AFRL-SA-WP-TR-2019-0011

Field Deployable Whole Blood Collection and Transfusion Set

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April 2019

Final Report
for July 2015 to June 2018

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
3 Apr 2019	Final Technical Report	July 2015 – June 2018

4. TITLE AND SUBTITLE

Field Deployable Whole Blood Collection and Transfusion Set

5a. CONTRACT NUMBER
FA8650-16-2-6B36

5b. GRANT NUMBER
FA8650-16-2-6B36

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER
14-132

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Cincinnati
Sponsored Research Services
51 Goodman Drive, Suite 530
Cincinnati, OH 45221-0222

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
USAF School of Aerospace Medicine
Aeromedical Research Dept/FHE
2510 Fifth St., Bldg. 840
Wright-Patterson AFB, OH 45433-7913

10. SPONSORING/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-SA-WP-TR-2019-0011

12. DISTRIBUTION / AVAILABILITY STATEMENT
DISTRIBUTION STATEMENT A. Approved for public release. Distribution is unlimited.

13. SUPPLEMENTARY NOTES
Cleared, 88PA, Case # 2019-2204, 7 May 2019

14. ABSTRACT

Damage control resuscitation principles advocate utilization of whole blood (WB) based resuscitation to treat shock and coagulopathy in an effort to decrease death from hemorrhage. However, white cells present in whole blood can cause adverse transfusion related events. This Terumo BCT ImuflexWB-SP FDA approved whole blood system was used containing a platelet sparing, leukoreducing filter and reconfigured capability to permit safer and easier WB transfusions for the user environment. To decrease filtration time 500 ml of WB from healthy blood donors was subjected to leukocyte filtration by gravitational, 150 and 300 mmHg forces and then tested. Additional testing then compared filtration at 1 or 4 hours after donation as well as changing the height of filtration from the standard of 85 ± 2 cm (33 inches) to 71 cm (28 inches) to evaluate the effects of time and height on the reduction of platelet aggregation over time. Gravity filtration took 35 ± 3 minutes and produced a consistently leukoreduced (LR) product ($<0.1\times10^6$/unit). Filtration at 150 mmHg significantly reduced filtration time to 14 ± 1 minutes ($p<0.001$), but resulted in 1 of 10 units with a residual WBC $>5.0\times10^6$/unit. Increasing pressure to 300 mmHg further reduced the time to 9 ± 0 minutes ($p<0.001$), but caused 4 of 10 units to exceed 5.0×10^6/unit. Platelet recovery decreased from 90 ± 9% using gravity to 84 ± 9% at 150 mmHg and 83 ± 15% at 300 mmHg. Gravity filtration 4 hours after donation at 33 inches height resulted in a slight decrease in the loss of platelet aggregability 24 hours after filtration (when stored at 4 degrees C), without any other changes over the subsequent three weeks in platelet performance compared to non-leukoreduced whole blood and whole blood leukoreduced after 1 hour at 33 inches or 4 hours at 28 inches. No change in standard quality parameters was seen at any pressure, height, or time of filtration compared to non-leukoreduced whole blood. Using viscoelastic analyses mild changes were seen in clotting time (slight increase), speed of clot formation and strength (decrease), however, all remained within normal ranges over time after filtration. No fibrinolysis was observed. Multiplate aggregometry showed an agonist-dependent 30-80% postfiltration decrease for all filtration pressures, times to filtration, and heights of filtration. Filtration also did not change the physiologic composition of the whole blood. LR time in remote arenas is of concern. Forced filtration at 150mmHg significantly reduced filtration time without significantly affecting quality. Increasing pressure caused an increase in residual leukocytes in the final product. Increased time from donation to filtration may mitigate the rapidity of reduction in platelet aggregability after filtration but does not affect overall coagulation or ongoing physiologic changes of leukoreduced whole blood.

15. SUBJECT TERMS
Damage control resuscitation, DCR, remote damage control resuscitation (RDCR), whole blood, leukoreduction, Imuflex

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			James Lehman
U	U	U	SAR	45	19b. TELEPHONE NUMBER *(include area code)*

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1.0 SUMMARY

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The Terumo BCT ImuflexWB-SP FDA approved whole blood system was used containing a platelet sparing, leukoreducing filter and reconfigured capability to permit safer and easier WB transfusions for the user environment. To decrease filtration time 500 ml of WB from healthy blood donors was subjected to leukocyte filtration by gravitational, 150 and 300 mmHg forces and then tested. Additional testing then compared filtration at 1 or 4 hours after donation as well as changing the height of filtration from the standard of 85 ± 2 cm (33 inches) to 71 cm (28 inches) to evaluate the effects of time and height on the reduction of platelet aggregation over time.

Gravity filtration took 35 ± 3 minutes and produced a consistently leukoreduced (LR) product ($<0.1\times10^6$/unit). Filtration at 150 mmHg significantly reduced filtration time to 14 ± 1 minutes ($p<0.001$), but resulted in 1 of 10 units with a residual WBC $>5.0\times10^6$/unit. Increasing pressure to 300 mmHg further reduced the time to 9 ± 0 minutes ($p<0.001$), but caused 4 of 10 units to exceed 5.0×10^6/unit. Platelet recovery decreased from 90 ± 9% using gravity to 84 ± 9% at 150 mmHg and 83 ± 15% at 300 mmHg. Gravity filtration 4 hours after donation at 33 inches height resulted in a slight decrease in the loss of platelet aggregability 24 hours after filtration (when stored at 4 degrees C), without any other changes over the subsequent three weeks in platelet performance compared to non-leukoreduced whole blood and whole blood leukoreduced after 1 hour at 33 inches or 4 hours at 28 inches. No change in standard quality parameters was seen at any pressure, height, or time of filtration compared to non-leukoreduced whole blood. Using viscoelastic analyses mild changes were seen in clotting time (slight increase), speed of clot formation and strength (decrease), however, all remained within normal ranges over time after filtration. No fibrinolysis was observed. Multiplate aggregometry showed an agonist-dependent 30-80% post filtration decrease for all filtration pressures, times to filtration, and heights of filtration. Filtration also did not change the physiologic composition of the whole blood. LR time in remote arenas is of concern. Forced filtration at 150mmHg significantly reduced filtration time without significantly affecting quality. Increasing pressure caused an increase in residual leukocytes in the final product. Increased time from donation to filtration may mitigate the rapidity of reduction in platelet aggregability after filtration but does not affect overall coagulation or ongoing physiologic changes of leukoreduced whole blood.

2.0 BACKGROUND

Among the group of potentially salvageable combat fatalities, hemorrhage is the leading cause of death. With ongoing blood loss, casualties lose not only red blood cells, but also the blood components required to properly form clot. Recognition of hemorrhage induced traumatic coagulopathy led to the development of hemostatic resuscitation: a resuscitative strategy targeting rapid restoration of normal clotting function with the goal of decreasing acute blood loss. Hemostatic resuscitation is a component of damage control resuscitation (DCR), the emergency stabilizing principles used to maximize survival by initially doing only the most essential interventions while continuing to resuscitate and stabilize a traumatically injured patient. DCR principles advocate for the use of blood components with red blood cells (RBCs),

plasma, and platelet units in addition to whole blood to manage shock and coagulopathy associated with traumatic hemorrhage.

Conventional transfusion practice separates donated blood into its constituent components: red blood cells, platelets, and plasma. In blood banks, donated blood is processed, separated, and preserved for future use. This allows specific transfusion of only the component(s) needed, extends the shelf-life for some of those products, and can increase the utilization of a single unit of donated blood to more than one patient. In recent decades, WB has not been traditionally transfused in adult civilian practice, but this resource had been used in combat conditions due to limited supply of stored blood products. The application of DCR in the prehospital setting is termed "remote damage control resuscitation" (RDCR) and has been a key focus of the Israeli Defense Force, the Committee on Tactical Combat Casualty Care, the Trauma Hemostasis and Oxygenation Research (THOR) network and others.

Fresh WB offers theoretical clinical advantages over preserved products: it replaces exactly what the hemorrhaging patient has lost in normal proportions, providing not only red blood cells but also the factors required to ensure adequate clot formation. Furthermore, those products have not been preserved or chilled, improving the physiologic utility of the blood cells. Over 10,000 units of fresh WB were transfused in Afghanistan Operation Enduring Freedom (OEF) and Iraq Operation Iraqi Freedom (OIF) since 2001. Its use in combat casualties has been independently associated with improved survival compared to standard blood components.

In a 10-year review of DCR and outcomes in combat casualties, component rations that came close to mimicking WB had improved outcomes: increased platelet to RBC unit ratios have been determined to be even more highly associated with increased survival than plasma to RBC unit ratios. Utilization of WB, however, dramatically reduced after the 1980's in part because of the inability to preserve platelets while filtering out the white blood cells. Use of WB for transfusion was also discouraged by the blood banking industry in part due to misunderstanding of the pathophysiology of patients with hemorrhagic shock secondary to traumatic injury. Based on initial work by researchers at the combat support hospital in Baghdad and then validated in numerous civilian studies, the trauma, critical care, and transfusion communities all now recognize that a patient in hemorrhagic shock requires a balanced resuscitation with RBCs, plasma, and platelets; similar to what comes from transfusing WB. This is evident in guidelines or position statements from each of these academic communities.

DoD research in the area of hemostatic resuscitation has changed hemorrhage resuscitation worldwide (both military and civilian) and is the most important medical lesson to originate out of the years of conflict in OEF and OIF. The Tactical Combat Casualty Care guidelines and the THOR Network thus recommend the use of whole blood as the preferred prehospital fluid for traumatic hemorrhagic shock. A combination of component blood products is a suitable alternative for many of these patients but not all of the blood products (especially plasma and platelets) are available at remote or austere locations or perhaps at any facility during a multiple casualty event. Making blood components available for RDCR presents logistical challenges, particularly with regard to platelets, as these have a limited shelf-life of 5-7 days and require room temperature storage with constant agitation (current U.S. Food and Drug Administration (FDA) requirement). In these situations WB may be the only option to treat these casualties to prevent death from hemorrhage.

One concern regarding WB transfusion is that white blood cells are also present in the donated blood. Although the risk is low, transfusion of these cells may cause viral transmission and induce inflammatory and immunologically mediated responses, which can cause adverse

transfusion related events, increasing morbidity and mortality. To reduce the risk of transfusion related immune modulation (TRIM), standard blood banking procedures now recommend leukoreduction (reduction of WBCs) to less than $5X10^6$ WBCs per unit of RBCs (FDA) or 1.0×10^6/unit (Council of Europe).

Unfortunately, most commercially available LR filters for WB also remove platelets. It is crucial to retain these platelets in WB since they are essential for hemostasis and have been demonstrated to be associated with improved survival and decreased death from bleeding in combat and civilian casualties with traumatic injury.

Despite the fact that LR of all blood products is a major goal of the Armed Forces Blood Program (AFBP), there was no suitable deployable filter available and configured for field use that removed potentially harmful white blood cells from WB while retaining clot forming platelets. As a consequence, when WB was transfused to combat casualties in the field, it was not leukoreduced, thereby increasing the risk for TRIM and associated morbidity and mortality. There has been an association of increased incidence of acute lung injury in combat casualties transfused with non-leukoreduced WB. While only one case of fatal graft versus host disease has been documented in a combat patient during OIF and OEF, it was determined that this disease may have been related to either non leukoreduced < 10 day old RBCs or the two units of fresh WB transfused to that patient.

The development and production of a field deployable WB collection and transfusion kit with an FDA approved, platelet sparing, LR filter has the potential to reduce morbidity and mortality for civilian and military casualties with severe hemorrhagic shock. This blood collection and transfusion kit will allow for both collection and storage of WB to be transfused at a later time or the collection and immediate transfusion of WB in the field environment.

For multiple reasons (to include hemostatic ability, minimizing dilution and acidosis, and logistical) the Committee on Tactical Combat Casualty Care made a recommendation in 2014 that whole blood (WB) should be the preferred resuscitative product for patients with traumatic hemorrhagic shock on the battlefield over all other fluids or blood product combinations. A 29 May 1996 Memorandum by the FDA recommended that blood products be leukoreduced; a September 2012 Memorandum by the FDA further clarifies this recommendation. In 2010 the Armed Services Blood Program (ASBP) began a coordinated effort to make all blood products leukoreduced. To implement these recommendations, all aspects of blood collection and storage would need to be evaluated, improved and/or augmented.

This project was to develop a FDA approved, ruggedized, field deployable, longer shelf-life, cost effective, and mass producible WB collection and transfusion system that would allow for the collection of leukoreduced (LR), platelet-sparing WB transfusion in austere and far forward settings.

The Terumo BCT IMUFLEX WB-SP (Terumo BCT; Lakewood, CO, USA) filter system is an FDA approved and CE marked (for use within the European Union) blood bag system used for the fractionation of blood products into components utilizing a novel LR but platelet-sparing filter. It has also been available since 2000 in many countries including Germany, Greece, Italy, Norway, Spain and Sweden, but was not approved by the FDA until 2006 (NDA BN880217/54). WB processed with the Terumo BCT IMUFLEX set is FDA approved for 21 days storage at 1-6 degrees Celsius.

Using this system, a unit of WB [500-mL in CPD (U.S.) or 450-mL (Europe)] can be leukoreduced by gravity in approximately 40 minutes. While acceptable in a blood bank for storage, such a delay may not be acceptable in a far forward combat scenario or other settings

where there is an urgent need for blood. (Preliminary work by the Norwegian Navy Special Operations research group had demonstrated preserved platelet counts with faster than gravity filtration.) The current system configuration is designed and used for collecting and separating WB into components (Figure 1).

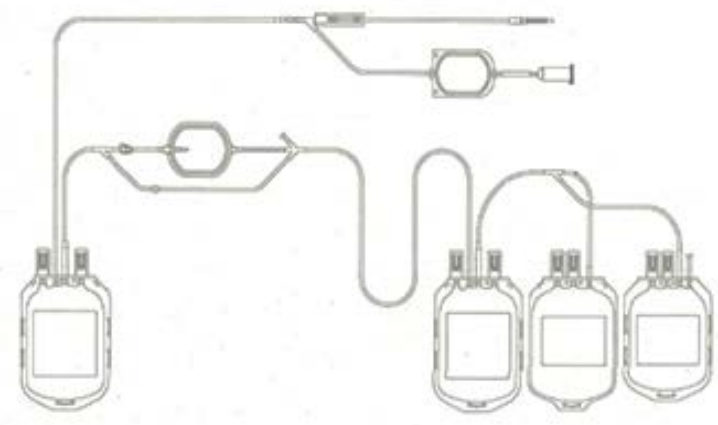

Figure 1. Terumo BCT IMUFLEX WB-SP.

Its configuration with multiple component collection bags was not deemed practical for field use by US Army Ranger Regiment medics. As it is designed for blood bank use, it is packaged from the manufacturer in 10 sets/sterile packaging; this was not practical for deployed use where it was desirable to process in lots of one to two-unit collections at a time. Packaging was not felt to be rugged enough to withstand the rigors of military deployment, handling and transport. Shelf-life of the product was approximately 30 months which was deemed too short and would create additional DoD logistic and cost challenges. Storage of the system was tested and reported to withstand only a narrow range of temperature (59-86 degrees Fahrenheit); these temperature ranges were clearly not practical for the deployed setting.

