing will be conducted using laboratory equipment and instruments that are in current calibration, according to site standard procedures or using a validated scientific method. Site research laboratory standard procedures for testing performed in the study are to be available for inspection by the Sponsor. When study testing is performed by a certified clinical laboratory, copies of current CLIA and/or CAP certification must be provided to the Sponsor.

Refrigerators used to store blood products will be equipped with a temperature recorder or linked to a temperature recording system that will alarm and initiate contact with site staff when out of the protocol-indicated temperature ranges.

6.2.11 Radiolabeled Red Cell Procedures

This study incorporates an in vivo cohort to evaluate Day 42 RBC recovery and subsequent survival. Measures to ensure the study donor receives his or her own (autologous) radiolabeled red cells begin at donation when the study donor's name, birth date and unique identifying number will be recorded on the donor registration form. Clinical sites must have a standard labeling and handling process intended to eliminate errors in linking packed RBC units with the correct study donor.

On the first day of each in vivo cohort (42-days following each donation for in vivo cohort participants), female study donors must not be pregnant as determined by a negative pregnancy test (whether urine and/or serum pregnancy tests are used will be dictated by local procedures; women who are post-menopausal or surgically sterile may be exempt from the pregnancy test requirement if allowed by local procedures). The outcome of the pregnancy test will be recorded in both the source documentation and EDC.

RBC survival testing will be performed at 24h (±1h), 48h (±4h), 72h (±4h), 7 days (±1day), 14 days (±1day), 21 days (±1day), and 28 days (±1day).

Radiolabeling RBC procedures are derived from the literature (Davey, 1987; Heaton, 1992). However, individual clinical sites will follow specific local labeling, infusion, sampling and recovery/mass determination procedures and SOPs.

6.2.12 Blood Product Disposition

Each whole blood unit collected in the study will generate one unit of RBC in additive solution and one unit of PPP. All additive RBC units are to be discarded per site procedure after completion of the study following receipt of Sponsor's written consent. 51Cr and 99mTc-labeled RBC samples will be discarded following receipt of written permission from the sponsor and will be discarded per study site-specific SOP relative to the destruction of radiolabeled biological materials. Written consent will not be required for any unit with a confirmed positive infectious disease result.

6.2.13 Endpoint Calculations

Study sites will perform real-time calculations (RBC mass) and batch testing (hemolysis) to determine whether study endpoints meet study criteria and specifications. Clinical sites will employ the formulas that follow to perform real-time calculations for the purpose of informing the study sponsor quickly when it appears that data relative to the study acceptance criteria appear to be out of the acceptable range. However, for the purpose of the database and resulting data, the raw values will be entered into the EDC database, and the reported values will be calculated separately.

24-hour in vivo RBC recovery and survival will occur per the procedures summarized in section 6.2.11, and clinical sites will use the raw data to perform their own 24-hour % RBC recovery and survival calculations. Only % Recovery and % Survival values will be entered into the study's EDC system. Formulas used and the means to perform calculations relative to RBC recovery are site-specific. Calculated values will be confirmed per data captured within the eCRF relative to confirmation of calculations during monitoring visits.

Upon performing calculations for recovery, should a calculated value fall outside of the protocol-specified endpoint criteria, then the sponsor will be notified as soon as possible and within 24 hours.

6.2.14 RBC Filtration Recovery Calculation

RBC filtration recovery will be calculated via both a standard and the alternative method (using post filtration and pre-filtration weights). These calculations will be performed during the data analysis phase of the study based on hematocrit, volume and pre-/post filtration weights provided by sites. To better ensure accurate data, it is recommended that unit volumes be calculated from the weight of the full product. The results from both calculations will be reported.

RBC filtration recovery will be determined according to Figure 4. The acceptance criteria will be determined by the "standard equation" below.

Figure 4: RBC Recovery calculations (Standard and Alternate equations)

Standard equation:

RBC Recovery = (A x Post-filtration unit volume) / (B x Pre-filtration unit volume) x 100

Where: 'A' = Post-Hemanext HCT or RBC count
'B' = Pre-filtration HCT or RBC count
Unit volume (mL) = unit weight (g) / 1.056 (g/mL)
1.056 = relative density of whole blood

Alternate equation:

RBC Recovery = (Post-filtration net unit weight) / (Pre-filtration net unit weight) x 100

6.2.15 Hemolysis Calculation

Supernatant hemoglobin will be tested and % hemolysis calculated.

% Hemolysis will be determined according to the equation in Figure 5 and using Spun Hematocrit only.

Figure 5: Hemolysis calculation

$$\% \text{ Hemolysis} = \frac{(\text{Supernatant Hgb (mg/dL)} \times 0.001) \times ((1-(\text{Hct} \times 0.01)) \times 100)}{\text{Total Hgb (g/dL)}}$$

6.2.16 Study Activities

Study donors who are eligible for study participation will be assigned to either the in vitro portion only or in vivo and in vitro groups. Study donors will be enrolled until the intended total of evaluable units has been collected. Note that cited days reference the number of days post collection.

See Tables 5-7for summary of analyses and data collection.

a) After signing the respective consent documentation, collect and record data relative to inclusion/exclusion criteria, demographics, concomitant medications and medical history.

b) Collect and record the collection set's identification numbers.

c) Collect a whole blood unit using the product per the IFU. Draw a sample of the study donor's native whole blood from the diversion bag and place aliquots in tubes for the indicated analyses.

d) As soon as reasonably possible after collection, place anticoagulated whole blood units into room temperature storage until filtration.

e) Immediately prior to filtration, weigh, sample and re-weigh each whole blood unit.

f) Initiate room temperature filtration for each unit (5-7 hours post-collection). Record filtration-associated data.

g) Weigh, sample and then re-weigh filtered whole blood units.

h) As soon as practical following the completion of filtration, the LRWB units will be centrifuged and processed at room temperature to separate the unit into PRBCs and PPP. Record centrifugation-associated data.

i) AS-3 will be mixed with the PRBCs as soon as practical following separation from the plasma.

j) Following processing, the additive RBC unit will be weighed.

k) Plasma will be discarded.

l) The unit will be sampled and weighed.

m) If the unit is in the CP arm then at this point it will be placed in refrigerated storage (1-6°C; within 8 hours of donation).

n) If the unit is in the IP arm then the AS-3 LR-RBC unit will be transferred to the Hemanext ORB (within 8 hours of donation) at which point the unit will be weighed to ensure that the volume of the unit is less than 350 mL.

o) The ORB will be placed on a Helmer Platelet Shaker for 3 hours + 15 minutes.

p) The O_2 reduced LR-RBC will be transferred to the HSB at a head height of up to 72 inches at which point the unit will be sampled and weighed again prior to refrigerated storage (1-6ºC; within 12 hours of donation) or on Day 1 until subsequent testing on Day 21 (+/- 1 day) and Day 42 (+ 1 day).

q) For study participants participating in the in vivo portion of the study, refer to the instructions in section 6.2.11. Dual radiolabeled RBCs will

be infused on Day 42 and post-infusion blood samples will be drawn per local procedure. Study Participants will return to the site periodically on Days 43-70, and blood samples will be drawn per local procedure such that they are drawn within the time specified in Section 6.2.10.

r) Study donors will return at ≥ 56 days following their first donation. If the study donor continues to meet the inclusion/exclusion criteria, then they will donate their second unit of whole blood using product alternate to their randomization.

s) Follow steps b) through m) if second unit is in the CP arm and follow steps b) through p) if second unit is in the IP arm..

Table 5: Procedure related data collection

			Donor	Collection		Leukoreduction		Separation	Post-Separation	
Donor, Donation & Sample Handling Data Collection			Selection	(Pre)Collection	Pre-filtration	Filtration	Post-filtration	Centrifugation	Baseline	Day 0,21 42 Storage
		Units / Blood Product	**WB**	**WB**	**WB**	**WB**	**LRWB**	**LRWB**	**RBC + AS**	**RBC + AS**
				+ anticoagulant						
	Date(s)	dd-MMM-yyyy	X						X	X
	Time(s)	hh:mm	X		X		X	X	X	X
	Temperature	°C / °F		X	X		X	X	X	
	IFU confirmation	Yes/No		X		X			X	
Study Donor	Informed consent	Y/N, date, time	X							
	Eligibility criteria	See protocol	X							
	Demographics	See protocol	X							
	Concomitant Medications	Donor-reported	X							
	Medical History	Donor-reported	X							
	Donation History	Donor-reported	X							
	Temperature	°C / °F	X							
	Blood pressure	mmHg	X							
	Pulse	BPM	X							
Donation	System Manufacturer	See product		X						
	Part #/ Product code/ Lot #	See product		X						
	Collection date	dd-MMM-yyyy		X						
	Needle in	hh:mm:ss		X						
	Needle out	hh:mm:ss		X						
	Adverse Events	Donor-reported		X						
Unit Handling	Pre-sample Gross Weight	g			X		X		X	X
	Post-sample Gross Weight	g			X		X		X	X
	Filtration start/end time	hh:mm:ss				X				
	Filtration temperature	°C				X				
	Filter weight	g				X				
	Centrifuge details	See protocol						X		
	Centrifuge settings	See protocol						X		
	Hemanext Gross Weight	g							X	
	Hemanext Processing Start	hh:mm							X	
	Hemanext Processing End	hh:mm							X	
	HSB Gross Weight	g								X
	Refrigerator Date/Time/Temp	standard							X	X
	Bacterial culture notification (if needed)	dd-MMM-yyyy								X

