Preparation of leukoreduced whole blood for transfusion in austere environments; effects of forced filtration, storage agitation, and high temperatures on hemostatic function

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BACKGROUND: Damage control resuscitation principles advocate the use of blood to treat traumatic hemorrhage. Hemorrhage is a leading cause of preventable death on the battlefield, but making blood components available far forward presents logistical challenges due to shelf life and storage requirements. Whole blood simplifies logistics and enables collection in the field but can cause leukocyte-related transfusion reactions. A field-adapted leukoreduction system must be fast and safe, and storage of whole blood should preserve hemostatic function.

METHODS: Blood was collected using Imuflex WB-SP and leukoreduced at 0, 150, or 300 mm Hg. Additional bags were stored at 4°C for 21 days unagitated, mixed daily, agitated or head-over-heel rotated, at 22°C for 3 days, or 32°C for 2 hours. Hematology, coagulation, CD62P/CD42b, thromboelastography (TEG)/thromboelastometry (ROTEM), and Multiplate was performed.

RESULTS: Filtration time was 35 ± 1, 14 ± 0, and 9 ± 0 minutes at 0, 150, and 300 mm Hg, respectively. One of 10 units at 150 mm Hg and 4 of 11 at 300 mm Hg had residual whole blood cells greater than 5.0×10^6 per unit. One of 11 at 300 mm Hg had platelet recovery of less than 80%. Hemolysis was less than 0.2%. Filtration decreased thromboelastography/thromboelastometry and Multiplate aggregation response. Stored at 4°C, α and MA/MCF moderately decreased regardless of mixing. Significant loss of aggregation response and increased CD62P expression was seen by Day 10. By Day 3, storage at 22°C caused loss of most aggregation. Two-hour storage at 32°C did not significantly affect hemostatic capacity.

CONCLUSION: Forced filtration reduced leukoreduction time, but increased residual whole blood cells reduced hemostatic function. Aggregation response deteriorated early in storage, while viscoelastic assays decreased more gradually. Mixing showed no benefits. (*J Trauma Acute Care Surg.* 2018;84: S93–S103. Copyright © 2018 Wolters Kluwer Health, Inc. All rights reserved.)

LEVEL OF EVIDENCE: Diagnostic study, level IV.

KEY WORDS: DCR; RDCR; whole blood; leukoreduction; Imuflex.

Among the group of potentially salvageable combat fatalities, hemorrhage is the leading cause of death.[1] Damage control resuscitation (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.[2] The application of DCR in the prehospital setting is termed *remote DCR*.[3] Making blood components available for remote DCR presents logistical challenges, particularly with regard to platelets, as these have a limited shelf life of 5 to 7 days and require room temperature storage with constant agitation. The Tactical Combat Casualty Care guidelines and the Trauma Hemostasis and Oxygenation Research Network thus recommend the use of whole blood (WB) as the preferred fluid for traumatic hemorrhagic shock.[3,4] A concern regarding the use of WB for transfusion is that white blood cells are also present in the donated blood. Transfusion of these cells may cause viral transmission and induce inflammatory and immunologically mediated responses, which can cause adverse transfusion-related events. To reduce the risk of transfusion-related immune modulation, standard blood banking procedures now recommend leukoreduction (LR) (reduction of white blood cells) to less than 5.0×10^6 white blood cell count (WBC) per unit of RBCs (US Food and Drug Administration (FDA)) or 1.0×10^6 per unit (Council of Europe).[5]

It is critical that an LR filter for WB preserves platelets and their function, as they are essential for hemostasis. There is currently only one FDA-approved platelet-sparing filter on the market for WB, as part of the Imuflex WB-SP collection set (Terumo BCT; Lakewood, CO, USA). Using this, LR of a 500-mL (US) or 450-mL (Europe) unit of WB can be performed in approximately 40 minutes. While acceptable in a blood bank, such a delay may not be acceptable in a far forward combat scenario or other settings where there is an urgent need for blood. The study evaluated the feasibility of reducing the filtration time

Submitted: November 24, 2017, Revised: February 23, 2018, Accepted: February 27, 2018, Published online: March 14, 2018.