This project collaborated with Terumo BCT to redesign and reconfigure its capability to permit easier and safer WB collections in far forward environments. (Figure 2).

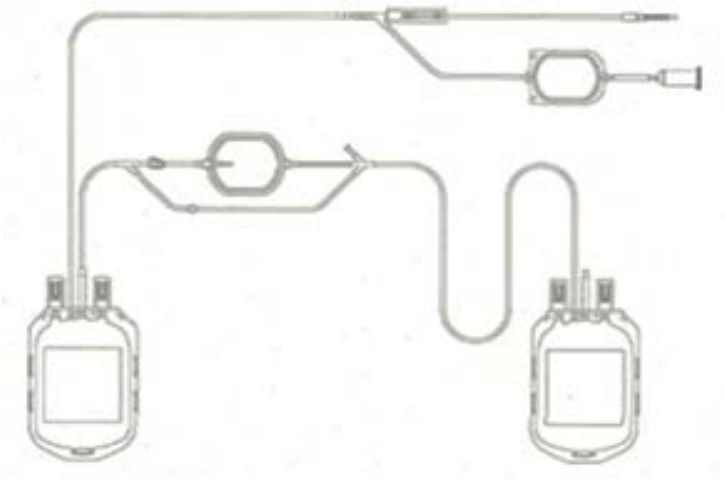

Figure 2. Reconfigured Terumo BCT IMUFLEX WB-SP.

In addition to the reconfiguration of the system that would be more applicable for the austere environment, it was also deemed important to determine and test the methods of deployment of the new product so that deployed units would have guidance and scientific evidence to guide its use. Some of the questions posed to the researchers included: how fast could blood be pushed through the system while still maintaining adequate platelet counts, adequate platelet function and adequate leukoreduction; what would be the effects if blood was stored before filtration; was agitation of the stored blood after filtration necessary to preserve platelet function and counts; what were the effects of storage of filtered blood at 4 °C versus 22 °C versus 32 °C; did the process of filtration affect the hemostatic properties or the platelet function of the filtered product.

The study was funded by Office of Secretary of Defense, Comparative Technology Office, Foreign Comparative Testing (FCT) and the United States Air Force. The FCT Program leverages items and technologies from foreign allies that have a high Technology Readiness Level (TRL) in order to satisfy valid defense requirements more quickly and economically.

The funds for the study were obligated by the Air Force Research Laboratory Contracting Office, United States Air Force School of Aerospace Medicine, Aeromedical Research Department, En Route Care Division utilizing a Basic Co-Operative Agreement “Expeditionary Medicine, Trauma, and En Route Care (EMTEC) Research and Technology Support" FA8650-10-2-6140, agreement Order FA8650-14-2-6B36 to the University of Cincinnati. The University of Cincinnati sub-contracted with: Haukeland University Hospital, Bergen, Norway; Terumo BCT, Tokyo, Japan; and Blood Solutions, LLC.

3.0 METHODS

This study included three major phases: I) Design and development for the modified WB transfusion set (with some specific military requirements) accomplished by Terumo BCT; II) Supplemental testing of the WB LR filter conducted by both Haukeland University Hospital and University of Cincinnati; and III) Submission to FDA for a Prior Approval Supplement (PAS). Many of the sub activities for Phase I and Phase II were worked on concurrently. Phase I data was compiled and analyzed in order to support FDA submission.

3.1 Design and Development for the Modified WB Transfusion Set

Terumo BCT began the process with a planning phase. This phase consisted of planning the design and development processes to include prototype design, mold creation, design of packaging materials, design of shipping containers, design of the label, in-vitro, accelerated and real-time age testing plan, and finally prototype design. During this phase, Terumo BCT also completed a market analysis in order to determine if the design and packaging was viable in the open market. This was an important action for Terumo BCT to support the project and create a manufacturing line for the modified Terumo BCT IMUFLEX WB-SP system. During the planning phase, there were many discussions between the research team and industry partner regarding Requirements Development and specifically the functional and performance requirements. While the specific functional and performance requirements were developed with input from the US special operation command tri-service medical community and highlighted in the original proposal to the Comparative Technology Office, Air Force Foreign Comparative Testing Program, they were not created through the official Army Requirements Development

Process. The functional and performance criteria were developed as collaboration between subject matter experts, stake holders and industry partners. The recommendations from the Committee on Tactical Combat Casualty Care, the AFMS Integrated Capabilities List (ICL) and specific MAJCOM Capability Based Assessment (CBA) reports were used as the demonstrated need for the product and supported the research project. The ICL and CBA are critical for AFMS to guide researchers to answer materiel solutions, research to materiel solutions, and research to knowledge. These documents may be considered as a partial Initial Capability Document by some reviewers as it does not meet the full Joint Capabilities Integration and Development System (JCIDS) process for research and acquisitions. After multiple efforts to secure administrative support for completing the JCIDS process from the military services failed, it was abandoned. Despite letters of endorsement (LOE) for the project from various tri-service stake holders, these LOE are not considered a financial agreement for purchase once the project is completed. The lack of a defined purchase quantity and commitment for acquiring the final WB filtration product forced Terumo BCT to temporarily stop the research planning and conduct a wider market analysis. As this filter system represents a technology and a capability not previously available, neither tested nor applied to the field environment, there were many questions which arose in regards to its applicability and function. Until there is further acceptance by the medical community of this new technology and capability (based in this projects data and future studies), immediate purchase and deployment by the DoD for fielding is not immediately imminent and may require some civilian use first to confirm the study's results in vivo. This inability to define when the research ends and the transition to an acquisition process appears problematic but is entirely within the norms of how new technology is implemented. While the transition and acquisitions plan included generalized actions to move from research into development/creation of an NSN and the ability for end users to purchase as a COTS item, the plan was not specific enough to the manufacturer to identify when the research teams hands off to the acquisitions process. The steps for these actions are defined in the JCIDS process, however not having a true ICD, and/or a user requirements document and not having immediate acceptance by the end users caused additional conversations with stakeholders to assist for the planning to gain an NSN.

The development phase included actions to execute the development plan to develop the product. During this phase Terumo BCT completed the product design, packaging design, manufacturing process design, stability testing, accelerated age testing, process validation and design verification. This action also completed the product specification to ensure the modified version met the needs of the research team. Accelerated age testing was an important step used to support the justification to release the product with an18-month shelf-life. Real-time age testing was initiated to support the eventual shelf-life of 36 months. If successful, the updated storage shelf-life would decrease procurement cost and allow for longer storage in far forward locations. The standard shelf-life for products approved by the FDA is 18 to 24 months. Currently no blood bags are approved for use over 24 months in the US. For this reason, it was determined that the FDA would only accept real-time age data to support a 36-month shelf-life claim, not accelerated age data. As part of this project the real-time aging test for 36-month was initiated but will not be completed before completion of this review. Terumo BCT currently manufactures and markets blood bags in other parts of the world with a shelf-life of 36 months.

The sample manufacture phase consisted of completing the process to manufacture the modified set. During this phase Terumo BCT manufactured samples for verification testing, testing of the manufacturing line, testing for capability to mass produce the set, samples for

stability testing, samples for accelerated and real-time age testing, and samples for additional clinical testing to be performed at the University of Cincinnati. Prior to performing the sample manufacturing phase, the research team received five prototype kits to ensure the modified version met usability and specification requirements. The research team approved the design allowing Terumo BCT to move forward with the manufacturing of the sets for in-house testing and clinical testing by the University of Cincinnati. Once approved Terumo BCT delivered 200 sets to the University of Cincinnati for their clinical testing.

The original design plan called for 2 sets of blood bags to be packaged in one aluminum pouch. However, it was found that this configuration resulted in the failure of some of the package with pinhole leaks occasionally found. Therefore, the design was modified, with the approval of the research team, to change to one blood bag set per aluminum pouch. This configuration successfully passed all packaging and ship test requirements. End users at the US Army Ranger Regiment and Special Operations Command provided input into this final packaging.

3.2 Supplemental Testing

In order to concurrently test the LR filter while Terumo BCT was completing the above actions. Terumo BCT shipped approved, standard non-modified 4 bag system to Haukeland University. There were four aims of this testing. Aim 1: To demonstrate that whole blood, stored at 4 degrees Celsius, leukoreduced by a platelet-sparing filter does not affect platelet function and hemostatic parameters compared to non-filtered whole blood over time. Aim 2: To demonstrate that whole blood, stored at 4 degrees Celsius, leukoreduced by a platelet-sparing filter is associated with improved platelet function and hemostatic parameters compared to non-filtered whole blood stored at 22 degrees Celsius over time. Aim 3: To demonstrate that a rapid filtration rate for whole blood, leukoreduced by a platelet sparing filter does not affect platelet function and other hemostatic parameters compared to filtration by gravity. Aim 4: To demonstrate that agitation of whole blood stored at 4C, leukocyte reduced with a platelet sparing filter, compared to non-agitation is associated with improved platelet function and hemostatic parameters compared to non-agitated whole blood over time.

Prior to testing, regulatory approvals were gained from The Regional Committee for Medical and Health Research Ethics, Section B, South East Norway, and the Air Force Research Lab (AFRL), Human Resource Protection Office (HRPO).

The Research Ethics Committee System in Norway consists of seven Independent Regional Committees for Medical and Health Research Ethics, with authority to either approve or disapprove Medical Research Studies conducted within Norway, or by Norwegian Institutions, in accordance with the Act on Medical and Health Research (2008). Only projects which fall within of the scope of the Health Research Act require approval from a Norwegian Regional Committee for Medical and Health Research Ethics (REC) according to Norwegian law. The scope of the Health Research Act is limited to “medical and health research on human beings, human biological material or personal health data”, where “medical and health research” is defined as “activity conducted using scientific methods to generate new knowledge about health and disease.”

The Norwegian System is based on a regional structure, where new project applications are distributed to a REC based on the geographical region of the Project Manager’s main

workplace. Since Haukeland University is located in the western region, its projects are reviewed by the Regional Committee for Medical and Health Research Ethics West.

However, in order to avoid uneven workloads between the Regions, the system is designed to avoid uneven distribution between regions. In other words, new applications will be distributed to other REC offices if a REC receives a disproportionate number of new applications. It is therefore possible that the same Research Institution could have projects assessed by different Regional Committees for Medical and Health Research Ethics. In the case of this study and as a result of this system of redistribution this research project was reviewed by the Regional Committee for Medical and Health Research Ethics, Section B, South East Norway, and not by the Regional Committee for Medical and Health Research Ethics West.

The Regional Committee for Medical and Health Research Ethics, Regional Committee for Medical and Health Research Ethics, Section B, South East Norway, Section B, first reviewed the Research Project at its Committee Review Meeting on the 28th of October 2015. The Committee assessed the project in light of Norway's Health Research Act and Norway's Act on Ethics and Integrity in Research.

The Committee concluded that the purpose of the project was not to generate new knowledge about health and disease, but to generate new knowledge about a medical device, although the project uses human blood to test this device.
The committee concluded that the Project can be conducted without approval as the project falls outside the Scope of the Health Research Act.

The approval and the protocol were submitted to the Air Force Research Lab, Human Research Protection Office for review. Upon review it was determined that the conclusion by The Regional Committee for Medical and Health Research Ethics, Regional Committee for Medical and Health Research Ethics, Section B, that the study could be conducted without approval was not consistent with U.S. Common Rule 32 CFR 219 standards. Air Force Research Lab, Human Research Protection Office requested a re-review of the protocol and supporting documents by Regional Committee for Medical and Health Research Ethics, Section B utilizing the U.S. Common Rule 32 CFR 219 standards.

The Regional Committee for Medical and Health Research Ethics, Regional Committee for Medical and Health Research Ethics, Section B found that the project satisfied the Common Rule's criteria for expedited review, as the project involved no more than minimal risk.

The Air Force Research Lab, Human Research Protection Official conducted an administrative review based on The Regional Committee for Medical and Health Research Ethics, Regional Committee for Medical and Health Research Ethics, Section B expedited review and approval. The Air Force Research Laboratory, Human Research Protections Office eventually approved the study.

When working with international partners for studies that may or may not involve human subjects, it is critical to ensure that all contracting/sub-contracting documents describe the regulatory requirements and approval process and the standards which apply. In this study the contract and sub contract stated that the regulatory review must include application of U.S standards.

Haukeland University is approved by the Office for Human Research Protections (OHRP) and listed in the Federal Wide Assurance (FWA). The FWA is the only type of assurance currently accepted and approved by OHRP. Through the FWA, an institution commits to HHS that it will comply with the requirements in the HHS Protection of Human Subjects

regulations at 45 CFR part 46. Having this affiliation supported the U.S standard application for research being conducted.

Human research activities involving international partners should ensure the institution has an assurance (FWA) or seeks to obtain one prior to research discussions. This action may alleviate delays when seeking the next level of DoD regulatory approval. In addition, an understanding of the regulatory requirements, approvals processes and definitions of human research by international partners will assist in the communication process between international partners, collaborators and U.S regulatory approving offices.

3.3 Testing Experiments

3.3.1 Collection. Five hundred milliliters of WB was collected from healthy volunteer donors with no known use of platelet-inhibiting medication or supplements using Imuflex WB-SP collection sets containing 70 mL of citrate-phosphate-dextrose (CPD). All ABO types and both genders were included. The collection set includes secondary bags for component production that were removed before filtration. In total, 103 units were collected. Three units were excluded due to low collection volume, two units were excluded due to an incubator malfunction resulting in incorrect storage temperature, and one unit was excluded due to an unexpectedly high reduction in platelet count during storage, leaving a total of 97 included WB units for study.

3.3.2 Sampling. Laboratory test sampling of the WB was performed by thoroughly mixing the bag by hand and then transferring 30 mL into 150-mL Teruflex transfer bags (Terumo BCT; Lakewood, CO, USA). An additional 8 mL was removed after filtration on day 0 for bacterial testing of units to be stored at room temperature.

3.3.3 Forced Filtration Testing. Immediately following donation, bags were sampled and filtered with the LR platelet sparing set extended to 85 ± 2 cm (length of tubing included in the set and deemed appropriate for gravity filtration) either according to the manufacturer's recommended procedure of gravity-assisted filtration (n=10) or at 150 mmHg (n=10) or 300 mmHg (n=11) using a 1000-mL irrigation pump (1PP110000, Unimax Medical Systems, Inc.; Taipei, Taiwan). Pressure was applied once the filter was saturated with blood, temporarily stopped for removal of air from the primary bag, and then reapplied to empty the filter. Sampling was then performed, and the remaining product was discarded.

3.3.4 Delayed Filtration. Five units were sampled immediately after donation and then stored for 7 days at 4 °C either unagitated or with daily mixing by hand. After a 2-hour hold at 22 °C on day 7, the bags were sampled and then gravity filtered with the set tubing extended to 85 ± 2 cm. The filtration process was aborted after 2 hours of filtration time. Postfiltration sampling of the filtered volume was then performed and the bag was discarded.