Table 6: Specimen acquisition and analyses for IP arm

Specimen Acquisition, Analysis & Data Collection		Study Donor Selection-Pre-collection	Pre-filtration	Post-filtration	Baseline (Day 0)	Post-Hemanext (RBC Day0-1)	During Storage (RBC 20-22d)	Post-Storage (RBC 42-43d)
	Units / Blood Product	**WB**	**WB**	**LR WB**	**LR-RBC**	**LR RBC + AS-3**	**LR RBC + AS-3**	**LR RBC + AS-3**
			+ Anticoagulant					
Hct/Hgb – finger stick	**% / g/dL**	x						
RBC Count	**cells x 10^6/µL**		x	x	x	x	x	x
WBC Count	**cells x 10^3/µL**		x	x	x	x	x	x
PLT Count	**cells x 10^3/µL**		x	x	x	x	x	x
Hemoglobin	**g/dL**		x	x	x	x	x	x
Hematocrit	**%**		x	x	x	x	x	x
MCV	**fL**		x	x	x	x	x	x
MCH	**pg**		x	x	x	x	x	x
MCHC	**g/dL**		x	x	x	x	x	x
RDW	**%**		x	x	x	x	x	x
Spun Hematocrit	**%**		x	x	x	x	x	x
Sickle Cell Trait	**+ / -**	x						
Viral markers	**+ / -**	x						
ABO w/Rh factor	**A/B/AB/O +/-**	x						
Supernatant Hgb	**mg/dL**		x	x	x	x	x	x
ATP	**µmol/L**				x		x	x
2,3-DPG	**µmol/L**				x		x	x
ATP Rejuvenation	**µmol/L**							x
2,3-DPG Rejuvenation	**µmol/L**							x
rWBC Count	**cells/µL**			x				
SO_2	**%**				x		x	x
pO_2	**mmHg**				x		x	x
pCO_2	**mmHg**				x		x	x
HCO_3	**mmHg**				x		x	x
pH	**at 37°C**				x		x	x
Potassium	***mmol/L or mEq/L***				x		x	x
Sodium	***mmol/L or mEq/L***				x		x	x
Lactate	***mmol/L or mg/dL***				x		x	x
Glucose	***mmol/L or mg/dL***				x		x	x
RBC Morphology	**0-100**				x		x	x
RBC Deformability	**MVA**				x		x	x
RBC Microparticles	**#MV (1000 beads^{-1})**				x		x	x
RBC Metabolomic Panel					x		x	x
Day 35-37 Bacterial culture	**+/- (& notification)**							x
Day 42 Pregnancy test	**+/- (♀only)**							x
Day 42 In vivo recovery	**%**							x
In vivo Survival	**%**							x*
		***Survival Calculated at 48h, 72h, 7d, 14d, 21d, 28d**						

Table 7: Specimen acquisition and analyses for CP arm

Specimen Acquisition, Analysis & Data Collection		Study Donor Selection-Pre-collection	Pre-filtration	Post-filtration	Baseline (Day 0)	During Storage (RBC 20-22d)	Post-Storage (RBC 42-43d)
	Units / Blood Product	**WB**	**WB**	**LR WB**	**LR-RBC**	**LR RBC + AS-3**	**LR RBC + AS-3**
			+ Anticoagulant				
Hct/Hgb – finger stick	**% / g/dL**	x					
RBC Count	**cells x 10^6/µL**		x	x	x	x	x
WBC Count	**cells x 10^3/µL**		x	x	x	x	x
PLT Count	**cells x 10^3/µL**		x	x	x	x	x
Hemoglobin	**g/dL**		x	x	x	x	x
Hematocrit	**%**		x	x	x	x	x
MCV	**fL**		x	x	x	x	x
MCH	**pg**		x	x	x	x	x
MCHC	**g/dL**		x	x	x	x	x
RDW	**%**		x	x	x	x	x
Spun Hematocrit	**%**		x	x	x	x	x
Sickle Cell Trait	**+ / -**	x					
Viral markers	**+ / -**	x					
ABO w/Rh factor	**A/B/AB/O +/-**	x					
Supernatant Hgb	**mg/dL**		x	x	x	x	x
ATP	**µmol/L**				x	x	x
2,3-DPG	**µmol/L**				x	x	x
ATP Rejuvenation	**µmol/L**						x
2,3-DPG Rejuvenation	**µmol/L**						x
rWBC Count	**cells/µL**			x			
SO_2	**%**				x	x	x
pO_2	**mmHg**				x	x	x
pCO_2	**mmHg**				x	x	x
HCO_3	**mmHg**				x	x	x
pH	**at 37°C**				x	x	x
Potassium	***mmol/L or mEq/L***				x	x	x
Sodium	***mmol/L or mEq/L***				x	x	x
Lactate	***mmol/L or mg/dL***				x	x	x
Glucose	***mmol/L or mg/dL***				x	x	x
RBC Morphology	**0-100**				x	x	x
RBC Deformability	**MVA**				x	x	x
RBC Microparticles	**#MV (1000 beads^{-1})**				x	x	x
RBC Metabolomic Panel					x	x	x
Day 35-37 Bacterial culture	**+/- (& notification)**						x
Day 42 Pregnancy test	**+/- (♀only)**						x
Day 42 In vivo recovery	**%**						x
In vivo Survival	**%**						x*
		***Survival Calculated at 48h, 72h, 7d, 14d, 21d, 28d**					

6.2.16.1 Detailed Data Collection and Study Analyses

Each data point and analysis is listed here in order to evaluate unmet acceptance criteria endpoints. Refer to the study-specific Operations Manual (separate from this protocol) for a concise listing of data collection and analyses per the various study scenarios.

Data in bold, black font will only be collected once in this study, and will not be re-collected when the study donor returns for their second donation.

6.2.16.2 Study Donor Selection: Consent and Demographics

Consent will be obtained from all study donors prior to the collection of demographic information and any study-related screening, tests or blood donation.

Data collected and samples processed during study donor selection are as follows:

- **Consent obtained (Yes/No)**
- **Informed Consent date and time**
- Inclusion/Exclusion criteria
- **Date of birth**
- **Gender**
- **Race**
- **Ethnicity**
- **Height**
- Weight
- Concomitant Medications
- Medical History
- Donation History
- Body temperature
- Blood Pressure
- Pulse
- Hct/Hgb (finger stick)
- **Randomization**

For the purpose of this study:

- Concomitant medications will include all study donor self-reported medications (e.g., prescription medication and/or over the counter medications and nutritional supplements) that the study donor takes or has taken within the last 14 days.
- Medical history will include all study donor self-reported chronic diseases for which the study donor is currently being treated and any surgeries the study donor experienced within the previous 6 months.
- Donation history will include whether the study donor is a first-time donor and, if not, when the study donor's last donation occurred. This data will be considered in the presence of a vaso-vagal-related adverse event.
- Sites will have the option to perform the hematocrit and/or the hemoglobin analysis via whole blood obtained by finger stick. Only the analysis performed will be recorded.

6.2.16.3 Collection: Pre-Collection, Collection and Unit Transfer

Data regarding whole blood donation and study donor sample collection and sample processing are listed below. The collection set's identifier numbers will be recorded. Routine phlebotomy will be conducted for venous access and blood donation per the IFU. A sample of the study donor's native (non-anti-coagulated) whole blood will be drawn from the diversion bag and aliquots will be placed in tubes for the indicated analyses.

Data in bold, black font will only be collected once in this study, and will not be re-collected when the study donor returns for their second donation.

Items followed by an "*" are analyses performed on the pre-collection study donor sample.

- System manufacturer
- Part number/Product code
- Lot #
- Filter ID
- Collection date
- Donation start time ("Needle in")
- Donation end time ("Needle out")

- Collection room temperature
- Procedural IFU confirmation (Yes/No)
- Adverse event documentation
- **Sickle Cell Trait ***
- Viral Markers *
- **Blood typing (ABO) with Rh factor ***

For the purpose of this study:

- For the procedural IFU confirmation, a response indicative of a deviation from the IFU will result in a prompt to record a protocol deviation in a separate Comments eCRF or other log.
- The study donor's evaluability confirmation will reflect the criteria listed in section 5.3.5.1 which defines each item that could impact evaluability.
- For the adverse event documentation, a response indicative of the occurrence of an adverse event will result in a prompt to record adverse event details in a separate Adverse Event eCRF.

6.2.16.4 Pre-filtration Storage and Sampling

Following transfer to the medical laboratory, whole blood mixed with anticoagulant will be stored prior to filtration. Samples for analysis will be obtained immediately prior to filtration. Two (2) plasma samples will be processed from the pre-filtration unit just prior to filtration and frozen for future analysis in instances of confounding data in subsequent analyses.

Pre-filtration data/specimen collection and sample processing are as follows:

- Unit placed in pre-filtration storage: time and temperature
- Time pre-filtration samples obtained
- WB NLR Unit Pre-sample Gross Weight
- WB NLR Unit Post-sample Gross Weight
- Spun Hematocrit
- CBC
    - WBC Count
    - RBC Count
    - MCV
    - MCH

  - MCHC
  - RDW
  - PLT Count
  - Total Hemoglobin
  - Hematocrit
- Supernatant Hemoglobin
- Two (2) reference plasma aliquots

6.2.16.5 Filtration (Leukoreduction)

The whole blood unit will be filtered for leukoreduction per the study IFU and under Study Arm-specific conditions.