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For publication in the Journal of Trauma and Acute Care Surgery's 2018 Trauma Hemostasis and Oxygenation Research (THOR) supplement. This manuscript has not been published elsewhere and the data not previously presented.

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DOI: 10.1097/TA.0000000000001896

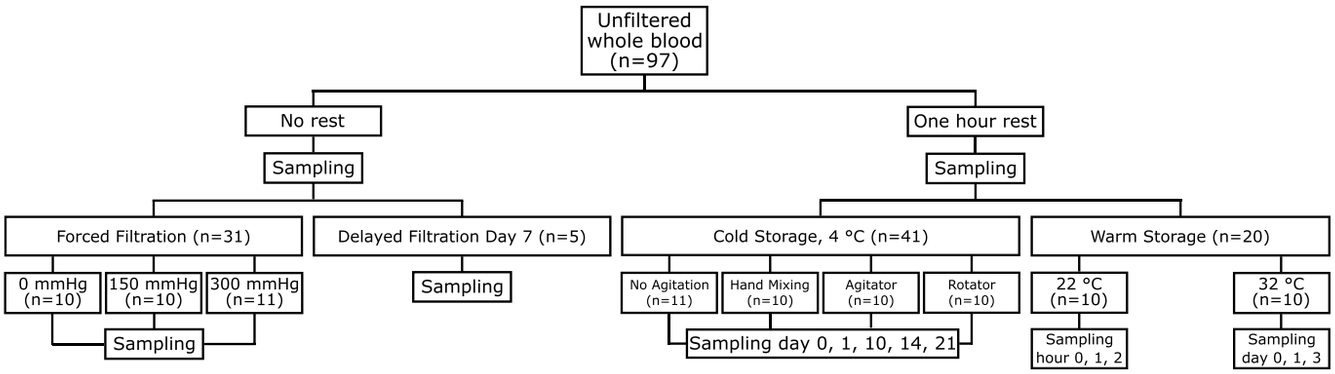

Figure 1. Disposition of units in the study. Whole blood was collected in Imuflex WB-SP collection sets (Terumo BCT, Lakewood, CO, USA) and subjected to different filtration or storage conditions as listed.

by applying pressure to the collection bag and forcing blood through the filter at a higher rate. In addition, the viability of storing the unfiltered blood at 4°C and delaying filtration until Day 7 was investigated.

Whereas platelet concentrates are stored with constant agitation to ensure adequate oxygenation, WB is currently stored unagitated at 4°C. To establish whether agitation or other means of mixing improves the hemostatic function of leukoreduced cold stored WB, the study compared current storage practice to daily mixing by hand, constant side-to-side agitation, and constant head-over-heel rotation over a 21-day period. Due to a lack of refrigeration capability, blood collected far forward may potentially be stored at or above room temperature before being transfused. The study also looked at the effects of 3-day storage at 22°C and 2-hour storage at 32°C.

METHODS

Collection

This project leveraged a currently FDA-approved blood bag system containing a platelet sparing, WB LR filter (Imuflex WB-SP, Terumo BCT) and simplifying its design to make it lighter and easier to use for WB transfusions for military environment. 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 ABO0 types and both sexes were included. The collection set includes secondary bags for component production that were removed before filtration. One hundred three units were collected in total. Three units were excluded due to low collection volume, two due to an incubator malfunction resulting in incorrect storage temperature, and one unit due to an unexpectedly high reduction in platelet count during storage, leaving a total of 97 included units. Figure 1 shows the disposition of units.

Sampling

Sampling was performed by thoroughly mixing the bag by hand and then transferring 30 mL into 150-mL Teruflex transfer bags (Terumo BCT). An additional 8 mL was removed after filtration on Day 0 for bacterial testing of units to be stored at room temperature.