3.3.5 Storage. After a 1-hour hold, bags were sampled and gravity filtered with the set tubing extended to 85 ± 2 cm according to the manufacturer's instructions. A postfiltration sample was collected and the bags were transferred to storage. Cold storage was examined by storing bags at 4 °C for 21 days either without agitation (n=11), with daily hand mixing (n=10), side-to-side agitated 3.8 cm at 60 Hz with daily hand mixing (n=10, PFS42, Helmer Scientific; Noblesville, IN, USA), or head-over-heel mixed at 3 rounds per minute with no daily mixing (n=10, SI-1002

Bag Rotator, Scientific Industries, Inc.; Bohemia, NY, USA). Sampling was performed on storage days 10, 14, and 21. Unagitated bags were additionally sampled on day 3 (n=8) for comparison with storage at 22 °C. An incubator was used to store bags unagitated at 22 °C for 3 days (n=10) with sampling on days 1 and 3 or at 32 °C for 2 hours after filtration (n=10) with samples taken after 1 and 2 hours of storage.

3.3.6 Bacterial Testing. Units stored at 22 °C were tested using the BacT/ALERT 3D system with BacT/ALERT FA Plus culture bottles (bioMérieux SA; Marcy l'Etoile, France).

3.3.7 Hemostatic Function and Aggregation. Hemostatic function was evaluated with kaolin-initiated thromboelastography (TEG) on a TEG 5000 analyzer (Haemonetics Corporation; Braintree, MA, USA). Additionally, rotational thromboelastometry (ROTEM) was performed using ROTEM delta (Tem International GmbH; Munich, Germany) with intrinsic (in-tem) and extrinsic (ex-tem) activation. Fibrinogen contribution was assessed by platelet-inhibited extrinsic activation (fib-tem). Platelet aggregation ability was measured using the Multiplate WB impedance aggregometer (Roche Diagnostics GmbH; Mannheim, Germany). Platelets were activated with 6.5 µM adenosine diphosphate (ADPtest), 0.5 mM arachidonic acid, 0.77 mg/mL ristocetin, and 32 µM thrombin receptor activating peptide 6 (TRAPtest). Due to the calcium-depleting effects of CPD, samples for ADPtest and TRAPtest were partially recalcified with 3 mM calcium chloride (CaCl2).

3.3.8 Flow Cytometry. The level of activated platelets and their adhesion capacity were investigated by flow cytometry. A premade antibody mix with monoclonal mouse anti-human antibodies from BD (BD Bioscience; San Jose, CA, USA) with PerCP CD61 (clone EUU-PL 7F12, cat. no. 347408), APC CD42b (cat. no. 551061), and PE CD62P (cat. no. 561921) was added to 50 µL of citrated whole blood (CWB) to give a volume of 2.5 µL, 1.25 µL, and 2.5 µL, respectively, per test. After vortex and 30 minutes of incubation at room temperature (dark), 465 µL of lysis buffer (Dako EasyLyse™, ref. no. S2364, Agilent; Santa Clara, CA, USA) was added. After 7 minutes of incubation at room temperature (dark), 2 mL of flow cytometry sheath fluid (FACSFlow, cat. no. 342003, BD Biosciences; San Jose, CA, USA) was added. The samples were run either immediately on a BD FACSCanto II cytometer using the FACSDiva software (v8.0.1) (BD Biosciences; San Jose, CA, USA) or stored at room temperature (dark) if analyzed within 2 hours after preparation. Gating was done with a fixed setup on FSC-H, FSC-A, and SSC, using CD61-positive cells as indicator of platelets and quantifying CD42b or CD62P-positive cells as a percentage of the CD61-positive population. The same gating was used for all samples.

3.3.9 Hematology and Clinical Chemistry. Platelet count (PLT), white blood cell count (WBC), hemoglobin (Hgb), and hematocrit (HCT) were analyzed in K2EDTA on a Cell-Dyn Sapphire analyzer (Abbott Diagnostics; Abbott Park, IL, USA). Residual WBC after filtration was analyzed on a BD FACSCanto II cytometer using the BD Leucocount Kit. Potassium levels were analyzed on the Cobas 8000 ISE module (Roche Diagnostics GmbH; Mannheim, Germany). Plasma was prepared by centrifugation at 1800 g for 10 minutes. Hgb in plasma (p-Hgb) was analyzed on a HemoCue Plasma/Low Hb photometer (HemoCue AB; Ängelholm, Sweden) and used to calculate percent hemolysis as follows: ((p-Hgb/10)×(100-%Hct))/Hgb. Prothrombin time/international normalized ratio (INR), activated partial thromboplastin time

(APTT), factor VIII, and fibrinogen were analyzed using the STA-R Evolution/STA-R Max platform with STA-SPA+, STA-PTT Automate 5, STA-Deficient VIII, 0.025M CaCl2, STA-Unicalibrator, and STA-Liquid Fib (Stago S.A.S; Asnières-sur-Seine, Paris, France).

3.3.10 Statistical Analysis. Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 24.0 (IBM Corp.; Armonk, NY, USA). Results were reported as mean ± standard deviation. Comparisons between pre- and postfiltration values were performed by use of independent samples t-test. The effects of forced filtration were compared by calculating the delta value from prefiltration to postfiltration and comparing the 150 and 300 mmHg groups to the gravity group. Platelet recovery was calculated as the difference in platelet count per unit before and after filtration. Changes between two sample points were compared using a paired-sample t-test. The changes in means during storage and between the different study groups were compared using repeated measures analysis of variance (ANOVA); p-values <0.05 were considered significant.

4.0 RESULTS

4.1 Blood Types and Gender

Measurements prior to filtration showed lower levels of factor VIII in group O donors (111 ± 26, n=35) compared to group A (134 ± 19, n=48) ($p<0.001$). Female donors (n=27) showed higher aggregation responses than male donors (n=65) to adenosine diphosphate (ADP) (52 ± 10 vs. 43 ± 11, $p<0.001$), arachidonic acid (AA) (43 ± 9 vs. 32 ± 11, $p<0.001$), and ristocetin (73 ± 18 vs. 61 ± 20, $p=0.009$). The difference for TRAP-6 was not statistically significant (93 ± 15 vs. 87 ± 17, $p=0.131$).

4.2 Forced Filtration

Gravity filtration took 35 ± 3 minutes and produced a consistent leukoreduced product ($<0.1\times10^6$/unit). Filtration at 150 mmHg significantly reduced filtration time to 14 ± 1 minutes ($p<0.001$), but resulted in 1 of 10 units with a residual WBC $>5.0\times10^6$/unit. Increasing pressure to 300 mmHg further reduced the time to 9 ± 1 minutes ($p<0.001$), but caused 4 of 10 units to exceed 5.0×10^6/unit. The highest residual WBC observed was 19.5×10^6/unit at 150 mmHg and 25.6×10^6/unit at 300 mmHg (Figure 3).

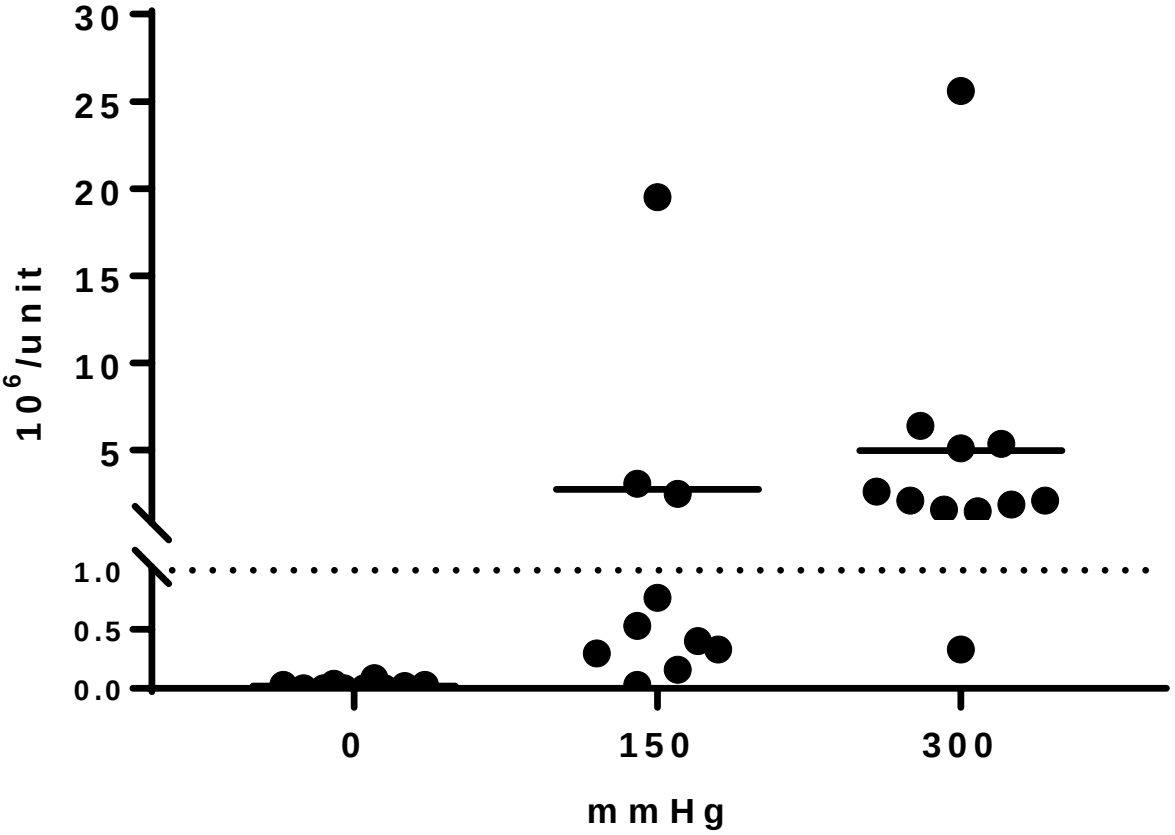

Figure 3. Residual white blood cell count of individual whole blood units after leukoreduction with the Imuflex WB-SP filter. An irrigation pump was used to apply 0 mmHg (n=10, mean=0.02), 150 mmHg (n=10, mean=2.76, p=0.182), or 300 mmHg (n=11, mean=4.97, p=0.043) pressure. Solid lines indicate means. Dotted line represents upper limit of 1.0×10^6/unit recommended by guidelines. The means for 150 and 300 mmHg were compared to 0 mmHg (gravity filtration) using the independent samples t-test with α=0.05.

Filtration yielded a platelet recovery of 90 ± 9% at gravity, 84 ± 9% at 150 mmHg, and 83 ± 15% at 300 mmHg. One bag filtered at 300 mmHg had a platelet recovery of 39%. There was no statistically significant difference in the change in PLT recovery from pre filtration to post filtration between gravity and 150 mmHg and gravity and 300 mmHg. Less decrease of Hgb was seen at 300 mmHg compared to gravity filtration (0.03 g/dL ± 0.11 vs. 0.14g/dL ± 0.12 g/dL, p=0.035). No change in hemolysis, HCT, mean corpuscular volume (MCV), potassium, INR, APTT, fibrinogen, or factor VIII was seen at any pressure (Table 1).

Table 1. Thromboelastography and Thromboelastometry Values in Whole Blood Before and After Leukoreduction with the Imuflex WB-SP Filter

	0 mmHg		150 mmHg		300 mmHg	
	Prefiltration	**Postfiltration**	**Prefiltration**	**Postfiltration**	**Prefiltration**	**Postfiltration**
TEG						
R (min)	5.8 ± 1.0	7.8 ± 1.6*	6.6 ± 1.1	8.5 ± 1.1**	5.5 ± 0.7	7.6 ± 1.1**
K (min)	1.5 ± 0.3	1.7 ± 0.3*	1.4 ± 0.2	1.9 ± 0.3**†	1.3 ± 0.2	1.9 ± 0.4**†
Angle (°)	68.5 ± 3.8	64.5 ± 4.1*	68.9 ± 3.2	63.2 ± 3.8**	70.1 ± 2.8	63.6 ± 3.4**
MA (mm)	63.6 ± 5.6	58.4 ± 6.2	63.7 ± 5.9	57 ± 4.9**	65.3 ± 4.1	58.2 ± 3.7**
LY30 (%)	1.3 ± 1.8	1.5 ± 2.1	2.4 ± 2.3	1.9 ± 2.7	1.1 ± 1.5	2.7 ± 3.1
in-tem						
CT (min)	167 ± 14	173 ± 17	176 ± 11	186 ± 11*	168 ± 9	174 ± 12
CFT (min)	83 ± 21	86 ± 20	77 ± 12	87 ± 18*†	79 ± 14	91 ± 29
Angle (°)	74 ± 3	74 ± 4	75 ± 2	73 ± 3*	74 ± 3	73 ± 3
MCF (mm)	58 ± 5	57 ± 5*	60 ± 3	58 ± 4*	60 ± 4	58 ± 4
LI30 (%)	100 ± 1	99 ± 1	100 ± 0	100 ± 1	100 ± 0	100 ± 0
ex-tem						
CT (min)	65 ± 8	61 ± 6*	61 ± 7	62 ± 8†	61 ± 6	61 ± 6
CFT (min)	94 ± 24	91 ± 25	84 ± 15	92 ± 22*†	87 ± 18	98 ± 30
Angle (°)	72 ± 5	73 ± 5	73 ± 3	72 ± 4*†	72 ± 3	72 ± 3
MCF (mm)	59 ± 6	59 ± 6	62 ± 3	60 ± 4*	61 ± 4	59 ± 4
LI30 (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
fib-tem						
CT (min)	63 ± 14	61 ± 5	60 ± 7	61 ± 9	65 ± 10	58 ± 6*
Angle (°)	67 ± 9	66 ± 9	68 ± 7	68 ± 7	65 ± 5	68 ± 7*††
MCF (mm)	13 ± 4	13 ± 4	13 ± 3	13 ± 3	12 ± 3	12 ± 3
LI30 (%)	100 ± 1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 1
Multiplate						
ADP (U)	42 ± 13	29 ± 9*	50 ± 14	29 ± 5*	43 ± 13	27 ± 11*
AA (U)	33 ± 14	21 ± 10*	35 ± 15	19 ± 7*	38 ± 10	23 ± 10*
Ristocetin (U)	57 ± 28	46 ± 20*	67 ± 18	51 ± 17*	68 ± 20	57 ± 19
TRAP-6 (U)	94 ± 20	35 ± 10**	95 ± 19	35 ± 5**	87 ± 19	35 ± 12**
Cytometry						
CD62P (%)	27.7 ± 9.6	21.9 ± 7.5*	19.5 ± 5.3	16.5 ± 6.5*	20.1 ± 10.6	17.3 ± 11.9
CD42b (%)	98.6 ± 1.3	99.2 ± 0.4	99.1 ± 0.6	99.4 ± 0.1	99.1 ± 0.3	99 ± 1.3

Filtration was performed using gravity (n=10) or with an irrigation pump at 150 mmHg (n=10) or 300 mmHg (n=11) pressure. Reported values are mean ± standard deviation. The independent samples t-test was performed to compare change from pre- to postfiltration (*$p<0.050$, **$p<0.001$) and Δprefiltration-postfiltration at 150 and 300 mmHg to gravity filtration (†$p<0.050$, ††$p<0.001$).