Filtration data collection is as follows:

- Filtration Start: time and temperature
- Head Height
- Prime Time
- Filtration End: date and time
- Drain Time
- Filter Weight (following filtration completion)
- Procedural IFU confirmation (Yes/No)

For the purpose of this study:

- For the procedural IFU confirmation, a response indicative of a deviation from the IFU will result in a prompt to record a protocol deviation in a separate Comments eCRF or other log.

6.2.16.6 Post-filtration

The unit will be weighed, sampled, and re-weighed.

- Sampling time and temperature
- Filtered WB Unit Pre-sample Gross Weight
- Filtered WB Unit Post-sample Gross Weight
- CBC
  - WBC Count
  - RBC Count

  - MCV
  - MCH
  - MCHC
  - RDW
  - PLT Count
  - Total Hemoglobin
  - Hematocrit
- Supernatant Hgb
- Spun Hematocrit
- rWBC Count

6.2.16.7 Filtered Whole Blood Separation

Following the completion of filtration, the filtered whole blood units are to be processed into additive PRBCs and PPP. The first step of this process involves centrifugation of the filtered whole blood unit using a hard spin method followed by expressing the PPP from the RBCs (no data is collected relative to plasma expressing).

Separation data collection is as follows:

- Centrifugation time and temperature
- Centrifuge Make
- Centrifuge Model
- Centrifuge Serial #
- Centrifuge Rotor #
- Centrifuge Cup Type
- Centrifuge Duration
- Centrifuge RCF
- Centrifuge Brake
- Centrifuge Temperature

For the purpose of this study:

- Standard information regarding each centrifuge used will be captured prior to the initiation of study activities. Only deviations from the standard equipment will be entered into the eCRF.

6.2.16.8 Baseline Sampling

The AS-3 is mixed with the RBCs per the IFU. The RBC units will be sampled for Baseline tests. If the LR-RBC is in the CP arm it willthen be placed in 1-6ºC storage for 42 days post-collection.

Baseline data collection and sample processing are as follows:

- Time AS-3 added to RBC unit
- IFU confirmation
- Sampling time
- Sampling room temperature
- Pre-sample Gross Weight
- Post-sample gross weight
- CBC
    - WBC Count
    - RBC Count
    - MCV
    - MCH
    - MCHC
    - RDW
    - PLT Count
    - Total Hemoglobin
    - Hematocrit
- Spun Hematocrit
- RBC morphology
- Supernatant Hemoglobin
- ATP
- 2,3-DPG
- SO_2
- pCO_2
- HCO_3
- pH
- Potassium

- Sodium
- Glucose
- Lactate
- RBC Morphology
- Time RBC placed in refrigerated storage
- Storage temperature

For the purpose of this study:

- For the procedural IFU confirmation, a response indicative of a deviation from the IFU will result in a prompt to record a protocol deviation in a separate Comments eCRF or other log.

6.2.16.9 Hemanext Processing (IP only)

If the unit is in the IP arm it will be transferred to the ORB and will undergo the Hemanext process per the Hemanext IFU.

Hemanext Processing data collection are as follows:

- Lot #
- ORB Gross Weight (net weight should be < 370g)
- Time ORB placed on agitator
- Time ORB removed from agitator
- IFU confirmation

For the purpose of this study:

- For the procedural IFU confirmation, a response indicative of a deviation from the IFU will result in a prompt to record a protocol deviation in a separate Comments eCRF or other log.

6.2.16.10 Post –Hemanext Processing (IP only)

After undergoing the Hemanext process the unit will be transferred to the HSB and placed in 1-6ºC storage for 42 days post-collection.

Post-Hemanext processing data collection and sample processing are as follows:

- Sampling date and time
- Sampling room temperature
- Pre-sample Gross Weight
- Post-sample gross weight
- CBC
    - WBC Count
    - RBC Count
    - MCV
    - MCH
    - MCHC
    - RDW
    - PLT Count
    - Total Hemoglobin
    - Hematocrit
- Spun Hematocrit
- Supernatant Hemoglobin
- Time RBC placed in refrigerated storage

For the purpose of this study:

- For the procedural IFU confirmation, a response indicative of a deviation from the IFU will result in a prompt to record a protocol deviation in a separate Comments eCRF or other log.

6.2.16.11 LR-RBC Bacterial Culture Samples (ONLY for units intended for in vivo autologous transfusion)

5-7 days prior to infusion, units intended for transfusion as part of the in vivo cohort must be cultured. Units evaluated as "+" will not be transfused. **Items in bold, underlined blue font refer specifically to in vivo cohort-specific data and analyses.**

- **Sample date**
- **Pre-sample unit gross weight**
- **Post-sample unit gross weight**
- **Outcome of bacterial culture (+/-)**

- **If "+", then date study donor notified not to return for in vivo study.**

6.2.16.12 21-day Analyses

On day 21 post baseline, RBC units will be analyzed.

21-day RBC data point collection and analyses are as follows:

- Sampling date and time
- Sampling room temperature
- Pre-sample Gross Weight
- Post-sample gross weight
- CBC
    - WBC Count
    - RBC Count
    - MCV
    - MCH
    - MCHC
    - RDW
    - PLT Count
    - Total Hemoglobin
    - Hematocrit
- Spun Hematocrit
- RBC morphology
- Supernatant Hemoglobin
- ATP
- 2,3-DPG
- SO_2
- pCO_2
- HCO_3
- pH
- Potassium
- Sodium
- Glucose

- Lactate
- RBC Morphology
- Time sample returned to refrigeration

6.2.16.13 LR-RBC 42-day Analyses

On day 42 post baseline, RBC units will be analyzed.

42-day RBC data point collection and analyses are as follows:

- Unit Sample date and time
- Pre-sample Gross Weight
- Post-sample gross weight
- CBC
    - WBC Count
    - RBC Count
    - MCV
    - MCH
    - MCHC
    - RDW
    - PLT Count
    - Total Hemoglobin
    - Hematocrit
- Supernatant Hemoglobin
- Spun Hematocrit
- SO_2
- pCO_2
- HCO_3
- pH
- Potassium
- Sodium
- Glucose
- Lactate
- ATP

- 2,3-DPG
- ATP Rejuvenation
- 2,3-DPG Rejuvenation
- RBC Morphology
- Time sample returned to refrigeration

6.2.16.14 RBC in vivo recovery and survival (ONLY for units selected for the in vivo study)

Items in bold, underlined blue font refer specifically to in vivo cohort-specific data and analyses.

Donors participating in the in vivo cohort will have an additional 2-4 weeks between donations to ensure washout from prior in vivo survival procedures.

- **Double radiolabel % recovery**
  - **24 hours (+/- 1hour)**
- **Double radiolabel % survival- 28days**
  - **24 hours (+/- 1hour)**
  - **48 hours (+/- 4 hours)**
  - **72 hours (+/- 8 hours)**
  - **1 week (+/- 1 day)**
  - **2 week (+/- 1 day)**
  - **3 week (+/- 1 day)**
  - **4 week (+/- 1 day)**
- **RBC Microparticles**
- **RBC Metabolomics panel**
- **Pregnancy test**
- **Bacterial Culture**

6.2.16.15 Blood Product Disposition

Each whole blood unit collected in the study will generate one unit of LR-RBC. All LR-RBC units will be stored by the site after completion of the study until written consent to discard the units is obtained from the Sponsor. Written consent to discard blood products will not be required for units with a confirmed positive infectious disease result. All Plasma units will be discarded.

7.0 Study Risks and Safety Reporting

7.1 Analysis of Risks

7.1.1 Test System

The IP is regulated as an investigational device and as such, risks associated with the use of the IP are those expected with manual whole blood donation. See sections 7.1.3 and 7.2.2.5 for more information regarding risks associated with manual whole blood donation.

7.1.2 Control System

Refer to the Precautions, Note(s), and Warnings in the Leukotrap® IFU. See sections 7.1.3 and 7.2.2.5 for more information regarding risks associated with manual whole blood donation.

7.1.3 Risk Assessment of Whole Blood Donation

The risks in the manual whole blood donation for this study are the same as any manual whole blood donation. The study donor may experience a vasovagal reaction. These occur in two to four percent (2-4%) of blood donations, regardless of the type. Very rarely, these progress to syncope and/or seizure activities (1:1000). In addition, as with any blood collection procedure, there is the risk of hematoma formation. Whole blood collections will be performed by trained staff at each site that will recognize the early signs of an adverse reaction and will discontinue the collection and administer appropriate aid to minimize the severity of a reaction under the direction of the Principal Investigator or designee. See section 7.2.2.5 for a list of expected adverse events.

7.1.4 Anemia

Current blood collection standard practice defines a maximum whole blood donation for a 110 pound donor within an eight week period as a single unit. Typically, the donor's body replenishes the fluid lost from donation within 24 hours, and replaces the lost red blood cells within two weeks.

With the exception of those study donors who participate in the in vivo cohort, donors will adhere to the collection standard practice. For the in vivo cohort participants who will have an additional ≤110 mL of whole blood drawn as part of the RBC recovery assessment at 6 weeks following each donation, they are still well below the two unit volume of red blood cells that would be donated via red cell apheresis which may occur every 16 weeks.

To maintain study donors' safety relative to the donation of whole blood for the purpose of this study, Hct or Hgb levels and other applicable inclusion/exclusion criteria will be confirmed prior to each donation.