Forced Filtration

Immediately following donation, bags were sampled and filtered with the set extended to 85 ± 2 cm either according to the manufacturer's recommended procedure of gravity-assisted filtration (n = 10), or at 150 mm Hg (n = 10) or 300 mm Hg (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.

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 extended to 85 ± 2 cm. The filtration process was aborted after 2 hours if no flow through the filter was observed. Postfiltration sampling of the filtered volume was then performed and the bag discarded.

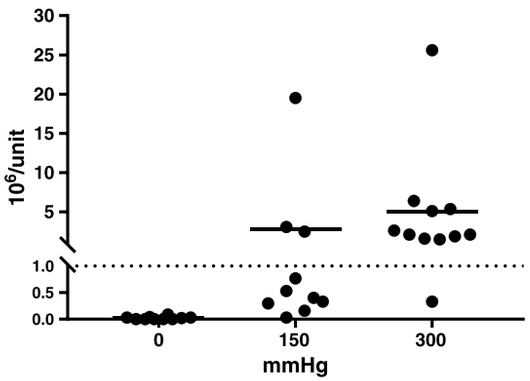

Figure 2. Residual WBC of individual whole blood units after LR with the Imuflex WB-SP filter (Terumo BCT). An irrigation pump was used to apply 0 mm Hg (n = 10, mean = 0.02), 150 mm Hg (n = 10, mean = 2.76, $p = 0.182$) or 300 mm Hg (n = 11, mean = 4.97, $p = 0.043$) pressure. *Solid lines* indicate means. *Dotted line* represents upper limit of 0.1×10^6 per unit recommended by guidelines. The mean values for 150 mm Hg and 300 mm Hg were compared to that of 0 mm Hg (gravity filtration) using the independent samples *t*-test with $\alpha = 0.05$.

TABLE 1. Thromboelastography (TEG) and Thromboelastometry (ROTEM) Values in Whole Blood Before and After Leukoreduction with the Imuflex WB-SP Filter

	0 mm Hg		150 mm Hg		300 mm Hg	
	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
ROTEM 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
ROTEM 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
ROTEM fib-tem						
CT, min	63 ± 14	61 ± 5	60 ± 7	61 ± 9	65 ± 10	58 ± 6*
Angle, degree	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 mm Hg (n = 10) or 300 mm Hg (n = 11) pressure. Reported values are mean ± standard deviation. The independent samples *t*-test was performed to compare change from prefiltration to postfiltration (*$p < 0.050$, **$p < 0.001$) and Δprefiltration-postfiltration at 150 mm Hg and 300 mm Hg to gravity filtration (†$p < 0.050$, ‡$p < 0.001$).

Storage

After a 1-hour hold, bags were sampled and gravity filtered with the set extended to 85 ± 2 cm according to the manufacturer's instructions. A Postfiltration sample was collected and the bags 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 three rounds per minute with no daily mixing (n = 10; SI-1002 Bag Rotator, Scientific Industries, Inc, Bohemia, NY, USA). Sampling was performed on 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.

Bacterial Testing

After 3 days of storage at 22°C, 8 mL was aseptically transferred into aerobic culture bottles (BacT/ALERT FA Plus) and incubated for 7 days using the BacT/ALERT 3D system (bioMérieux SA, Marcy l'Etoile, France). Bacterial testing was not performed on bags stored at 4°C or 32°C.