For all groups combined, filtration increased TEG time to initial clot formation (R) from 5.9 ± 1.0 to 8.0 ± 1.3 minutes ($p<0.001$) and decreased speed of clot formation (α) from 69.2 ± 3.2 to 63.7°± 3.7 ($p<0.001$) and clot strength (MA) from 64.2 ± 5.1 to 57.9 ± 4.9 mm ($p<0.001$). ROTEM clot strength (MCF) was reduced from 60 ± 4 to 58 ± 4 mm (in-tem) ($p<0.001$) and 61 ± 4 to 59 ± 4 mm (ex-tem) ($p=0.002$). At 300 mmHg, ROTEM platelet-inhibited clot formation speed (fib-tem α) increased from 65 ± 1 to 68 ± 7 mm ($p=0.001$) and time to initial clotting time (CT) decreased from 65 ± 10 to 58 ± 6 seconds ($p=0.004$). Fibrinolysis was not seen on TEG or ROTEM. Platelet aggregation ability significantly decreased after filtration in all groups. The

percentage of activated platelets (CD62P) decreased from 22.4 ± 9.3 to 18.5% ± 9.0 ($p< 0.05$) after filtration. See Table 1 for detailed data.

4.3 Delayed Filtration

Prefiltration measurements on day 7 showed a small decrease in PLT from 184×109/l ± 28 on day 0 to 148×109/l ± 45 on day 7 ($p=0.015$) and an increase in potassium from 3.3 ± 0.1 to 13.1 ± 1.3 mmol/L ($p<0.001$). Multiplate platelet function was reduced, but remained (ADP: 49 ± 13 to 15 ± 4, $p=0.003$, AA: 38 ± 10 to 12 ± 6, $p=0.002$, ristocetin: 73 ± 10 to 31 ± 14, $p=0.005$, TRAP: 85 ± 7 to 18 ± 6, $p<0.001$). A small decrease in hemostatic function was seen on TEG (K 1.5 ± 0.3 to 2.4 ± 0.2, $p=0.008$, Angle 68 ± 3 to 58 ± 3, $p=0.022$) and ROTEM in-tem (CT 176 ± 10 to 230 ± 22, $p=0.003$, CFT 74 ± 8 to 154 ± 30, $p=0.002$, α 76 ± 2 to 66 ± 3, $p=0.002$) and ex-tem (CT 56 ± 5 to 70 ± 5, $p=0.031$, CFT 81 ± 7 to 167 ± 34, $p=0.003$, MCF 61 ± 1 to 54 ± 3, $p=0.010$), combined with an increase in APTT (34 ± 4 to 44 ± 5 s, $p<0.001$) and reduction in factor VIII (130 ± 30 to 47% ± 25, $p=0.001$) and CD42b (98 ± 1 to 75 ± 7, $p=0.019$). Other parameters did not show statistically significant changes. Filtration on day 7 was unsuccessful in all units, with less than half of the volume completed before the filter was clogged. Post filtration PLT was reduced to 45×10^9/l ± 11 ($p=0.003$).

4.4 Cold Storage Conditions

Hemolysis did not exceed a mean of 0.2% on day 21. Platelet loss was greatest during the first 10 days with a reduction from 169 ± 27 to 137 ± 28×109/l ($p<0.001$), followed by a leveling out. There was no relevant change in Hgb, RBC, or HCT over the 21 days. There were no statistically significant differences between the groups. MCV remained stable until day 10 and then started increasing, with the greatest change seen in the hand-mixing group. Potassium levels increased at a steady rate in all groups, reaching a mean of 17.1 ± 2.9 on day 10, 19.3 ± 2.8 on day 14, and 23.2 ± 3.1 mmol/L on day 21 ($p<0.001$).

INR increased from 1.0 on day 1 to 1.2 on day 10 and then remained stable until day 21. APTT increased until day 10, followed by a decline until day 21. The increase in APTT from day 0 to 21 was 3 seconds longer in the no mixing group compared to the head-over-heel group. Fibrinogen levels remained stable throughout in all groups, while factor VIII went down until day 10 and then stabilized at around 50%. Platelet-inhibited clot strength (fib-tem MCF) was stable during the whole storage period ($p=0.355$).

All groups showed a significant loss of platelet aggregation response by day 10 (Figure 4). CD62P and CD42b expression showed that 16.5 ± 8.5% of platelets were in an activated state on day 0, rising to 76.7 ± 10.5% on day 10, 84.4 ± 6.0% on day 14, and 85.4 ± 6.0% on day 21 ($p<0.001$). For more details, see Table 2.

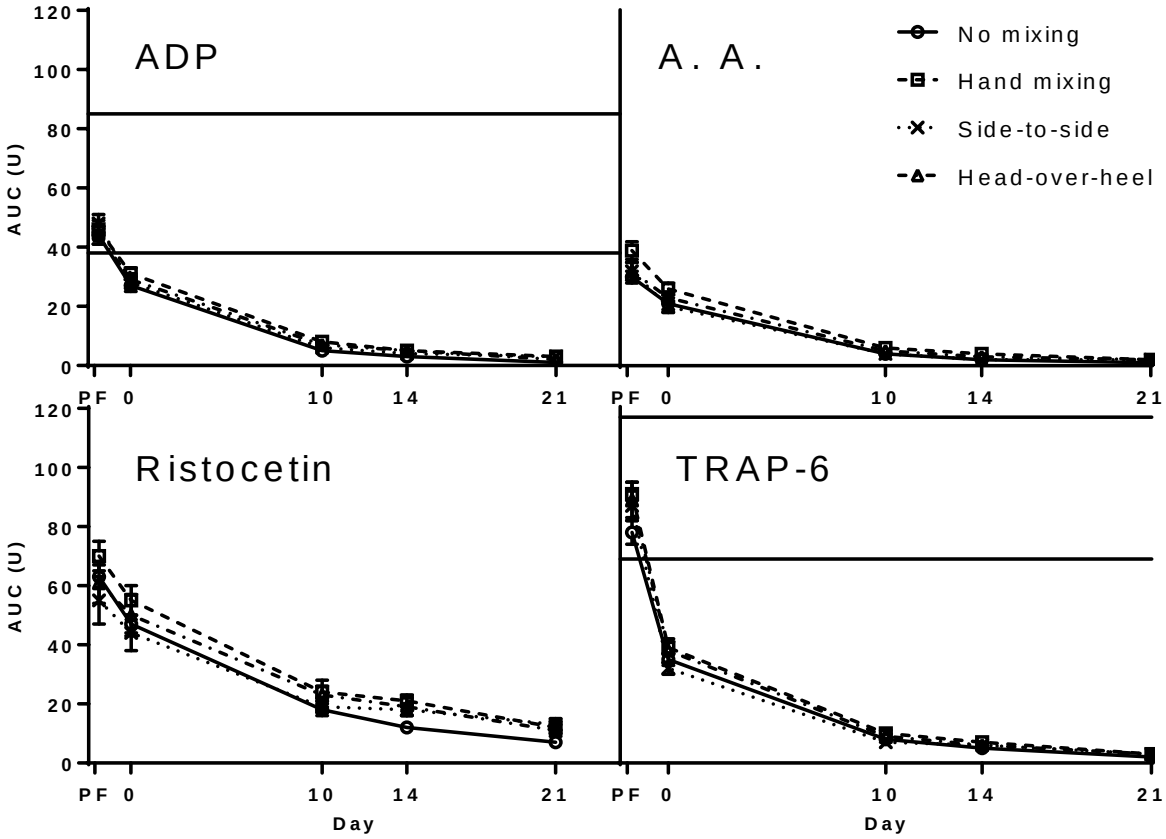

Figure 4. Multiplate impedance aggregometry response in whole blood leukoreduced with the Imuflex WB-SP filter and stored at 4 °C following filtration. PF indicates response prior to leukoreduction. Error bars show standard error of the mean. Horizontal lines indicate normal range for citrated samples. Repeated measures ANOVA showed a decreasing response to all agonists during storage ($p<0.001$). Differences between groups were not statistically significant (ADP: $p=0.787$, A. A.: $p=0.563$, ristocetin: $p=0.484$, TRAP-6: $p=0.206$).

Table 2. Storage at 4 °C of Whole Blood Leukoreduced Using the Imuflex WB-SP Filter

TEG and ROTEM showed a reduction of clot formation speed (α) and maximum clot strength (MA/MCF) during storage. ROTEM additionally showed an increase in time to first clot formation (Figure 5). There was no relevant level of fibrinolysis. Measurements were not statistically significant different between the groups.

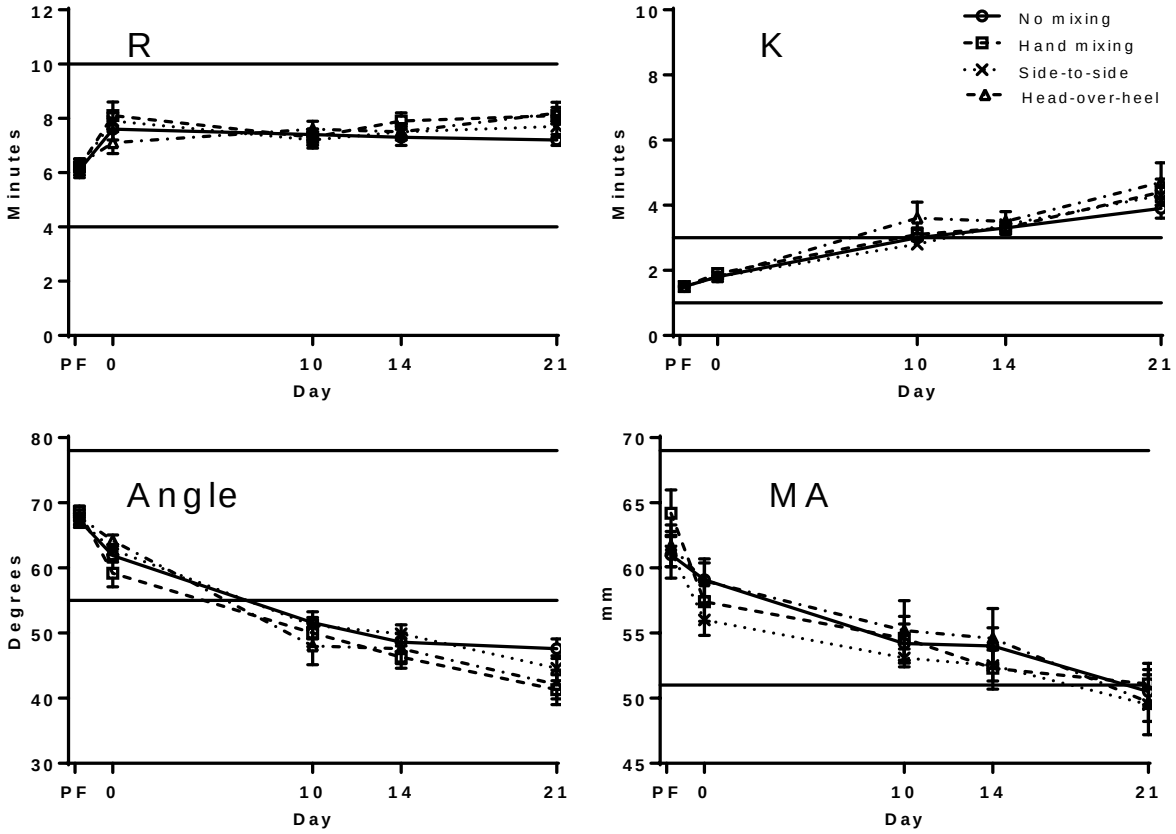

Figure 5. TEG thromboelastography response in whole blood leukoreduced with the Imuflex WB-SP filter and stored at 4°C. PF indicates response prior to leukoreduction. Error bars show standard error of the mean. Horizontal lines indicate normal range. Repeated measures ANOVA showed an increase in K and decrease in angle and MA during storage ($p<0.001$). The change in R was not statistically significant ($p=0.152$). Differences between groups were not statistically significant (R: $p=0.126$, K: $p=0.409$, Angle: $p=0.142$, MA: $p=0.755$).

4.5 Storage at or Above Room Temperature

Storage at 22 °C did not cause hemolysis beyond 0.1%. There was no relevant change in PLT, Hgb, or RBC over the 3-day period. HCT increased from 0.38 ± 0.02 on day 0 to 0.41 ± 0.02 on day 3 ($p<0.001$). MCV increased from 95 ± 4 to 100 ± 3 fL ($p<0.001$) in the same period. This change was not seen with cold storage. Potassium increased at a slower rate than for cold storage and peaked at 6.9 ± 0.8 mmol/L on day 3 compared to 8.2 ± 1.1 mmol/L in the cold storage group ($p=0.011$). Bacterial tests were negative for all units.

INR increased from 1.0 ± 0.1 to 1.1 ± 0.1 on day 3 at 22 °C ($p=0.015$), while no statistically significant change was observed for APTT and fibrinogen. There was a slight decrease in platelet-inhibited clot strength (fib-tem MCF) from 14 ± 4 to 13 ± 3 ($p=0.001$). Factor VIII decreased from 125 ± 25% on day 1 to 76 ± 19% in the 22 °C group ($p<0.001$), compared with a reduction from 131 ± 24 to 48 ± 15% at 4 °C ($p<0.001$).

Most aggregation responses to ADP, AA, and ristocetin were lost on day 3 (Figure 6). The loss was greater than the cold storage group, with the biggest difference observed for ristocetin. TRAP response remained relatively stable and was higher than the cold group on day 3. Storage at 22 °C (Figure 7) increased time to initial clot formation (R/CT), but preserved the speed of clot formation (α) better than cold storage when measured by TEG and ROTEM. Maximum clot strength (in-tem MCF) increased from 59 ± 4 to 64 ± 3 ($p<0.001$) at 22 °C, but not at 4 °C. Fibrinolysis was insignificant. See Table 3 for detailed data.