7.1.5 Risk to Those Conducting the Research

Chosen investigative sites all have long experience in blood collection, storage clinical trials and in vitro studies. All participating staff at the study sites has had training and experience in the handling of potentially biohazardous materials and has been offered hepatitis B vaccine. All phlebotomies are performed in a well-equipped area and only staff with phlebotomy training and experience will perform the whole blood collections. Each of the sites has in-place SOPs for drawing whole blood units and producing and testing blood and blood products. Site staff members are required to have documented SOP training relative to study-related activities. Study donors will be screened with the same criteria used for whole blood donation.

Site staff performing the in vivo portion of the study will be exposed to the radiation attendant to performing the in vivo measurement procedures. Site staff involved in performing the radiolabeled red cell recovery procedures has been trained in handling radioactive materials and wear radiation collection badges that are periodically evaluated. Each investigative site has more than 10 years of experience in the performance of 51Cr and 99mTc radiolabeled red cell recovery procedures and has SOPs in place for the performance of all required procedures. These procedures are well recognized as being safe.

7.1.6 Radiation Exposure

Subjects participating in the in vivo portion of the trial will be exposed to the radiation concomitant to the in vivo measurement procedures. Approximately 15-20 µCi of radioactivity will be administered to in vivo subjects in the autologous, radiolabeled packed RBC + whole blood mixture one on day 42. Red cells labeled with 51Cr are frequently used in immunohematology to assess the survival of either allogeneic or autologous red cells. 99mTc labeled red cells are used to measure total blood volume. Each investigative site is experienced in the performance of 51Cr and 99mTc radiolabeled red cell recovery and survival procedures. These procedures are well recognized as being safe. Standard procedures at both sites for radiolabeled red cell recovery measurement include a determination of the radiation exposure history of each subject that will participate in radiolabeled red cell recovery procedures to ensure that, with the addition of the expected exposure in the study, the subject total exposure will not exceed legal limits. In addition, site procedures are expected to minimize the subject radiation dose in the study. There are no known adverse events associated with the low radiation dose that will be administered in this study. Female in vivo subjects must have a negative pregnancy test prior to each reinfusion, be postmenopausal (> 1 year), or surgically sterile.

7.1.7 Receiving Allogeneic Blood

Subjects participating in the in vivo portion of the study will receive a reinfusion of a small aliquot of their own (autologous) radiolabeled red cells. The possibility exists that in the case of protocol deviation, the study donor could receive allogeneic (from a different donor) red cells. Possible adverse events that could occur from the infusion of allogeneic blood include:

- Infectious disease transmission
- Hemolytic reaction to non-compatible red cells

Each clinical site has more than 15 years' experience in performing autologous, in vivo, radiolabeled red cell recovery and survival procedures and has current standard procedures that include redundant subject identification procedures to ensure that only autologous blood is reinfused.

7.1.8 Study Donor Benefits

Study donors will not receive any direct benefit from study participation.

7.2 Adverse Events

7.2.1 Monitoring

This multiple site study is intended to enroll healthy individuals to serve as study donors. Study donors' study activity will be limited to the donation of two units of blood and that the active study duration is expected to be less than 24 hours for each unit. As such, a Data Safety Monitoring Board (DSMB) will not be appointed. Each site will serve as its own primary safety monitor responsible for identifying any adverse events, providing any necessary immediate medical care for a study-related adverse event and the accurate and timely reporting of any adverse events.

Study-related adverse events are defined as those study participant-reported adverse events occurring within 24-hours of donation and/or transfusion. Non-study-related adverse events outside of these periods will not be recorded unless they are of a nature to impact study participant evaluability, safety or data validity.

Reports for events determined by the investigator to be possibly or definitely related to study participation and reports of events resulting in death must be promptly verbally reported to the sponsor within 24 hours, formally reported to the sponsor within 72 hours, and forwarded to the relative site's IRB within the timing dictated by the respective IRB (typically within 3 days).

7.2.2 Definitions

The following definitions are based on 21 CFR 314.80, ISO 14155-2011 Clinical investigation of medical devices for human subjects, MEDDEV 2.7/3 Guidelines on Clinical Investigations: Serious Adverse Event Reporting under Directives 90/385/EEEC and 93/42/EEC and ICH E6 Good Clinical Practice: Consolidated Guidance.

7.2.2.1 Adverse Event (AE)

An AE is defined as any untoward medical occurrence, unintended disease or injury or untoward clinical sign (including abnormal laboratory finding) in a study donor while enrolled in the clinical investigation, whether or not considered investigational product- related.

Study-related adverse events are defined as those study participant-reported adverse events occurring within 24-hours of donation and/or transfusion. Non-study-related adverse events outside of these periods will not be recorded unless they are of a nature to impact study participant evaluability, safety or data validity.

7.2.2.2 Serious Adverse Event (SAE)

An adverse event is defined as serious, if it:

- led to death;
- led to a serious deterioration in the health of the patient that:
- resulted in a life-threatening illness or injury,
- resulted in a permanent impairment of a body structure or a body function,
- required in-patient hospitalization or prolongation of existing hospitalization,
- resulted in medical or surgical intervention to prevent permanent impairment to a body structure or a body function;
- led to fetal distress, fetal death, congenital abnormality or birth defect.

The term “life-threatening” is an event that places a patient or study donor at immediate risk of death from the event as it occurred; it does not include an event that, had it occurred in more severe form, might have caused death.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event

when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention.

"Non-serious adverse events" are all events that do not meet the criteria for a "serious" adverse event.

All serious and/or unexpected AEs shall be reported to NHSi within 1 business day of learning of their occurrence. They shall be reported by telephone to a study monitor or other designated individual and recorded within the EDC system on the provided AE electronic form. Additional documentation requested by NHSi will be e-mailed or faxed to NHSi within 1 business day following the request.

7.2.2.3 Unexpected Adverse Event

AE's for which the nature or severity of which is not consistent with the applicable product information (e.g., Investigator's Brochure or IFU) or been previously identified in the risk analysis report. This includes events that may be symptomatically and pathophysiologically related to an event listed in the labeling, but differ from the event because of greater severity or specificity.

The investigator must determine if the AE was related to the investigational product using the following scoring system:

- Unrelated — The AE is clearly NOT related to the investigational product. The AE has no temporal relationship to the administration or use of the investigational product, follows no known or suspected pattern of response, and an alternative cause is present.
- Unlikely related — The AE is unlikely to be related to the investigational product. The AE has a temporal relationship to the administration or use of the investigational product, but follows no known or suspected pattern of response, and an alternative cause is present.
- Possibly related — There is a reasonable possibility that the event may have been caused by or is linked in a significant way to the research; the AE has a temporal relationship to the administration or use of the investigational product, follows a suspected pattern of response, but an alternative cause is present.

- Probably related — The adverse event is likely related to the investigational product. The AE has a temporal relationship to the administration of the investigational product or research intervention, follows a known or suspected pattern of response, but an alternative cause may be present.
- Definitely related — The AE is clearly related to the investigational product or research intervention. The adverse event has a temporal relationship to the administration of the investigational product or research intervention, follows a known pattern of response, and no alternative cause is present.

Each AE should be evaluated as either expected or unexpected. If the risks for entering the study described in the informed consent, clinical protocol or risk analysis reflect the severity or specificity of the AE, then it was expected. If not, then the AE is unexpected.

7.2.2.4 Adverse Event Severity

The investigator shall determine the AE Severity using the following scoring system:

- "Mild" – An adverse event which is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities.
- "Moderate"- An adverse event, which is sufficiently discomforting to interfere with normal everyday activities.
- "Severe"- An adverse event, which is incapacitating and prevents normal everyday activities.

7.2.2.5 Adverse Event Expectedness

Expected AEs for this study would include the following:

- Vasovagal response
- Thirst
- Arm pain
- Hives / Itching
- Hypotension
- Tingling
- Unusual taste
- Bruising / Hematoma

- Arterial puncture
- Nerve injury
- Iron depletion
- Weakness / Lightheadedness
- Pallor / Sweating / Chills / Febrile reaction
- Shortness of breath / Hyperventilation
- Nausea / Vomiting / Abdominal cramps
- Syncope - transient loss of consciousness
- Headache
- Tiredness
- Severe reactions – convulsions/ Chest pains / Cardiac arrest

Study donors participating in the in vivo portion of the study will receive an autologous, radiolabeled RBC transfusion. The radiation exposure is very small and the level used in this trial has not been found to cause cancer. These study donors would be further subject to experiencing the following expected AEs in addition to those listed above:

- Infection from bacterial contamination

Each AE will be evaluated to determine if it meets serious criteria.

8.0 Study Sites

8.1 Study Sites

Three clinical sites will participate in this study. American Red Cross Mid-Atlantic Research Facility (Site A) and Hoxworth Blood Center (Site H) and Blood Center of Wisconsin (Site B) will all participate as primary research sites.

8.2 Central Laboratory

Analyses of blood products will occur at the laboratories associated with each of the clinical sites.

9.0 Data Management

9.1 Case Report Forms

All original source data will be contained in original laboratory printouts, site laboratory notebooks, site data sheets and/or study-specific source documents. Data transmittal to the Sponsor will be accomplished using electronic Case Report Forms (eCRFs). Original source data will not be entered directly on the eCRFs. Original data is to be transcribed

from source data records to the eCRFs by site study staff. Data entered into the eCRFs will then be verified against the source data during Sponsor on-site monitoring visits. At the study site visits the verified and corrected CRFs must be reviewed and electronically signed by the respective Principal Investigator to finalize the data. Investigative site study data and records for this protocol are subject to monitoring or inspection by the Sponsor, FDA and relevant state or federal government agencies.