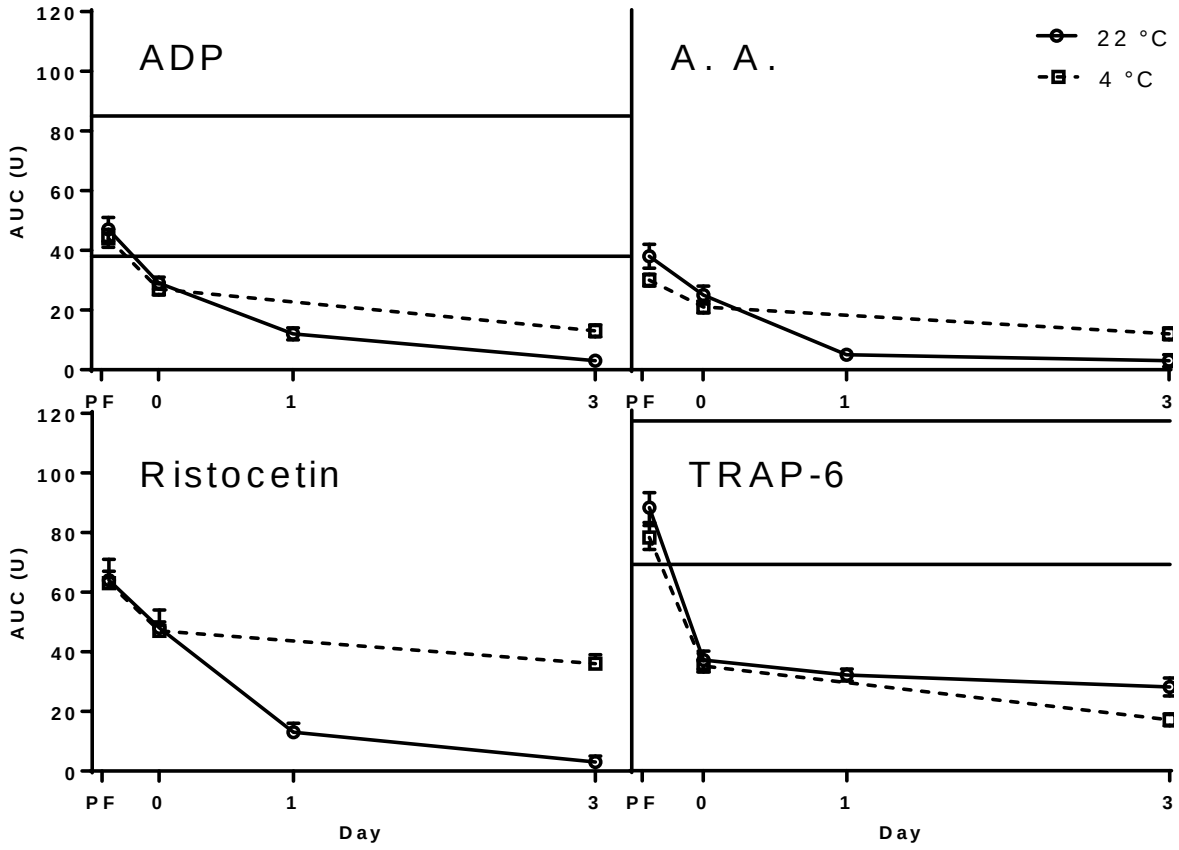

Figure 6. Multiplate impedance aggregometry response in whole blood leukoreduced with the Imuflex WB-SP filter and stored at 22°C vs. 4°C. PF indicates response prior to leukoreduction. Error bars show standard error of the mean. Horizontal lines indicate normal range for citrated samples. Repeated measures ANOVA showed a decreasing response to all agonists during storage ($p<0.001$). The decrease was greater at 22 °C compared to 4 °C for ADP ($p=0.007$), AA ($p=0.007$), and ristocetin ($p=0.001$). TRAP-induced aggregation was better preserved at 22 °C ($p=0.002$).

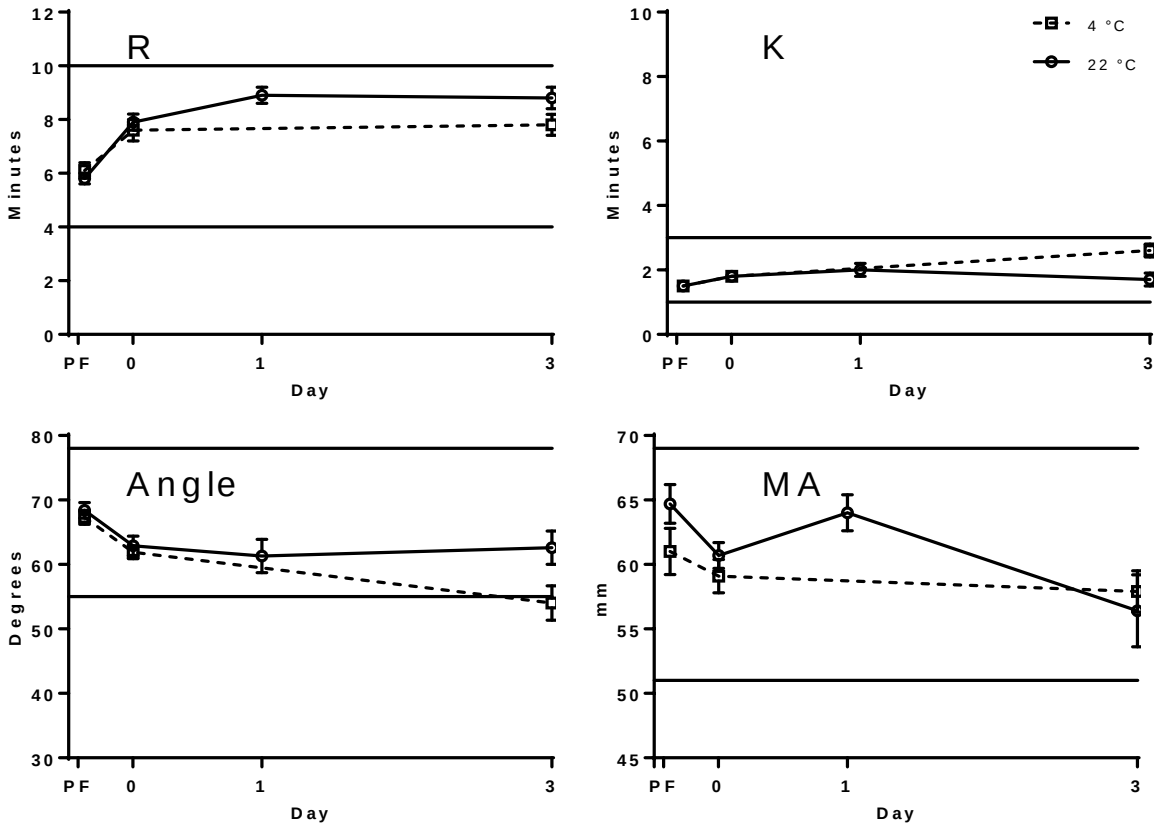

Figure 7. TEG thromboelastography response in whole blood leukoreduced with the Imuflex WB-SP filter and stored at 22°C. PF indicates response prior to leukoreduction. Error bars show standard error of the mean. Horizontal lines indicate normal range. Repeated measures ANOVA showed an increase in K (p=0.012) and decrease in angle (p=0.037). The change in R and MA was not statistically significant (R: p=0.152, MA: p=0.060). With the exception of K (p=0.002), the differences between groups were not statistically significant (R: p=0.410, Angle: p=0.052, MA: p=0.504).

Table 3. Storage at 22 °C of Whole Blood Leukoreduced Using the Imuflex WB-SP Filter

TEG/TEM parameters	PF	Day 0	Day 1	Day 3
General				
PLT (10^9/L)	185 ± 47	180 ± 48	190 ± 46	189 ± 46
Hgb (g/dL)	12.8 ± 0.9	12.8 ± 0.8	12.7 ± 0.8	12.7 ± 0.8
RBC (10^12/L)	4 ± 0.3	4 ± 0.3	4 ± 0.3	4 ± 0.3
HCT (%)	0.38 ± 0.02	0.38 ± 0.02	0.39 ± 0.02	0.41 ± 0.02
MCV (fL)	96 ± 4	95 ± 4	98 ± 4	100 ± 3
Hemolysis (%)	0.1 ± 0	0 ± 0	0.1 ± 0	0.1 ± 0
K (mmol/L)	3.3 ± 0.2	3.3 ± 0.2	4.1 ± 0.3	6.9 ± 0.8
Coagulation				
INR	1 ± 0.1	1 ± 0.1	1 ± 0	1.1 ± 0.1
APTT (s)	34 ± 2	34 ± 2	35 ± 2	32 ± 2
Fibrinogen (g/dL)	3.1 ± 0.5	3.1 ± 0.5	3.2 ± 0.5	3.1 ± 0.5
Factor VIII (%)	122 ± 26	125 ± 25	95 ± 27	76 ± 19
TEG				
R (mins)	5.8 ± 0.6	7.9 ± 0.9	8.9 ± 0.9	8.8 ± 1.3
K (min)	1.5 ± 0.4	1.8 ± 0.3	2 ± 0.6	1.7 ± 0.5
Angle (°)	68.4 ± 3.8	62.9 ± 4.9	61.3 ± 8.3	62.6 ± 8.3
MA (mm)	64.7 ± 4.8	60.7 ± 3.3	64 ± 4.4	56.4 ± 7.8
LY30 (%)	1.1 ± 2.4	1.7 ± 2	0.6 ± 0.9	1.7 ± 1.9
ROTEM in-tem				
CT (min)	176 ± 7	186 ± 14	199 ± 23	227 ± 34
CFT (min)	73 ± 11	82 ± 13	80 ± 13	87 ± 14
Angle (°)	76 ± 2	74 ± 2	74 ± 2	72 ± 3
MCF (mm)	61 ± 3	59 ± 4	61 ± 3	64 ± 3
LI30 (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0
ROTEM ex-tem				
CT (min)	63 ± 5	61 ± 5	63 ± 7	74 ± 13
CFT (min)	81 ± 14	87 ± 14	86 ± 12	89 ± 19
Angle (°)	74 ± 3	74 ± 3	74 ± 2	73 ± 3
MCF (mm)	62 ± 4	61 ± 4	62 ± 3	62 ± 4
LI30 (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0
ROTEM fib-tem				
CT (min)	57 ± 4	58 ± 8	62 ± 8	67 ± 10
Angle (°)	73 ± 3	70 ± 6	67 ± 6	63 ± 9
MCF (mm)	15 ± 3	14 ± 4	13 ± 3	13 ± 3
LI30 (%)	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Multiplate				
ADP (U)	47 ± 12	29 ± 7	12 ± 6	3 ± 3
AA (U)	38 ± 14	25 ± 8	5 ± 5	3 ± 6
Ristocetin (U)	64 ± 22	48 ± 17	13 ± 8	3 ± 5
TRAP-6 (U)	88 ± 17	37 ± 9	32 ± 7	28 ± 10
Cytometry				
CD62P (%)	25.5 ± 7	20 ± 5.3	19.8 ± 7.2	46.8 ± 13.4
CD42b (%)	99 ± 0.3	99.2 ± 0.2	99.2 ± 0.5	97.8 ± 1.7

At 32 °C, a minor decrease in aggregation response to ADP (31 ± 7 to 27 ± 6, p=0.027) and A. A. (24 ± 9 to 19 ± 7, p=0.028) and a minor increase for TRAP (38 ± 10 to 41 ± 10, p=0.037) was observed from after filtration to hour 2 of storage. A greater reduction was seen for ristocetin (62 ± 17 to 49 ± 13, p=0.006). A slight increase in time to first coagulation was seen on

TEG (R: 7.8 ± 1.2 to 8.6 ± 1.5, p=0.028) and ROTEM in-tem (CT: 181 ± 12 to 189 ± 15, p=0.001). The number of platelets expressing CD62P was reduced from 17 ± 6 to 10% ± 4 (p<0.001). No other parameters were affected. See Table 4 for detailed data.

Table 4. Storage at 32 °C of Whole Blood Leukoreduced Using the Imuflex WB-SP Filter

TEG/TEM parameters	PF	Hour 0	Hour 1	Hour 2
General				
PLT (10^9/L)	185 ± 47	174 ± 45	180 ± 44	180 ± 45
Hgb (g/dL)	13.1 ± 0.9	13 ± 0.9	13 ± 0.9	13 ± 0.9
RBC (10^12/L)	4.2 ± 0.3	4.1 ± 0.3	4.2 ± 0.3	4.2 ± 0.3
HCT (%)	0.39 ± 0.03	0.39 ± 0.03	0.39 ± 0.03	0.39 ± 0.03
MCV (fL)	94 ± 5	94 ± 5	94 ± 4	94 ± 5
Hemolysis (%)	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
K (mmol/L)	3.2 ± 0.2	3.2 ± 0.2	3.2 ± 0.2	3.2 ± 0.2
Coagulation				
INR	1 ± 0.1	1 ± 0	1 ± 0	1 ± 0
APTT (s)	34 ± 3	34 ± 3	34 ± 3	34 ± 3
Fibrinogen (g/dL)	2.9 ± 0.5	2.9 ± 0.4	2.9 ± 0.5	2.9 ± 0.4
Factor VIII (%)	124 ± 24	124 ± 22	123 ± 23	125 ± 23
TEG				
R (mins)	6.7 ± 1.5	7.8 ± 1.2	7.8 ± 1.1	8.6 ± 1.5
K (min)	1.6 ± 0.5	1.8 ± 0.3	1.7 ± 0.3	1.9 ± 0.5
Angle (°)	67.2 ± 5.4	63.4 ± 3.7	64.4 ± 4	63.1 ± 5
MA (mm)	64.1 ± 3.6	61.7 ± 4.6	61.1 ± 4.1	60.2 ± 4.4
LY30 (%)	1.3 ± 0.9	1.3 ± 1.6	1.2 ± 0.8	1.5 ± 2.1
ROTEM in-tem				
CT (min)	177 ± 13	181 ± 12	185 ± 10	189 ± 15
CFT (min)	69 ± 10	74 ± 11	74 ± 10	76 ± 13
Angle (°)	76 ± 2	75 ± 2	75 ± 2	75 ± 2
MCF (mm)	61 ± 4	59 ± 3	59 ± 4	59 ± 4
LI30 (%)	100 ± 1	99 ± 1	99 ± 1	99 ± 1
ROTEM ex-tem				
CT (min)	60 ± 5	60 ± 3	60 ± 5	58 ± 5
CFT (min)	78 ± 10	81 ± 10	87 ± 10	84 ± 8
Angle (°)	74 ± 2	74 ± 2	72 ± 2	73 ± 2
MCF (mm)	62 ± 4	60 ± 4	60 ± 4	61 ± 4
LI30 (%)	100 ± 0	100 ± 0	100 ± 1	100 ± 1
ROTEM fib-tem				
CT (min)	58 ± 7	58 ± 6	60 ± 4	53 ± 9
Angle (°)	67 ± 8	67 ± 8	68 ± 4	67 ± 8
MCF (mm)	13 ± 2	13 ± 2	12 ± 2	13 ± 2
LI30 (%)	100 ± 0	100 ± 0	99 ± 1	100 ± 0
Multiplate				
ADP (U)	50 ± 12	31 ± 7	29 ± 5	27 ± 6
AA (U)	37 ± 14	24 ± 9	21 ± 9	19 ± 7
Ristocetin (U)	74 ± 14	62 ± 17	56 ± 16	49 ± 13
TRAP-6 (U)	91 ± 19	38 ± 10	39 ± 9	41 ± 10
Cytometry				
CD62P (%)	19.3 ± 5.6	17.4 ± 6.2	13.1 ± 5.3	9.9 ± 4.1
CD42b (%)	97.1 ± 1.1	97.6 ± 0.9	97.9 ± 0.7	97.9 ± 0.9

5.0 DISCUSSION

The effects of leukoreduction on WB have been researched previously, but no studies on forced leukoreduction are published [1-3]. This is easily explained by the fact that collection and production of blood products usually are within the domain of the blood bank: a setting where time is less of a concern. While forced filtration is an unlabeled use of the Imuflex WB-SP set, filtration time was significantly reduced without affecting the recovery of platelets or hemoglobin, except for one unit filtered at 300 mmHg where platelet recovery did not meet the manufacturer's performance criteria. Prefiltration measurements for this unit were not significantly different from the other units and no problems were noted during filtration. Increasing filtration pressure did, however, cause an increase in residual leukocytes in the final product, but most units were below 1.0×10^6/unit at 150 mmHg and 5.0×10^6/unit at 300 mmHg (Figure 1). While forced filtration yielded higher residual WBC than gravity filtration, the highest value observed still represents a significant reduction and may be acceptable for use in trauma patients who are unlikely to be immunocompromised. However, even a 9-minute delay to transfusion by forced filtration at 300 mmHg may be substantial in an acute situation in the field. This could be mitigated by having available a limited number of cold stored units and starting transfusion of these immediately. If necessary, collection and filtration of additional units could then be carried out while the transfusion is in progress.

When evaluated by viscoelastic tests and aggregometry, forced filtration affected the blood product by increasing the time it took to form a stable clot, reducing the strength of the clot, and reducing the aggregation ability of the platelets. This occurred irrespective of increased filtration pressure. The clinical relevance of this change, if the product is transfused immediately after filtration, is uncertain. Platelet function measured by Multiplate aggregometry appears to be affected negatively by filtration to an extent that does not seem to correlate with TEG/ROTEM, CD62P-expression, or light transmission aggregometry [3].

The Multiplate analyzer is designed to measure platelet function in fresh WB samples. It is possible that the filtration process induces changes that interfere with the electrode impedance measurement method used, and that the results do not reflect the in vivo function of the leukoreduced WB. Further studies directly comparing Multiplate to other aggregation tests such as light transmission aggregometry or flow cytometry would be beneficial to establish whether this analysis is an appropriate quality measure for blood products, ideally in combination with in vivo testing of transfused patients.

Platelet counts remained relatively high throughout unagitated storage at 4 °C, while hemostatic function of the leukoreduced WB deteriorated. The rate of reduction mirrors that previously shown [4]. Removing the blood from unagitated storage once a day and mixing it thoroughly by hand appeared less beneficial to RBCs without improving platelet function. Adding continuous mixing in the form of side-to-side agitation or head-over-heel rotation did not improve function either, other than a marginal improvement in APTT. Mixing had no effect on hemolysis, other hematological parameters, or potassium leakage. Additionally, Stubbs et al. have previously shown that orbital agitation does not improve the loss of collagen-induced aggregation [5]. While the starting point at storage on day 0 is lower, the function of leukoreduced WB does not appear to degrade faster than what previously has been shown in non-leukoreduced blood [6]. The effects of filtration on storage could perhaps be alleviated by using CPDA-1 instead of CPD as anticoagulant, as there are data to suggest that the addition of adenine leads to better preservation of hemostatic function [7]. However, there is presently no

collection set commercially available with an in-line platelet-sparing filter and CPDA-1 as additive. That said, the demand for WB is growing and, if studies show a benefit, manufacturers may respond.

Storing the leukoreduced blood at 22 °C seems to cause a complete loss of Multiplate aggregation response to ADP, A. A., and ristocetin after the first day. However, ambient temperature storage preserved TRAP-induced aggregation and TEG/ROTEM hemostatic function better. Two-hour storage at 32 °C did not induce any notable reduction in quality. Forced leukofiltration of WB through the Imuflex WB-SP collection set resulted in significant decrease of filtration time, but minor filtration failure at 150 mmHg pressure and major at 300 mmHg. Recovery of platelets in all the bags was acceptable (there is no FDA regulatory requirement for platelet recovery) and averaged > 80%.

Storage mixing conditions demonstrated no benefit to manipulation/mixing of WB bags through the 21-day cold storage period, neither for platelet content or function, hemolysis, or Hgb content. Significant decrease in Multiplate aggregation was noted but this is contrasted with the thromboelastographic and thromboelastometric results, which were all around lower normal ranges, and a minor decrease in the expression of CD62P. Any clinical significance of these in vitro findings can only be resolved by clinical studies.

5.1 Clinical Testing: University of Cincinnati

Following the initial testing of filter pressure and storage conditions, further testing was applied to determine the optimal timing and height of filtration to achieve leukoreduction and platelet sparing. Five hundred milliliters of whole blood was obtained from male donors in a standard donation bag set containing CPD, which was immediately spliced into the IMUFLEX WB-SP blood bag system maintaining a constant distance from the filter. The whole blood units were transported from the blood center to the laboratory. The units were randomly assigned to one of four different groups: non-leukoreduced (NLR), leukoreduced at 1 hour following collection at standard height of 83 cm or 33 inches (LR-1), leukoreduced at 4 hours following collection at 83 cm or 33 inches (LR-4(33)) or leukoreduced at 4 hours at a lower height of 71 cm or 28 inches (LR-4(28)). The units were gravity filtered at the assigned timepoint according to the manufacturer's instructions then stored at 4°C. Twenty milliliter samples were taken from each whole blood unit on the day of collection (day 0) before and after leukoreduction, as well as days 1, 7, 14 and 21 after filtration. Prior to sampling, the units were gently massaged to ensure adequate mixing and homogenous samples.

5.2 Complete Blood Count and Arterial Blood Gas Analysis

A complete blood count for each unit was obtained utilizing a Coulter AcT 10 Hematology Analyzer (Beckman Coulter, Brea, California) to assess white blood cell count, hemoglobin, and platelet counts. A blood gas was performed utilizing iSTAT (Abaxis, Union City, CA). Whole blood prothrombin time was attempted to be evaluated with the iSTAT, but was unable to be performed in any sample, potentially due to the underlying CPD anticoagulant.

5.3 Thromboelastometry: EXTEM, FIBTEM

Viscoelastic coagulation testing was performed using the ROTEM delta WB analyzer (Tem Innovations GmbH, Munich, Germany) following the manufacturer's instructions. Three hundred microliters of whole blood was aliquoted into Eppendorf tubes. The test was initiated with twenty microliters of each respective reagent. EXTEM testing was initiated with STAR-TEM (recalcification) and EXTEM (tissue factor) reagents. FIBTEM testing was performed with EXTEM testing and the addition of Cytochalasin D for platelet inhibition. The temperature was maintained at 37°C and the samples were allowed to run until the 60 minutes after the maximal clot firmness (MCF) was reached. Platelet contribution to clot strength (%MCFPlatelet) was calculated by the equation: (EXTEM MCF – FIBTEM MCF)/ (EXTEM MCF) similar to the methods previously described.

5.4 Platelet Aggregation Analysis

Platelet aggregation was measured by using a Multiplate impedance aggregometer by Roche Diagnostics (Risch-Rotkreuz, Switzerland) according to the manufacturer's instructions. Four different agonists were used including adenosine diphosphate (ADP), arachidonic acid (ASPi), collagen (COL) and thrombin receptor agonist peptide (TRAP). The test was performed by incubating 300 µL of whole blood with 300 µL of 0.9% sodium chloride + CaCl2 for 3 minutes. After incubation, 20 µL of the respective agonist was added (6.5 µmol/L ADP, 0.5 mmol/L ASPi, 3.2 µg/mL collagen, or 32 µmol/L TRAP). Platelet aggregation velocity, total platelet aggregation, and the area under the curve (AUC) were measured.

5.5 Thrombin Generation

Thrombin generation was measured using a calibrated automated thrombinoscope from Diagnostica Stago (Parsippany, NJ) according to the manufacturer's instructions. The platelet poor plasma (PPP) and platelet rich plasma (PRP) were prepared immediately following collection. For PPP, whole blood was centrifuged at 4600 rpm (Eppendorf Mini Spin centrifuge) for ten minutes at room temperature. The plasma was then collected and centrifuged again at 10,200 rpm for another ten minutes. The plasma was then aliquoted for testing. For PRP, whole blood was centrifuged at 260 g for 6 minutes. The plasma was collected and centrifuged for another 6 minutes at 640 g. For the evaluation of PPP, the plasma was aliquoted with 20 µL of each reagent was added (PPP low, PPP high and MP). For PRP, the plasma to be tested was aliquoted with 20 µL of the PRP reagent. The samples were incubated at 37°C for 10 minutes and 20 µL of the FluCa reagent was added. Each sample was run as in triplicate as recommended by the manufacturer.

5.6 Platelet Factor Measurement by ELISA

Whole blood was collected and placed in serum separator tubes (BD Bioscience, San Diego, California), centrifuged at 1,000 g for 10 min, and serum was collected. Serum levels of fibrinogen, CD40L, P Selectin and Platelet factor-4 (PF4) were measured by ELISA according to the manufacturer's protocol (Abcam, Cambridge, MA). Thromboxane A2 (TXA2) was measured by ELISA according to the manufacturer's protocol (Antibody Research Corporation, St. Peters,

MO). The lower limits of detection were: fibrinogen 1.56 ng/mL, CD40L 8.23 pg/mL, P selectin 0.041 ng/mL, PF4 0.04 ng/mL and TXA2 62.5 pg/mL.

5.7 Statistical Analysis

Prism 6 (GraphPad Software, La Jolla, California) was used for all statistical analyses. All data is presented as the median (interquartile range). The Kruskal-Wallis test was used to compare continuous nonparametric data between the different groups. Analysis was performed to compare non-LR vs. LR-1 vs. LR-4(33) vs. LR-4(28) at day 0 pre-LR and post LR, day 1, day 7, day 14 and day 21. A p value of less than 0.05 was considered significant.

5.8 Effect of Leukoreduction on Complete Blood Count

The WBC count for non-leukoreduced (NLR) group was significantly higher at day 1 compared to the whole blood leukoreduced at 4 hours and 28 inches. The NLR group also showed a significant difference in WBC count compared to the LR-1 group on day 7 and day 21 (using Coulter counter). No other differences were found between groups (Figure 8). Hemoglobin was similar between groups. The percentage of platelet recovery following leukoreduction is shown in Table 5. Platelet recovery was similar between groups, with some variability demonstrated amongst the five units in each group.

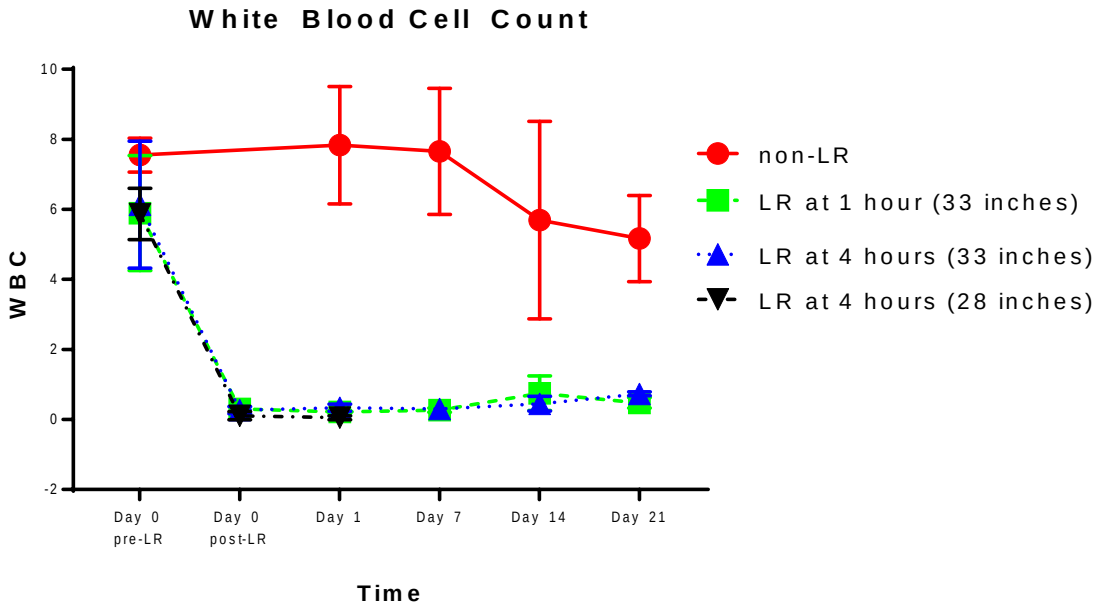

Figure 8. White blood cell count over the study period.

5.9 Effect of Leukoreduction on Blood Gas Analysis

The pH and potassium levels were not significantly different between groups. However, the pH significantly decreased in each group by day 21 compared to pre-LR. Although the base excess did not have any significant differences between groups, there was a trend over time

showing worsening base deficit. (Figures 9-11) Sodium level did not differ between filtration groups, but did significantly decrease over time in all groups.

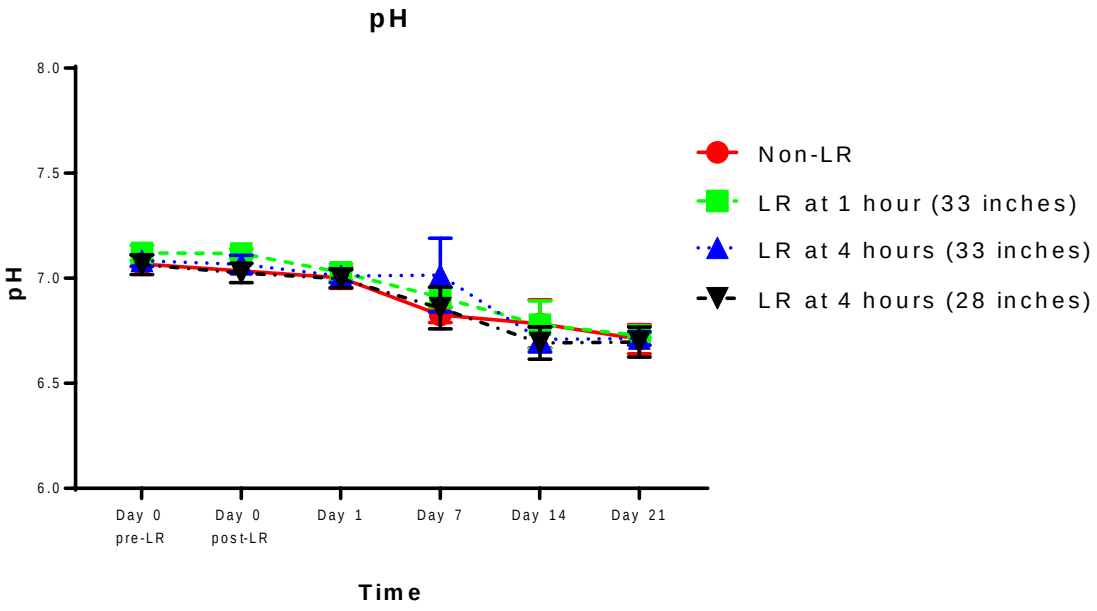

Figure 9. pH over the study period.