9.2 Monitoring

Monitoring will be conducted by the sponsor or delegate according to clinical monitoring SOPs to assure that the protocol is followed, any adverse events are reported, data collected are scientifically sound, and that the study is conducted in compliance with federal and ICH guidelines for GCP. On-site monitoring visits will occur at study initiation, during the study performance as needed (minimum one interim visit) and at the study close.

9.3 Protocol Deviations

Sites are required to perform the study as described in the protocol and informed consent. All protocol deviations are to be documented in source records and transcribed to the appropriate eCRF page with a description of the deviation and reason(s) for the deviation.

Any protocol deviation that impacts study donor safety or the scientific integrity of the study must be reported within 24 hours of notification to the Sponsor, and within 48 hours to the site IRB where the deviation occurred.

Issuance of a revised protocol is required for a protocol deviation/modification that substantially affects the design of the study or study donor safety (including but not limited to a change in, principal investigator, inclusion/exclusion criteria, number of study donors to be enrolled, study sites or study procedures). Each protocol addendum must be signed by the Sponsor and the principal investigators and be submitted for local IRB review and approval before implementation of the protocol addendum. The study can continue under the previous protocol during this process, if appropriate. Once the Sponsor and principal investigator signature, IRB approval has been obtained, performance of the protocol addendum can begin at that site.

Minor deviations such as a missed measurement must be documented as described but do not require immediate reporting to the IRB. Minor deviations will be reported to local IRB in continuing review reports and the study final report.

10.0 Data Use

10.1 Test Report

A final report will be prepared by NHSi and be reviewed by the study sites. This final report will include data reported on all study donors enrolled including cases where they

are excluded from the total evaluable study donors. This report will be made available to all persons responsible for the conduct of the study.

10.2 Confidentiality

Confidentiality of each study donor shall be preserved in the report and any publication of the clinical investigation data.

10.3 Positive TTD Results – Result Reporting Requirements and Unit Disposition

In the event of a positive TTD testing report or other significant abnormality found during subject testing, the subject will be confidentially informed by the site principal investigator in person and/or by mail. The result will be explained with tact and understanding. A written record of the finding will be provided to the subject's personal physician with permission of the subject. The subject may be referred to an appropriate health care source. At each site positive infectious disease results are required to be reported to the appropriate state agencies. Subjects, and their blood products, yielding a positive infectious disease result or displaying another significant abnormality will be discontinued from the study and replaced with a new subject. Note that some form of documentation must exist within the study records and/or other site records that demonstrate that these requirements have been fulfilled.

10.4 Publication

Publication of the trial results is subject to the mutual agreement of each research site and the sponsor.

10.5 Anticipated Study Schedule

IRB submission:	September, 2017
Procedures start date:	November, 2017
Enrollment completed:	May, 2018
Test report completed:	July, 2018

11.0 Responsibilities

Sponsor/promoter: NHSi Corporation
161 First St., Suite 1A
Cambridge, MA 02142

12.0 References

1) Davey, R.J. Red Cell Radiolabeling in Transfusion Medicine. In: Davey RJ, Wallace ME, eds. Diagnostic and investigational uses of radiolabeled blood elements. Arlington, VA: AABB, 1987:93-114.

2) Heaton, W. Evaluation of Post Transfusion Recovery and Survival of Transfused Red Cells. Transfus Med Rev 1992:6:153-169.

3) Thomas, S. Ambient overnight hold of whole blood prior to the manufacture of blood components. Transfus Med. 2012 Dec;20(6):361-8.

4) Wilsher C, Garwood M, Sutherland J, Turner, C and Cardigan R. The effect of storing whole blood at 22°C for up to 24 hours with and without rapid cooling on the quality of red cell concentrates and fresh-frozen plasma. Transfusion. 2008, Nov:4(11):2338-2347.

13.0 Appendices

13.1 Study Flow Chart

Highlevel flowsheet Pivotal Crossover.pdf

13.2 Control Product/Leukotrap System – Instructions for Use

Manufactured for:
Haemonetics Corporation
400 Wood Road
Braintree, MA 02184, USA

By: Haemonetics Manufacturing Inc.
1630 Industrial Park Street
Covina, CA 91722, USA

www.haemonetics.com
888-489-5938

HAEMONETICS®
THE Blood Management Company™

Leukotrap® WB System

CP2D/AS-3 Blood Bag Unit with In-Line WBF Filter* and Sampling System

For collection of blood and preparation of red blood cells and plasma with pre-storage leukocyte reduction. Use aseptic technique. Note: Platelet concentrates are not intended to be made with this product.

Instruction for Use for Systems Containing a Y Sampling Site (YSS) or Sample Diversion Pouch (with or without a pre-attached SampLok® Vacuum Tube Holder) - see unit foil package label for specific product description being used.

Precaution: Use only if solutions are clear. Sterile, nonpyrogenic fluid path. Sterilized by steam. **Warning:** Failure to achieve and maintain a closed system during processing would result in a product that must be transfused within 24 hours. **Rx only.** This product is free of natural rubber latex.

I. Blood Collection Instructions for Systems Containing a Y Sampling Site (YSS) Only

1. Load blood agitation device or suspend blood bag on donor scale and adjust donor scale to desired collection gross weight as per manufacturer's instructions.
2. Clamp donor tubing between DonorCare® Needle Guard (DCNG) and Y Sampling Site.
3. Secure donor tubing above the Y connector and disinfect site of phlebotomy.
4. If using blood pressure cuff, inflate to not more than 60 mm Hg.
5. Remove donor needle cover and accomplish phlebotomy.
6. Release clamp and ensure there is blood flow. Reduce pressure as required.
7. Slide the DCNG midway over the needle hub and securely tape DCNG to the donor's arm as close to the top of the DCNG as possible. Note: If blood flow is slow, slide DCNG away from the needle hub, adjust and re-engage DCNG. If repeated needle adjustment is necessary, slide DCNG away from the needle hub and re-engage at the end of blood collection.
8. Collect appropriate volume of blood into collection bag, as indicated on packaging. Note: Mix blood and anticoagulant frequently during collection, for example, once every 45 seconds, and immediately after collection. If blood agitation device is used, follow manufacturer's operating instructions.
9. After required amount of blood has been collected, seal donor tubing between Y Sampling Site and collection bag.** Note: If pre-filtration quality control is to be performed, leave an adequate length (~10 inches) of QC tubing containing anticoagulated blood attached to the collection bag.
10. For blood sampling, remove the Y Sampling Site needle cover. Ensure the protective sheath is in place over the sampling needle.
11. Fasten the vacuum tube holder on to the base of the sampling needle.
12. Collect blood samples into vacuum tubes.
13. Ensure the vacuum tubes are centered within the vacuum tube holder.
14. Maintain forward pressure on the vacuum tubes during sample collection. Note: After the last tube is collected, it is recommended that the vacuum tube holder be left in place.
15. After blood samples are collected, clamp donor tubing between the Y Sampling Site and as close to the DCNG as possible.
16. Release any remaining pressure from the donor's arm.
17. DCNG must be held stationary while the needle is withdrawn into it. While holding sides of DCNG near the front, grasp the tubing below the clamp and pull the needle into the DCNG until it locks into place, and the needle hub engages the bottom of the DCNG.
18. Insert the DCNG into the vacuum tube holder. Note: It is recommended that the DCNG be inserted securely into the vacuum tube holder, prior to discarding.
19. Seal donor tubing adjacent to DCNG.** Detach and discard needle, DCNG, Y Sampling Site and tubing.**
20. Strip tubing between seal and collection bag.
21. Continue to "Filtration Instructions", Section IV, Step 1.

II. Blood Collection Instruction for Systems Containing a Sample Diversion Pouch with or without a pre-attached SampLok® Vacuum Tube Holder

When using systems with pre-attached SampLok vacuum tube holder, follow instructions as noted below, but refer to Section III when indicated to do so.

1. Load blood agitation device or suspend blood bag on donor scale and adjust donor scale to desired collection gross weight as per manufacturer's instructions.
2. Clamp donor tubing between DonorCare Needle Guard (DCNG) and Sampling Site.
3. Secure donor tubing above the Y connector and disinfect site of phlebotomy.
4. If using blood pressure cuff, inflate to not more than 60 mm Hg.
5. Remove donor needle cover and accomplish phlebotomy.
6. Release clamp and ensure there is blood flow.
7. Slide the DCNG midway over the needle hub and securely tape DCNG to the donor's arm as close to the top of the DCNG as possible. Note: If blood flow is slow, slide DCNG away from the needle hub, adjust and re-engage DCNG. If repeated needle adjustment is necessary, slide DCNG away from the needle hub and re-engage at the end of blood collection.
8. The donor blood will be automatically diverted to the sample diversion pouch. Once the sample diversion pouch is filled, close clamp immediately on tubing between the sample diversion pouch and Y connector. Warning: To avoid risk of air embolism to donor, do not squeeze sample diversion pouch while tubing is open.
9. Open snap-open closure between the Y connector and the collection bag to initiate blood collection. Reduce pressure as needed.
10. Permanently seal tubing between the sample diversion pouch and the Y connector to maintain sterility of the system prior to collecting blood samples.** Note: When using systems with a pre-attached SampLok vacuum tube holder, go to Section III.
11. For blood sampling, remove the Sampling Site needle cover. Ensure the protective sheath is in place over the sampling needle.
12. Fasten the vacuum tube holder on to the base of the sampling needle.
13. Position the sample diversion pouch downwards so that the air rises to the top of the pouch and away from the vacuum tube holder. Note: Drawing air into the vacuum tube may cause hemolysis.
14. Collect blood samples from the sample diversion pouch within approximately four minutes to avoid possible clot formation.
15. Ensure the vacuum tubes are centered within the vacuum tube holder during sample collection.
16. Maintain forward pressure on the vacuum tubes during sample collection. Note: After the last tube is collected, it is recommended that the vacuum tube holder be left in place.
17. Collect appropriate volume of blood into collection bag as indicated on packaging. Note: Mix blood and anticoagulant frequently during collection, for example, once every 45 seconds, and immediately after collection. If blood agitation device is used, follow manufacturer's operating instructions.
18. After required amount of blood has been collected, seal donor tubing between snap-open closure and collection bag.** Note: If pre-filtration quality control is to be performed, leave an adequate length (~10 inches) of QC tubing containing anticoagulated blood attached to the collection bag.
19. Clamp donor tubing between the Y connector and DCNG as close as possible to the DCNG.
20. Release any remaining pressure from donor's arm.
21. DCNG must be held stationary while the needle is withdrawn into it. While holding sides of DCNG near the front, grasp the tubing below the clamp and pull the needle into the DCNG until it locks into place, and the needle hub engages the bottom of the DCNG.
22. Insert the DCNG into the vacuum tube holder, if desired. Note: It is recommended that the DCNG be inserted securely into the vacuum tube holder, prior to discarding.
23. Seal donor tubing adjacent to DCNG.** Detach and discard needle, DCNG, sample diversion pouch and tubing.**
24. Strip tubing between seal and collection bag.
25. Continue to "Filtration Instructions", Section IV, Step 1.

III. When Using Systems with a pre-attached SampLok® Vacuum Tube Holder

1. To collect blood samples, open lid from SampLok vacuum tube holder.
2. Open snap-open closure between sample diversion pouch and SampLok vacuum tube holder.
3. Position the sample diversion pouch downwards so that the air rises to the top of the pouch and away from the SampLok vacuum tube holder. Note: Drawing air into the vacuum tube may cause hemolysis.
4. Collect blood samples from the sample diversion pouch into vacuum tubes within approximately four minutes to avoid possible clot formation.
5. Ensure the vacuum tubes are centered within the SampLok vacuum tube holder during sample collection.
6. Maintain forward pressure on the vacuum tubes during sample collection.
7. The lid may be closed on the SampLok vacuum tube holder after sample collection.
8. Return to Section II, Step 17. Note: When collection of unit is complete, and the donor needle is engaged in the DCNG, open the lid of the SampLok vacuum tube holder and insert the DCNG into the holder. Twist until it locks into place. An audible click will confirm that it is locked.

IV. Filtration Instructions

1. Filter and process whole blood within 72 hours of collection.
2. Mix whole blood/anticoagulant thoroughly.
3. Place empty red cell storage bag on a horizontal surface.
4. Ensure cap of blood recovery vent above the filter is tightly closed.
5. Hang whole blood bag up to 60 inches (1.52 meters) above empty red cell storage bag and ensure filter is vertical. Note: The maximum head height should be 60 inches (1.52 meters) for whole blood filtered at room temperature and at 1—6 °C.
6. Open snap-open closure of whole blood bag. Priming will occur automatically by gravity.
7. After blood fills one side of the air vent below the filter, open snap-open closure immediately below the air vent to begin filtration.
8. Allow blood to filter by gravity. Note: Do not apply mechanical or manual pressure to increase flow rate. Filtration is complete when whole blood bag is empty.
9. Remove cap from blood recovery vent above the filter and allow the upstream side (non-printed side) of the filter to drain.
10. When the upstream side of the filter is empty, clamp tubing below the filter. Note: Filtration times can be influenced by collection and processing conditions and biological variability of donors. Experimental data with some filter products indicate that a prolonged filtration can be an indication of sub-optimal leukocyte reduction.
11. Seal tubing just below the snap-open closure.**
12. Detach and discard collection bag and filter.** Note: Do not strip tubing prior to sealing the tubing distal to the filter. If it is desired to strip blood from numbered tubing, do so only after tubing has been sealed close to the snap-open closure and detached.
13. If desired, seal at or adjacent to "X" marks on the tubing to provide numbered segments of anticoagulated blood for typing or crossmatching.** Notes: If it is necessary to strip blood from numbered tubing for re-suspension, care should be taken when stripping is performed. Increased (mechanical) hemolysis has been associated with stripping when blood is cold and has a higher hematocrit. Do not strip forcefully or frequently against a snap-open closure.

V. Processing Instructions

1. Load filtered blood and remaining bags into centrifuge cup, ensuring that the tubing stays in the top half of the cup.
2. Centrifuge at appropriate conditions to produce desired components.
3. Carefully remove the unit from the centrifuge and place the red cell storage bag in the plasma expressor.
4. Clamp tubing to extra satellite bags, if present.
5. Gently apply expressor pressure.
6. Open snap-open closure to satellite bag and express plasma.
7. After plasma is expressed, clamp tubing between red cell storage bag and Y connector, and release expressor pressure.
8. Clamp tubing between the Y connector and plasma bag.
9. Hang AS-3 bag above red cell storage bag and remove clamp from tubing to the red cell storage bag.
10. Open snap-open closure on the bag containing the AS-3 additive solution and transfer to the bag containing the red cells. Note: AS-3 solution should be added to the packed red blood cells immediately after removal of plasma. Transfer AS-3 solution under one of the following processing conditions:
    a. within 8 hours of collection if whole blood is held at room temperature.
    b. within 72 hours of collection if whole blood is refrigerated.
11. Seal tubing and detach the bag containing packed red cells, and set aside plasma for further processing.** Note: If quality control is to be performed on postfiltration sample, leave an adequate length (~10 inches) of tubing attached to the bag containing red cells.
12. Gently mix packed red cells and AS-3 solution.
13. Store CP2D/AS-3 preserved red blood cells at 1—6 °C for up to 42 days and use as indicated. Note: If AS-3 is not used, whole blood or red blood cells in CP2D alone may be stored at 1—6 °C for up to 21 days.

* WBF2

** During processing, always observe the following precautions:
1. Sealing should be done in a manner that avoids fluid splatter.
2. Always dispose of blood-contaminated products in a manner consistent with established **BIOHAZARD** safety procedures.

147126928, issued Jan. 2013

Leukotrap® WB System

CP2D/AS-3 Blood Bag Unit with In-Line WBF Filter* and Sampling System

For collection of blood and preparation of red blood cells and plasma with pre-storage leukocyte reduction.

Use aseptic technique. Note: Platelet concentrates are not intended to be made with this product.

Instruction for Use for Systems Containing a Y Sampling Site (YSS) or Sample Diversion Pouch (with or without a pre-attached Samp*Lok*® Vacuum Tube Holder) - see unit foil package label for specific product description being used.

Precaution: Use only if solutions are clear. Sterile, nonpyrogenic fluid path. Sterilized by steam.

Warning: Failure to achieve and maintain a closed system during processing would result in a product that must be transfused within 24 hours.

Rx only. This product is free of natural rubber latex.

I. Blood Collection Instructions for Systems Containing a Y Sampling Site (YSS) Only

1. Load blood agitation device or suspend blood bag on donor scale and adjust donor scale to desired collection gross weight as per manufacturer's instructions.

2. Clamp donor tubing between Donor*Care*® Needle Guard (DCNG) and Y Sampling Site.

3. Secure donor tubing above the Y connector and disinfect site of phlebotomy.

4. If using blood pressure cuff, inflate to not more than 60 mm Hg.

5. Remove donor needle cover and accomplish phlebotomy.

6. Release clamp and ensure there is blood flow. Reduce pressure as required.

7. Slide the DCNG midway over the needle hub and securely tape DCNG to the donor's arm as close to the top of the DCNG as possible.

Note: If blood flow is slow, slide DCNG away from the needle hub, adjust and re-engage DCNG. If repeated needle adjustment is necessary, slide DCNG away from the needle hub and re-engage at the end of blood collection.

8. Collect appropriate volume of blood into collection bag, as indicated on packaging.

Note: Mix blood and anticoagulant frequently during collection, for example, once every 45 seconds, and immediately after collection. If blood agitation device is used, follow manufacturer's operating instructions.

9. After required amount of blood has been collected, seal donor tubing between Y Sampling Site and collection bag.

** **Note**: If pre-filtration quality control is to be performed, leave an adequate length (~10 inches) of QC tubing containing anticoagulated blood attached to the collection bag.

10. For blood sampling, remove the Y Sampling Site needle cover. Ensure the protective sheath is in place over the sampling needle.

11. Fasten the vacuum tube holder on to the base of the sampling needle.

12. Collect blood samples into vacuum tubes.

13. Ensure the vacuum tubes are centered within the vacuum tube holder.

14. Maintain forward pressure on the vacuum tubes during sample collection.

Note: After the last tube is collected, it is recommended that the vacuum tube holder be left in place.

15. After blood samples are collected, clamp donor tubing between the Y Sampling Site and as close to the DCNG as possible.

16. Release any remaining pressure from the donor's arm.

17. DCNG must be held stationary while the needle is withdrawn into it. While holding sides of DCNG near the front, grasp the tubing below the clamp and pull the needle into the DCNG until it locks into place, and the needle hub engages the bottom of the DCNG.

18. Insert the DCNG into the vacuum tube holder.

Note: It is recommended that the DCNG be inserted securely into the vacuum tube holder, prior to discarding.