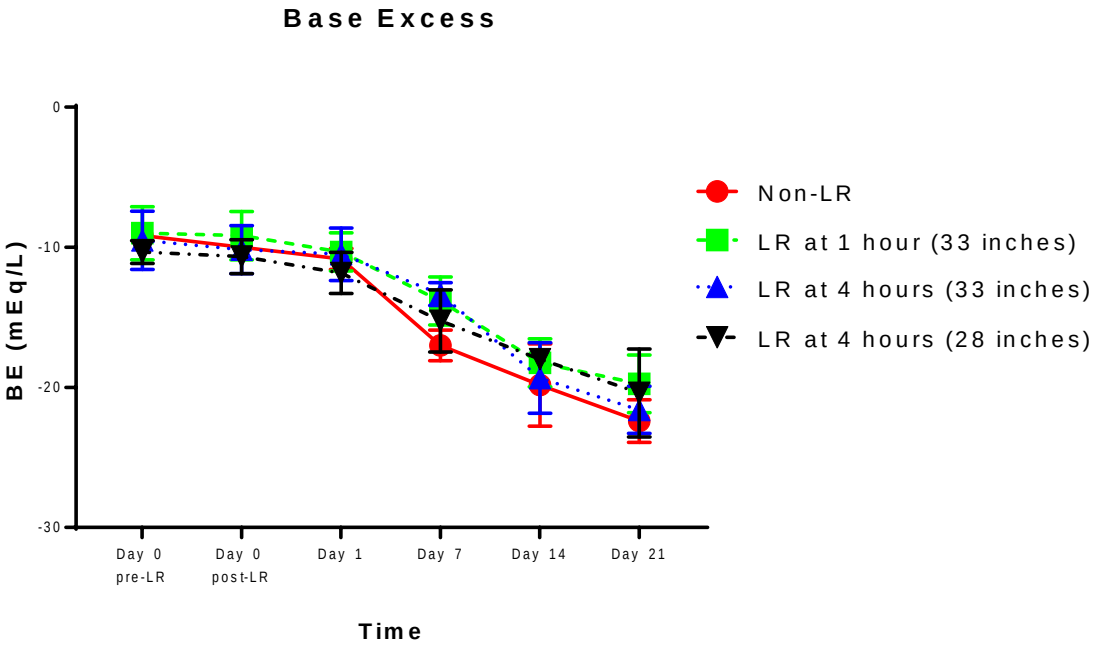

Figure 10. Base excess over study period.

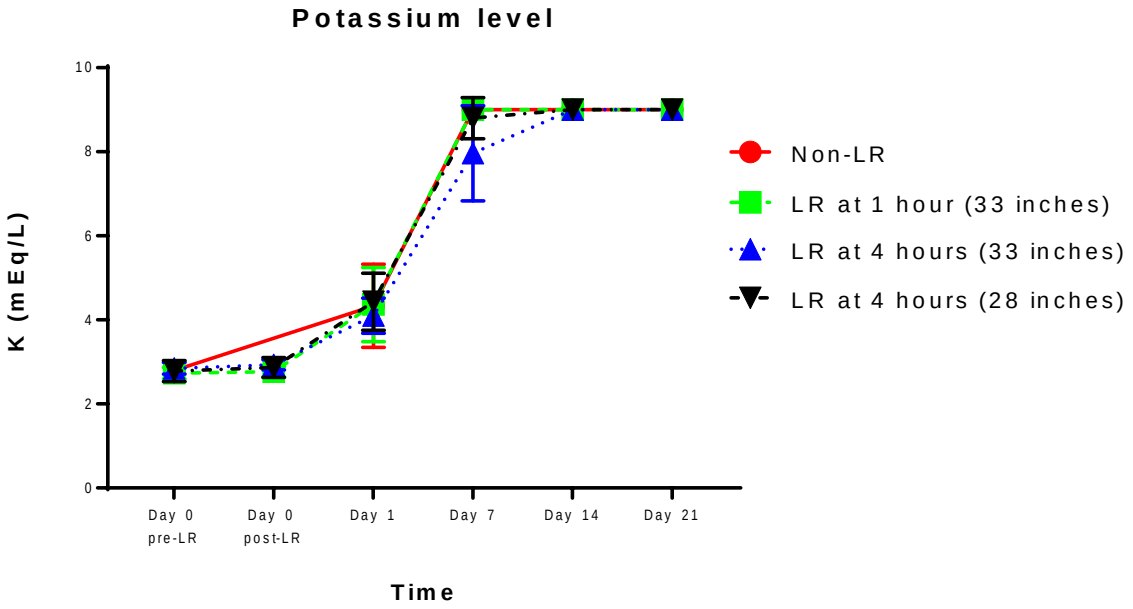

Figure 11. Potassium levels over the study period.

5.10 Effect of Leukoreduction on Platelet Aggregation

The AUC for aggregation with all agonists was significantly reduced in the LR-1 group compared to the NLR group on day 14 and 21. On day 1, the LR-1 group was reduced compared to the NLR group with ADP, COL and TRAP. On day 7, the LR-1 group was reduced compared to the NLR group with COL, TRAP and ASPi.

On days 1 and 21, the AUC was reduced in the LR-4(28) group compared to NLR group after ADP addition (Figure 12). On day 1, the AUC was reduced in the LR-4(28) compared to NLR using ASPi. On days 1, 7 and 21, the AUC was reduced in the LR-4(28) group compared to NLR using TRAP (Figure 13). On days 14 and 21, the AUC was reduced in the LR-4(28) group compared to NLR using COL. Importantly, the AUC in the LR-4(28) group was also significantly reduced at day 21 compared to prior to leukoreduction in all four agonist groups. The AUC for the LR-4(33) group was only reduced at day 21 compared to prior to leukoreduction using TRAP. Notably, the NLR, LR-1, LR-4(33), and LR-(28) groups all demonstrated significantly reduced platelet aggregation in response to all four agonists at day 21 compared to day 0 pre-leukoreduction. Overall, platelet aggregation decreased by day 7 in all four groups over time and remained stable over the next two weeks, with small, statistically but not clinically significant, differences between NLR and leukoreduced groups.

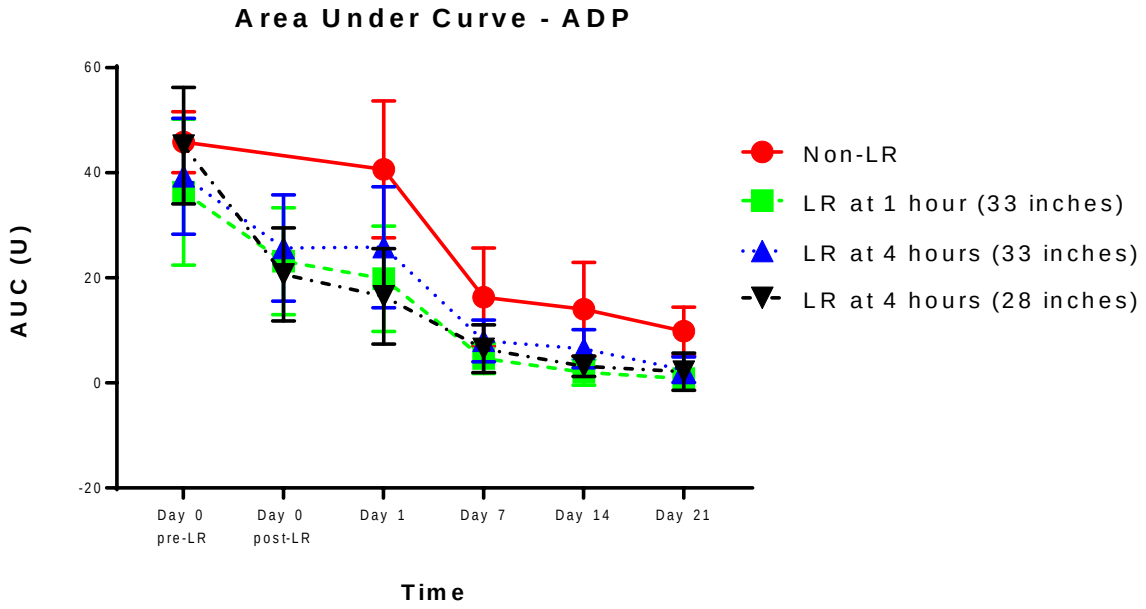

Figure 12. AUC for ADP over the study period.

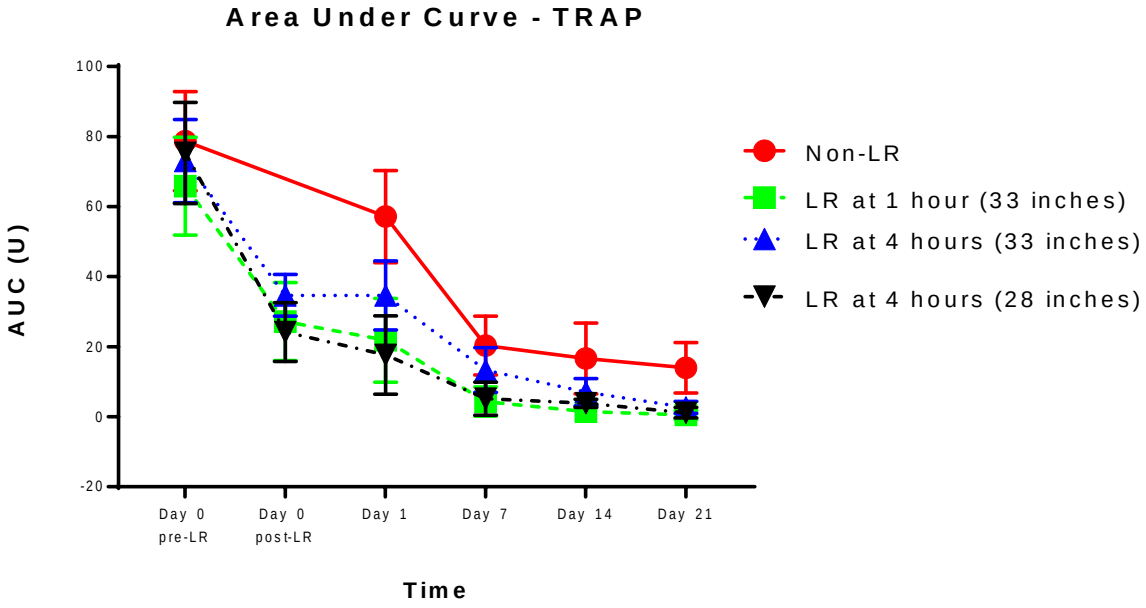

Figure 13. AUC for TRAP agonist over the study period.

5.11 Effect of Leukoreduction on Viscoelastic Coagulation Parameters

There were no differences in EXTEM clotting time, clot formation time, alpha angle, or MCF between groups at any time points. The EXTEM MCF was reduced in all groups from day 0 to day 21 (Figure 14). However, the percentage of platelet contribution to MCF did not show a decrease from day 0 to day 21 (Figure 15). The FIBTEM CT, MCF and alpha angle were similar

between all groups. No notable fibrinolysis was demonstrated in any of the groups in EXTEM or FIBTEM testing.

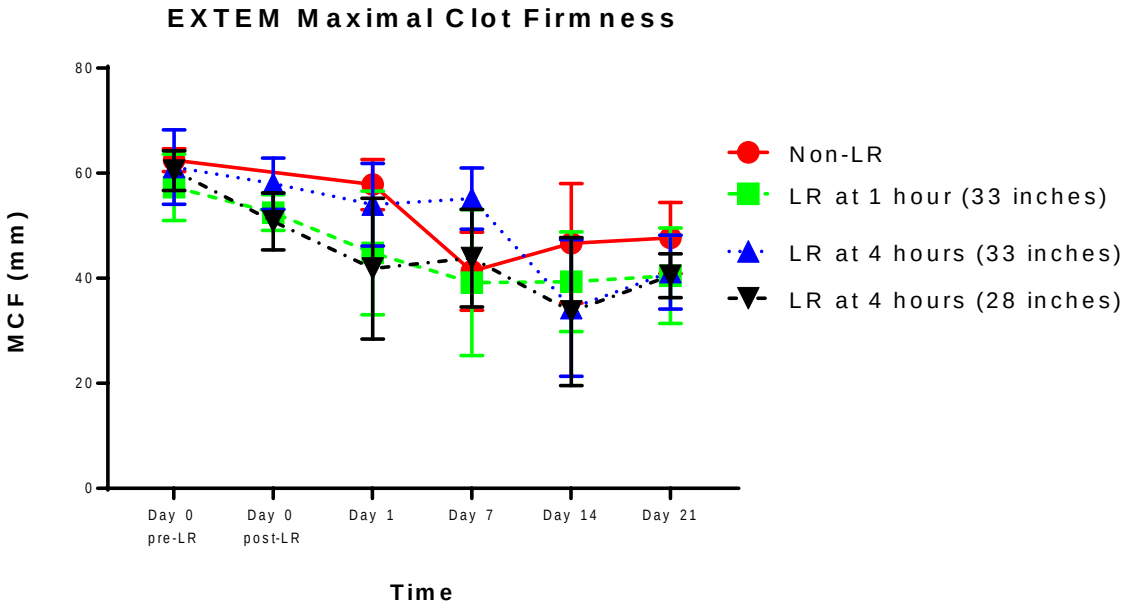

Figure 14. EXTEM MCF over the study period.

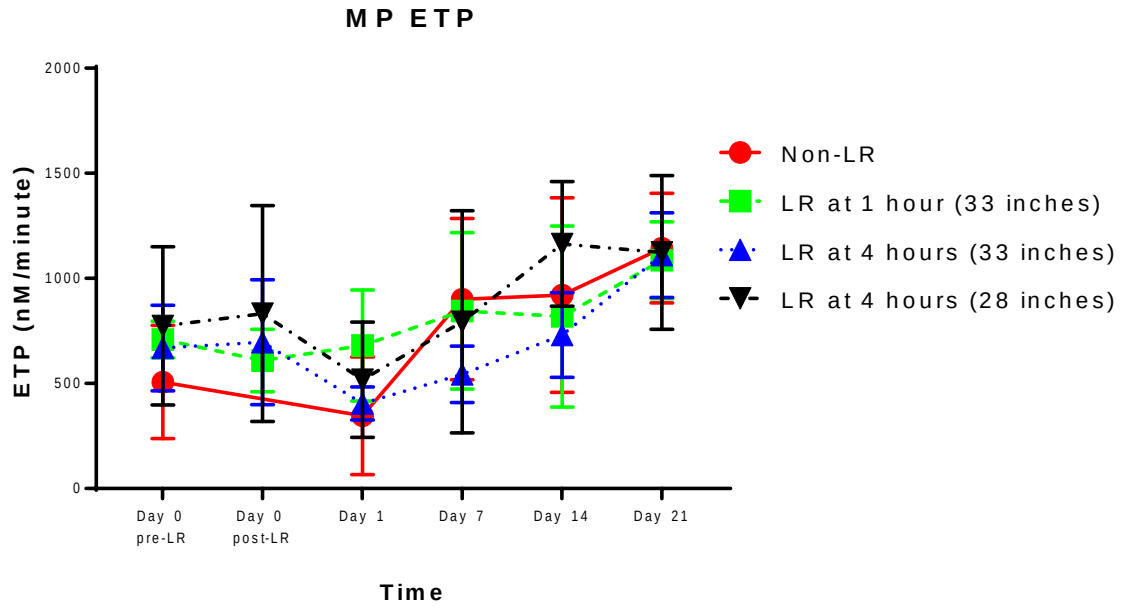

Figure 15. Percentage of platelet contribution to MCF over the study period.