19. Seal donor tubing adjacent to DCNG.** Detach and discard needle, DCNG, Y Sampling Site and tubing.**

20. Strip tubing between seal and collection bag.

21. Continue to "Filtration Instructions", Section IV, Step 1.

II. Blood Collection Instructions for Systems Containing a Sample Diversion Pouch with or without a pre-attached Samplok® Vacuum Tube Holder

When using systems with pre-attached Samp*Lok* vacuum tube holder, follow instructions as noted below, but refer to Section III when indicated to do so.

1. Load blood agitation device or suspend blood bag on donor scale and adjust donor scale to desired collection gross weight as per manufacturer's instructions.

2. Clamp donor tubing between Donor*Care* Needle Guard (DCNG) and Sampling Site.

3. Secure donor tubing above the Y connector and disinfect site of phlebotomy.

4. If using blood pressure cuff, inflate to not more than 60 mm Hg.

5. Remove donor needle cover and accomplish phlebotomy.

6. Release clamp and ensure there is blood flow.

7. Slide the DCNG midway over the needle hub and securely tape DCNG to the donor's arm as close to the top of the DCNG as possible.

Note: If blood flow is slow, slide DCNG away from the needle hub, adjust and re-engage DCNG. If repeated needle adjustment is necessary, slide DCNG away from the needle hub and re-engage at the end of blood collection.

8. The donor blood will be automatically diverted to the sample diversion pouch. Once the sample diversion pouch is filled, close clamp immediately on tubing between the sample diversion pouch and Y connector.

Warning: To avoid risk of air embolism to donor, do not squeeze sample diversion pouch while tubing is open.

9. Open snap-open closure between the Y connector and the collection bag to initiate blood collection. Reduce pressure as needed.

10. Permanently seal tubing between the sample diversion pouch and the Y connector to maintain sterility of the system prior to collecting blood samples.

** **Note:** When using systems with a pre-attached Samp*Lok* vacuum tube holder, go to Section III.

11. For blood sampling, remove the Sampling Site needle cover. Ensure the protective sheath is in place over the sampling needle.

12. Fasten the vacuum tube holder on to the base of the sampling needle.

13. Position the sample diversion pouch downwards so that the air rises to the top of the pouch and away from the vacuum tube holder.

Note: Drawing air into the vacuum tube may cause hemolysis.

14. Collect blood samples from the sample diversion pouch within approximately four minutes to avoid possible clot formation.

15. Ensure the vacuum tubes are centered within the vacuum tube holder during sample collection.

16. Maintain forward pressure on the vacuum tubes during sample collection.

Note: After the last tube is collected, it is recommended that the vacuum tube holder be left in place.

17. Collect appropriate volume of blood into collection bag as indicated on packaging.

Note: Mix blood and anticoagulant frequently during collection, for example, once every 45 seconds, and immediately after collection. If blood agitation device is used, follow manufacturer's operating instructions.

18. After required amount of blood has been collected, seal donor tubing between snap-open closure and collection bag.

** **Note**: If pre-filtration quality control is to be performed, leave an adequate length (~10 inches) of QC tubing containing anticoagulated blood attached to the collection bag.

19. Clamp donor tubing between the Y connector and DCNG as close as possible to the DCNG.

20. Release any remaining pressure from donor's arm.

21. DCNG must be held stationary while the needle is withdrawn into it. While holding sides of DCNG near the front, grasp the tubing below the clamp and pull the needle into the DCNG until it locks into place, and the needle hub engages the bottom of the DCNG.

22. Insert the DCNG into the vacuum tube holder, if desired.

Note: It is recommended that the DCNG be inserted securely into the vacuum tube holder, prior to discarding.

23. Seal donor tubing adjacent to DCNG.** Detach and discard needle, DCNG, sample diversion pouch and tubing.**

24. Strip tubing between seal and collection bag.

25. Continue to "Filtration Instructions", Section IV, Step 1.

III. When Using Systems with a Pre-attached Samplock® Vacuum Tube Holder

1. To collect blood samples, open lid from Samp*Lok* vacuum tube holder.

2. Open snap-open closure between sample diversion pouch and Samp*Lok* vacuum tube holder.

3. Position the sample diversion pouch downwards so that the air rises to the top of the pouch and away from the Samp*Lok* vacuum tube holder.

Note: Drawing air into the vacuum tube may cause hemolysis.

4. Collect blood samples from the sample diversion pouch into vacuum tubes within approximately four minutes to avoid possible clot formation.

5. Ensure the vacuum tubes are centered within the Samp*Lok* vacuum tube holder during sample collection.

6. Maintain forward pressure on the vacuum tubes during sample collection.

7. The lid may be closed on the Samp*Lok* vacuum tube holder after sample collection.

8. Return to Section II, Step 17.

Note: When collection of unit is complete, and the donor needle is engaged in the DCNG, open the lid of the Samp*Lok* vacuum tube holder and insert the DCNG into the holder. Twist until it locks into place. An audible click will confirm that it is locked.

IV. Filtration Instructions

1. Filter and process whole blood within 72 hours of collection.

2. Mix whole blood/anticoagulant thoroughly.

3. Place empty red cell storage bag on a horizontal surface.

4. Ensure cap of blood recovery vent above the filter is tightly closed.

5. Hang whole blood bag up to 60 inches (1.52 meters) above empty red cell storage bag and ensure filter is vertical.

Note: The maximum head height should be 60 inches (1.52 meters) for whole blood filtered at room temperature and at 1-6 °C.

6. Open snap-open closure of whole blood bag. Priming will occur automatically by gravity.

7. After blood fills one side of the air vent below the filter, open snap-open closure immediately below the air vent to begin filtration.

8. Allow blood to filter by gravity.

Note: Do not apply mechanical or manual pressure to increase flow rate. Filtration is complete when whole blood bag is empty.

9. Remove cap from blood recovery vent above the filter and allow the upstream side (non-printed side) of the filter to drain.

10. When the upstream side of the filter is empty, clamp tubing below the filter.

Note: Filtration times can be influenced by collection and processing conditions and biological variability of donors. Experimental data with some filter products indicate that a prolonged filtration can be an indication of sub-optimal leukocyte reduction.

11. Seal tubing just below the snap-open closure.**

12. Detach and discard collection bag and filter.

** **Note:** Do not strip tubing prior to sealing the tubing distal to the filter. If it is desired to strip blood from numbered tubing, do so only after tubing has been sealed close to the snap-open closure and detached.

13. If desired, seal at or adjacent to "X" marks on the tubing to provide numbered segments of anticoagulated blood for typing or crossmatching.

** **Notes:** If it is necessary to strip blood from numbered tubing for re-suspension, care should be taken when stripping is performed. Increased (mechanical) hemolysis has been associated with stripping when blood is cold and has a higher hematocrit. Do not strip forcefully or frequently against a snap-open closure.

V. Processing Instructions

1. Load filtered blood and remaining bags into centrifuge cup, ensuring that the tubing stays in the top half of the cup.

2. Centrifuge at appropriate conditions to produce desired components.

3. Carefully remove the unit from the centrifuge and place the red cell storage bag in the plasma expressor.

4. Clamp tubing to extra satellite bags, if present.

5. Gently apply expressor pressure.

6. Open snap-open closure to satellite bag and express plasma.

7. After plasma is expressed, clamp tubing between red cell storage bag and Y connector, and release expressor pressure.

8. Clamp tubing between the Y connector and plasma bag.

9. Hang AS-3 bag above red cell storage bag and remove clamp from tubing to the red cell storage bag.

10. Open snap-open closure on the bag containing the AS-3 additive solution and transfer to the bag containing the red cells.

Note: AS-3 solution should be added to the packed red blood cells immediately after removal of plasma. Transfer AS-3 solution under one of the following processing conditions:

a. within 8 hours of collection if whole blood is held at room temperature.

b. within 72 hours of collection if whole blood is refrigerated.

11. Seal tubing and detach the bag containing packed red cells, and set aside plasma for further processing.

** **Note:** If quality control is to be performed on post filtration sample, leave an adequate length (~10 inches) of tubing attached to the bag containing red cells.

12. Gently mix packed red cells and AS-3 solution.

13. Store CP2D/AS-3 preserved red blood cells at 1—6 °C for up to 42 days and use as indicated.

Note: If AS-3 is not used, whole blood or red blood cells in CP2D alone may be stored at 1-6 °C for up to 21 days.

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** During processing, always observe the following precautions:
1. Sealing should be done in a manner that avoids fluid splatter.
2. Always dispose of blood-contaminated products in a manner consistent with established BIOHAZARD safety procedures.

13.3 Investigational Product/Hemanext RBC Processing System – Instructions for Use

Hemanext™ RBC Processing System

CAUTION – Investigational Device. Limited by Federal (or United States) Law to Investigational Use.

DEVICE DESCRIPTION:

Blood container set used to process and store CP2D/AS-3 Red Blood Cells, Leukocytes Reduced, and O_2/CO_2 Reduced.

Each system consists of:

- One Oxygen Reduction Bag (ORB): empty oxygen impermeable container for processing of CP2D/AS-3 Red Blood Cells, Leukocytes Reduced.
- One Hemanext Storage Bag (HSB): empty oxygen impermeable container for storage of CP2D/AS-3 Red Blood Cells, Leukocytes Reduced, and O_2/CO_2 Reduced.
- One LR RBC blood line
- Three flow control blood line clips

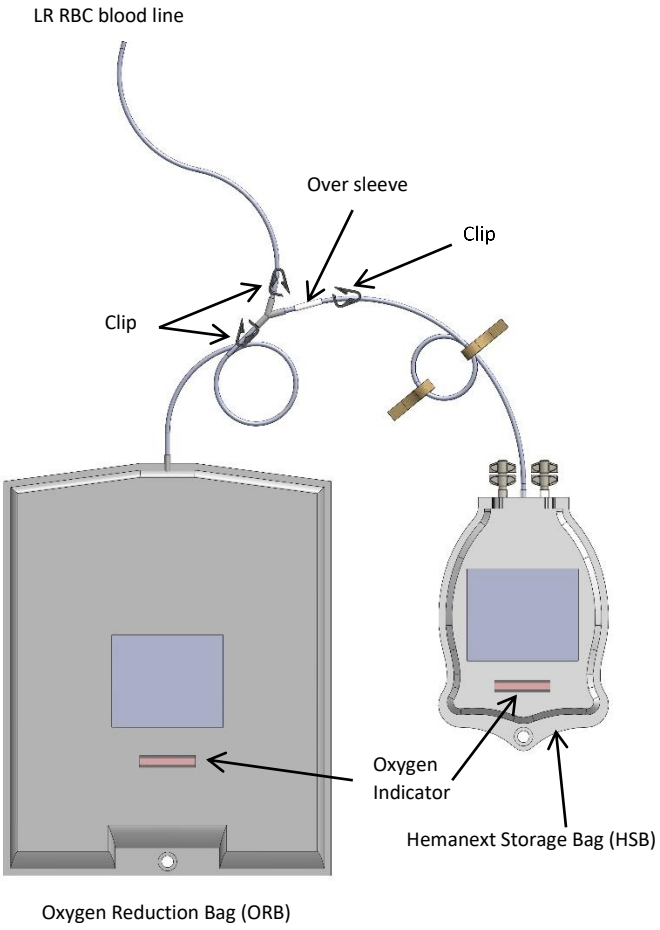


Figure 1: Hemanext RBC Processing System

INDICATIONS FOR USE:

Blood container set used to process and store Red Blood Cells Leukocytes Reduced, O_2/CO_2 Reduced.

The Hemanext system is intended to process and store CP2D/AS-3 Red Blood Cells, Leukocytes Reduced that have been prepared within the standard 8-hour hold time. The Red Blood Cells will be processed using the Hemanext system within 12 hours of collection. The Hemanext RBC Processing System limits the O_2 and CO_2 levels in the storage environment. Red Blood Cells Leukocytes Reduced, O_2/CO_2 Reduced may be stored for up to 42 days at 1-6°C. The Hemanext RBC Processing System is used for volumes no greater than 350 ml of LR RBC.

CONTRAINDICATIONS:

- Sickle Cell Trait blood cannot be used for Hemanext RBC processing.

PRECAUTIONS:

- The Hemanext RBC Processing System is not intended for manufacture of red blood cells using other additive solutions than AS-3.
- Not intended for non-leukocytes reduced red blood cells.
- Not intended for processing of apheresis red blood cells.
- Dispose of used components using acceptable biohazard disposal methods.
- Units must be used within 24-hours of opening individual packaging.
- Studies have not been performed to allow irradiation and/or freezing of Hemanext red blood cells.
- Studies have not been performed to allow cell washing.
- Processing with Hemanext RBC Processing System must be completed with red cells at room temperature prior to refrigeration.

INSTRUCTIONS FOR USE

REMOVAL FROM PACKAGING:

1) Remove Hemanext RBC Processing System from the individual package and inspect for damage. **Warning: Do not use if package or disposable set is damaged**.
2) Smooth out any kinks observed in the tubing prior to processing.
3) Close all 3 clips on the LR RBC, the ORB, and the HSB blood lines.
4) Confirm the color indicators on the ORB and HSB are pink.
   Caution: If an indicator is purple, the product may not be used.

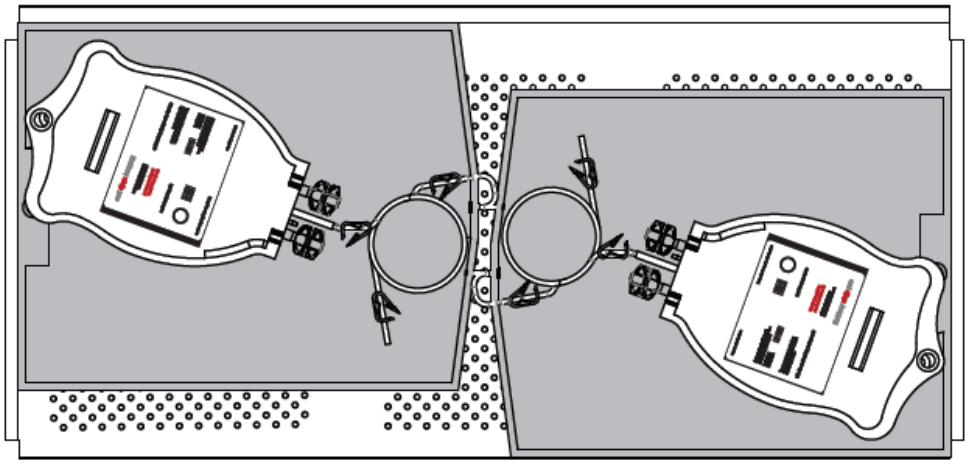
HEMANEXT PROCESSING OF RED BLOOD CELLS:

1) Confirm all clips are closed on blood lines.

2) Ensure incoming CP2D/AS-3 Leukocytes Reduced RBC blood line is not segmented. (**Caution: unit must be no more than 350 ml)**
3) Sterile dock the CP2D/AS-3, Leukocytes Reduced RBC bag to the LR RBC blood line.
4) Confirm that the O_2 indicator of the ORB is pink.
5) At room temperature (22±2°C), hang LR RBC bag at a head height of no greater than 72 inches.
6) Open clip on LR RBC blood line and ORB blood line.
7) Pinch the sterile connection on the LR RBC blood line to allow red blood cells to flow into the ORB.
8) Transfer the LR RBC by gravity to the ORB.
9) Close the clip on the ORB blood line.
10) Move the clip on the Hemanext LR RBC blood line close to the Y connector. Close the clip on the LR RBC blood line.
11) Seal and separate the LR RBC blood line between the Y connector and the ORB.

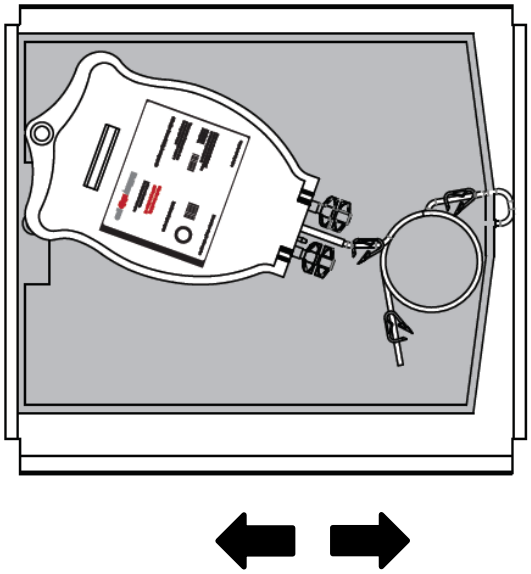
12) Transfer the Hemanext RBC Processing System to a flatbed agitator shelf at room temperature (22±2°C).
13) When placing on the flatbed agitator shelf, be sure to place no more than two Hemanext RBC Processing Systems per shelf with the ORB parallel to the axis of motion and the HSB on top of the ORB as shown in the diagram below (see Figure 2). Note: ORB must lay flat on shelf surface and ensure HSB and tubing is placed completely within the shelf.

Figure 2



Note: If flatbed agitator shelf is less 36 x 76 x 2.5 cm, please place one Hemanext RBC Processing System as shown in Figure 3 below.

Figure 3



14) Make sure the agitator shelf is fully closed. If applicable, make sure the incubator doors are closed. Note: limit the amount of times the agitator doors are opened and remain open during processing.
15) Leave Hemanext RBC Processing System on the agitator shelf for 3 hours +/- 15 minutes for O_2/CO_2 reduction.
16) After 3 hours, remove the Hemanext RBC Processing System from the agitator shelf. Note: confirm that the O_2 indicator in the ORB is pink.
17) Hang ORB at head height of no greater than 72 inches.
18) Open clip on the ORB blood line and the HSB blood line. Transfer the LR RBC O_2/CO_2 reduced into the HSB.
19) Once all volume has been transferred to the HSB, close the clip on the HSB blood line and heat seal the tubing immediately after the white over sleeve section. (Caution: Do not close clip on white over sleeve section. Do not heat seal tubing in the white over sleeve section)
20) Remove the ORB and dispose accordingly.
21) Segment bloodline as needed.
22) Place HSB into cold storage of 1 to 6° C for up to 42 days after collection.
23) When pulling from cold storage, confirm that the O_2 indicator in the HSB is pink.

STORAGE AND HANDLING

- Do not use if:

- Tamper evident package has been opened
- Signs of deterioration are visible
- The fluid path closures are loose or not intact
- The package is damaged
- The ORB, which contains RBC, is dropped on a hard surface (e.g. floor, lab bench, etc.)

- Store at room temperature
  - Avoid exposure to excessive heat
  - Protect from freezing

SYMBOL LEGEND	
	Symbol for Manufacturer
	Symbol for Date of Manufacture
	Lot Number
	Symbol for Sterile Fluid Path
	Symbol for Single Use Only
	Symbol for Refer to Instructions for Use

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