5.12 Effect of Leukoreduction on Thrombin Generation

The median ETP in both high and low tissue factor experiments showed no difference between the groups. In the PRP and MP experiments, the median ETP was also similar between groups. However, the median ETP in the MP experiment was increased from day 0 to 21 in all groups except LR-4(28) (Figure 16). The Peak, ttPeak and velocities were similar between all groups for PRP, PPP high, PPP low, and MP.

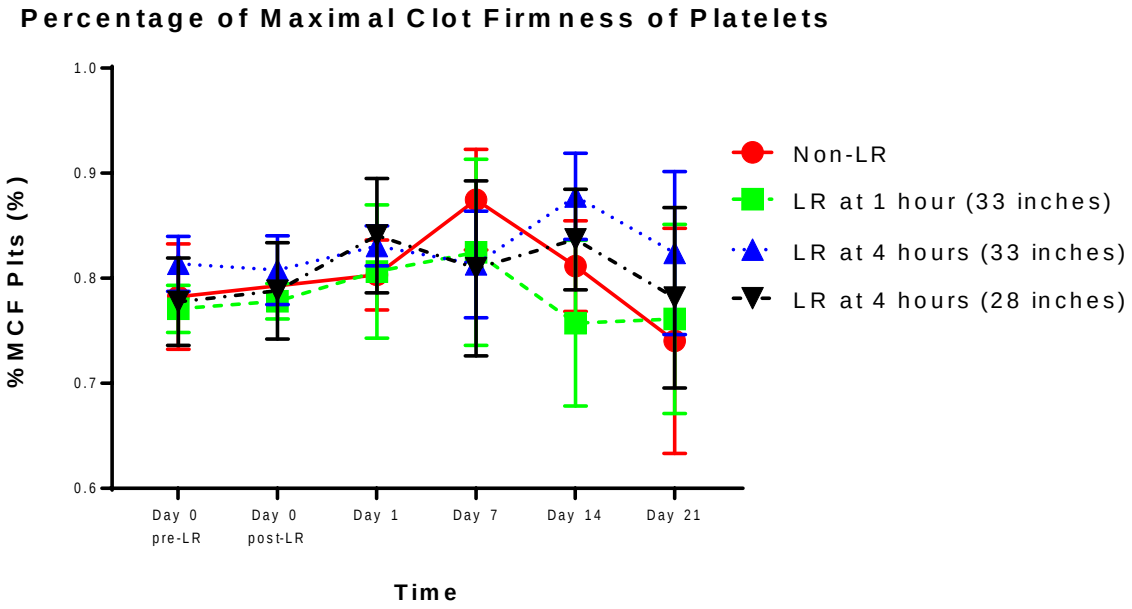

Figure 16. MP ETP over the study period.

5.13 Effect of Leukoreduction on Platelet Factors and Degradation Products

The PF4 levels were significantly increased in the NLR group compared to LR-4(28) group at days 14 and 21. The PF4 levels significantly decreased over time in all groups (Figure 17). Thromboxane A2 and fibrinogen levels were similar between all groups. Fibrinogen levels were also constant from day 0 to day 21 in all groups. The P selectin and CD40L levels had no differences between groups. However, both were increased in each group at day 21 compared to day 0 (Figures 18 and 19).

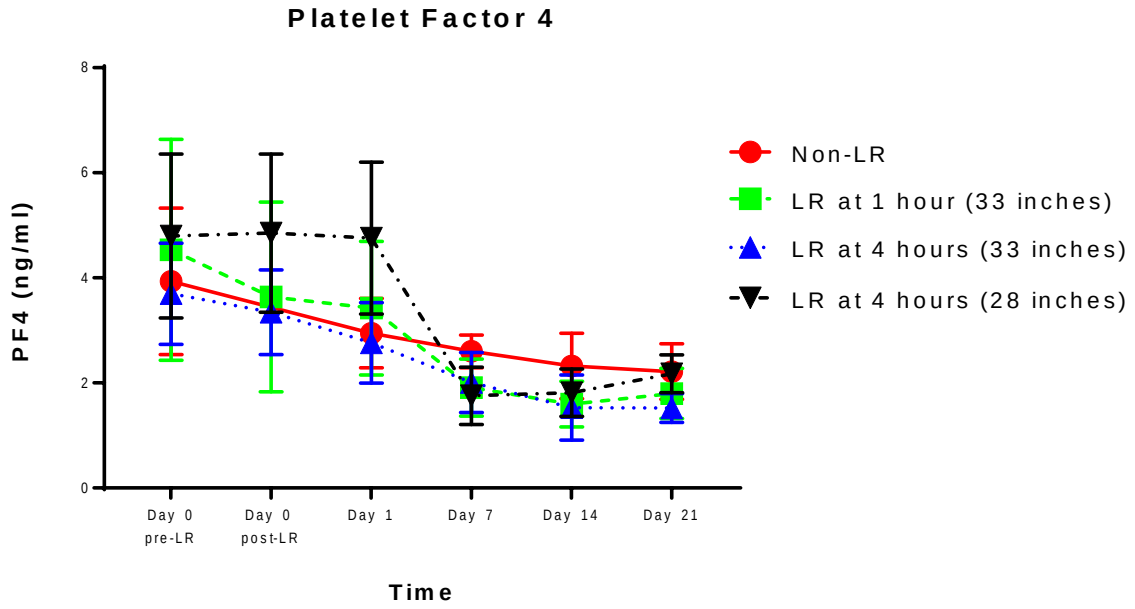

Figure 17. Platelet Factor 4 levels over the study period.

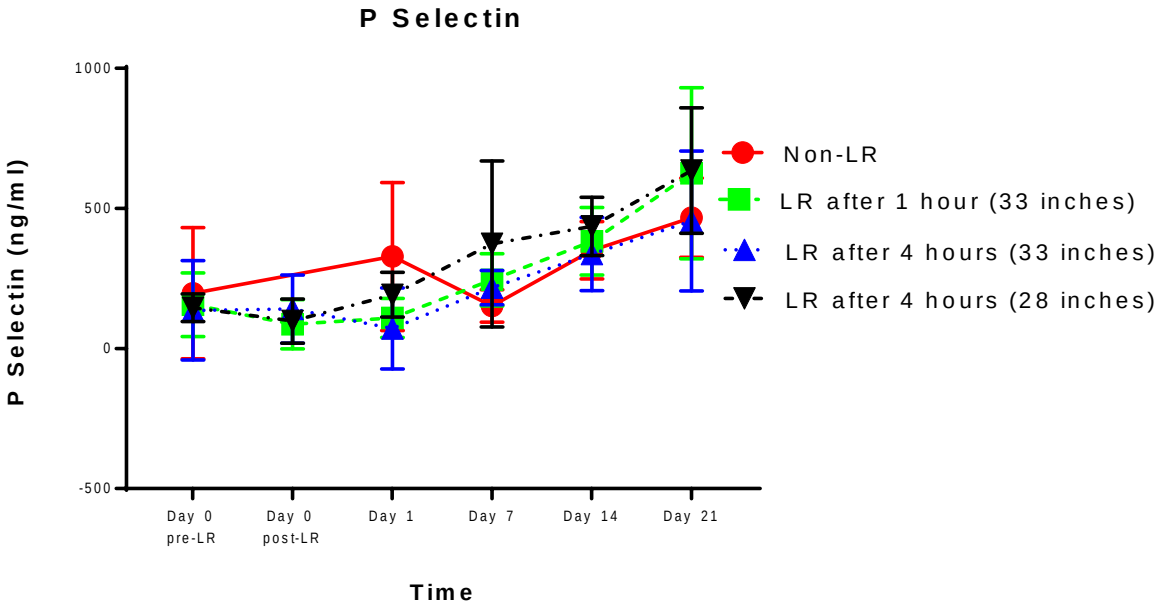

Figure 18. P Selectin levels over the study period.

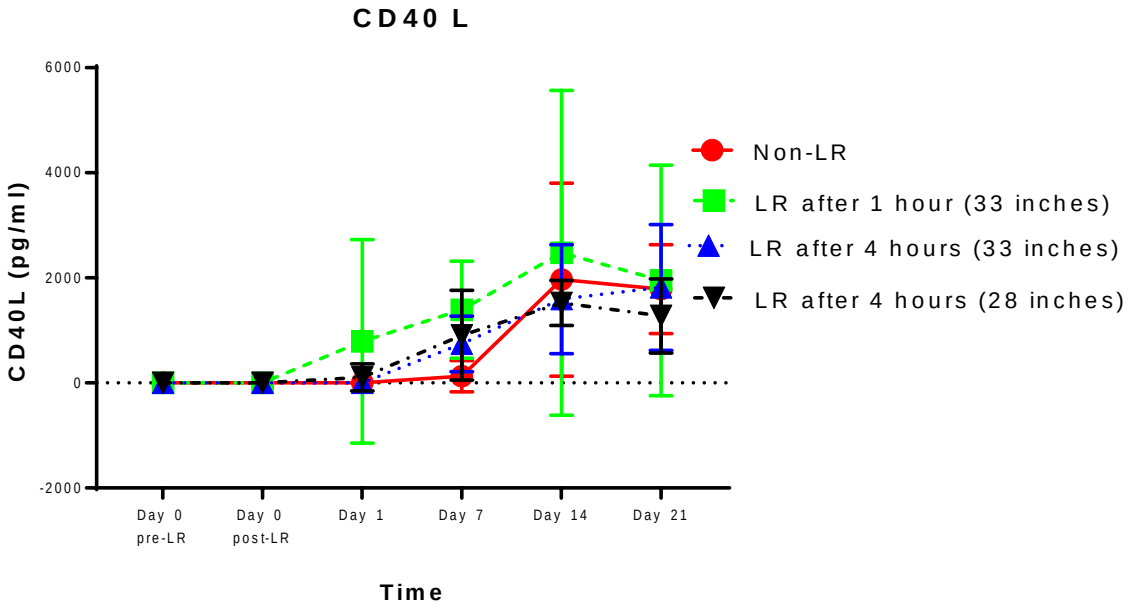

Figure 19. CD40L levels over the study period.

Table 5. Average Platelet and RBC Recovery Following Leukoreduction in Each Group

LR at 1 hour (33 inches)	**69.5 ± 19.9**	**92.7**
LR at 4 hours (33 inches)	90.1 ± 6.3	94.9
LR at 4 hours (28 inches)	73.7 ± 16.7	97.4

5.14 FDA Submission

Date of submission by Terumo BCT: June 5, 2018. Date of acknowledgement of receipt by FDA: June 7, 2018. Screening for completeness of the submission by the FDA is expected to take approximately 60 days (August 7, 2018). Assuming we meet the expectations of the FDA, approval of the product is expected approximately 90 days after that (December 7, 2018). The FDA may come back with additional questions which may result in a longer approval period.

6.0 CONCLUSIONS

While this study had many challenging aspects to its implementation, the end result is having a materiel solution for the end user to be able to leukoreduce WB using a platelet sparing filter in the pre-hospital or forward setting. This capability could increase the potential to improve survival and safety for combat casualties with the most common cause of preventable death – non compressible hemorrhagic shock and allows for adherence to FDA and DoD blood utilization guidelines for leukoreduction.

Once the final environmental Terumo testing phase is completed and submitted to the FDA, this project should lead to an FDA approved, ruggedized packaging, field deployable (light weight, single unit sterile packaging), 36 month shelf-life, cost effective (< $34.00 per unit), and mass producible (manufacturing process established) WB collection and transfusion system that

would allow for the collection of leukoreduced (LR), platelet-sparing WB transfusion in austere and far forward settings.

Testing efforts in Bergen, St Louis and Cincinnati have further validated or clarified methods for the systems use in the deployed setting:

- Gravity filtration (using the tubing length provided by the system) should be employed to preserve maximum platelet count and adhere to leukoreduction guidelines.
- Units should be filtered before storage; attempts at filtration after 7 days of storage resulted in clogging of the filter.
- Cold-stored WB does not need to be agitated since there is no effect of agitation on hemostatic function.
- Storage of WB after filtration is best at 4 C
- Leukoreduction of WB with platelet sparing filter caused a reduction in some measures of hemostatic potential as measured with thromboelastography (TEG) and rotational thromboelastometry (ROTEM). This effect was most evident during the early period of storage (days 0-10)
- Platelet aggregation was measured by using a Multiplate impedance aggregometer and decreased throughout the storage period.
- Thrombin generation was measured using a thrombinoscope and was unaffected by filtration.
- Serum levels of fibrinogen, CD40L, P Selectin and Platelet factor-4 (PF4) were measured by ELISA and were unaffected by filtration.
- Optimal in vitro platelet function measurements or testing is unknown.
- Hemolysis was minimal in blood stored at 4 C to 21 days.
- Platelet loss was greatest during the first 10 days but then leveled out until day 21 of storage.
- There was no relevant change in Hgb, RBC, or HCT over the 21 days of storage.

Since initiation of this development process and as of Sept 2018, 23 civilian trauma centers have initiated WB programs.

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LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

ADP	adenosine diphosphate
AFBP	Armed Forces Blood Program
ANOVA	analysis of variance
APTT	activated partial thromboplastin time
ASBP	Armed Services Blood Program
ASPi	arachidonic acid
AUC	area under the curve
CaCl2	calcium chloride
CBA	Capability Based Assessment
COL	collagen
CPD	citrate-phosphate-dextrose
CT	clotting time
CWB	citrated whole blood
DCR	damage control resuscitation
FCT	Foreign Comparative Testing
FDA	Food and Drug Administration
FWA	Federal Wide Assurance
HCT	hematocrit
Hgb	hemoglobin
ICL	Integrated Capabilities List
INR	international normalized ratio
JCIDS	Joint Capabilities Integration and Development System
LOE	letter of endorsement
LR	leukoreduced
MA	clot strength
MCF	ROTEM clot strength
MCV	mean corpuscular volume
NLR	non-leukoreduced
OEF	Operation Enduring Freedom
OHRP	Office for Human Research Protections

OIF	Operation Iraqi Freedom
PDP	platelet poor plasma
PLT	platelet
PRP	platelet rich plasma
RBC	red blood cells
RDCR	remote damage control resuscitation
REC	Regional Committee for Medical and Health Research Ethics
ROTEM	rotational thromboelastometry
TEG	thromboelastography
THOR	Trauma Hemostasis and Oxygenation Research
TRAP	thrombin receptor activating peptide
TRIM	transfusion related immune modulation
TRL	Technology Readiness Level
WB	whole blood
WBC	white blood cell