Hemostatic Function and Aggregation

Hemostatic function was evaluated with kaolin-initiated thromboelastography on a thromboelastography (TEG) 5000 analyzer (Haemonetics Corporation, Braintree, MA, USA). Additionally, thromboelastometry was performed using thromboelastometry (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,

TABLE 2. Storage at 4°C of Nonleukoreduced Whole Blood in Imuflex WB-SP Collection Sets

	Day 0	Day 7
General		
PLT, 10^9/L*	184 ± 28	148 ± 45
HGB, g/dL*	12.9 ± 1.2	12.9 ± 1.2
Hemolysis, %	0.1 ± 0.0	0.1 ± 0.1
K, mmol/L**	3.3 ± 0.1	13.1 ± 1.3
Coagulation		
APTT, s**	34 ± 4	44 ± 5
FVIII, %*	130 ± 30	47 ± 25
TEG		
R, min	6.4 ± 1.0	7.6 ± 1.1
K, min*	1.5 ± 0.3	2.4 ± 0.2
Angle, degree*	68 ± 3	58 ± 3
MA, mm	62.1 ± 5.5	56.4 ± 4.3
ROTEM in-tem		
CT, min*	176 ± 10	230 ± 22
CFT, min*	74 ± 8	154 ± 30
Angle, degree	76 ± 2	66 ± 3
MCF, mm	60 ± 2	55 ± 3
ROTEM ex-tem		
CT, min*	56 ± 5	70 ± 5
CFT, min*	81 ± 7	167 ± 34
Angle	74 ± 2	69 ± 5
MCF, mm*	61 ± 1	54 ± 3
Multiplate		
ADP, U*	49 ± 13	15 ± 4
AA, U*	38 ± 10	12 ± 6
Ristocetin, U*	73 ± 10	31 ± 14
TRAP-6, U**	85 ± 7	18 ± 6
Cytometry		
CD62P, %*	22 ± 14	87 ± 6
CD42b, %*	98 ± 1	75 ± 7

Measurements on Day 0 and Day 7 were compared using a paired sample *t*-test (*$p < 0.05$, **$p < 0.001$).

Mannheim, Germany). Platelets were activated with 6.5-µM adenosine diphosphate (ADP, ADPtest), 0.5 mmol/L of arachidonic acid (AA, ASPItest), 0.77 mg/mL of ristocetin (RISTOtest), and 32 µmol/L of thrombin receptor activating peptide 6 (TRAP-6, TRAPtest). Due to the calcium-depleting effects of CPD, samples for ADPtest and TRAPtest were partially recalcified with 3 mmol/L of $CaCl_2$.

Flow Cytometry

The level of activated platelets and their adhesion capacity was investigated by flow cytometry. A premade antibody mix with monoclonal mouse antihuman antibodies from BD (BD Bioscience; San Jose, CA, USA) with PerCP CD61 (clone EUU-PL 7 F12, cat. no. 347408), APC CD42b (cat. no. 551061), and PE CD62P (cat. no. 561921) was added to 50 µL of blood 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) was added. The samples were run either immediately on a BD FACSCanto II cytometer using the FACSDiva software (Version 8.0.1) (BD Biosciences) or stored at room temperature (dark) if analyzed within 2 hours after preparation. Gating was done with a fixed setup on forward scatter height/area and side scatter, 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.

Hematology and Clinical Chemistry

Platelet count (PLT), white blood cell count (WBC), hemoglobin (Hgb), and hematocrit (HCT) were analyzed in K_2EDTA on a Cell-Dyn Sapphire analyzer (Abbott Diagnostics; Abbott Park, IL, USA). Residual WBC (rWBCs) 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). Plasma was prepared by centrifugation at 1800 G for 10 minutes. Hemoglobin 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, 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, 0.025 mol/L of CaCl2, and STA-Unicalibrator and STA-Liquid Fib (Stago S.A.S; Asnières-sur-Seine, Paris, France).

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 prefiltration and postfiltration values were performed by the 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 mm Hg and 300 mm Hg 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 mean values during storage and between the different study groups were compared using repeated-measures analysis of variance (ANOVA); $p < 0.05$ was considered significant.

Ethical Approval

The study was granted an exemption of ethical approval by the Norwegian Regional Ethical Committee on October 28, 2015 (2015/1916b).

RESULTS

Blood Types and Sex

Measurements before 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 ADP (52 ± 10 vs 43 ± 11; $p < 0.001$), AA (43 ± 9 vs 32 ± 11;

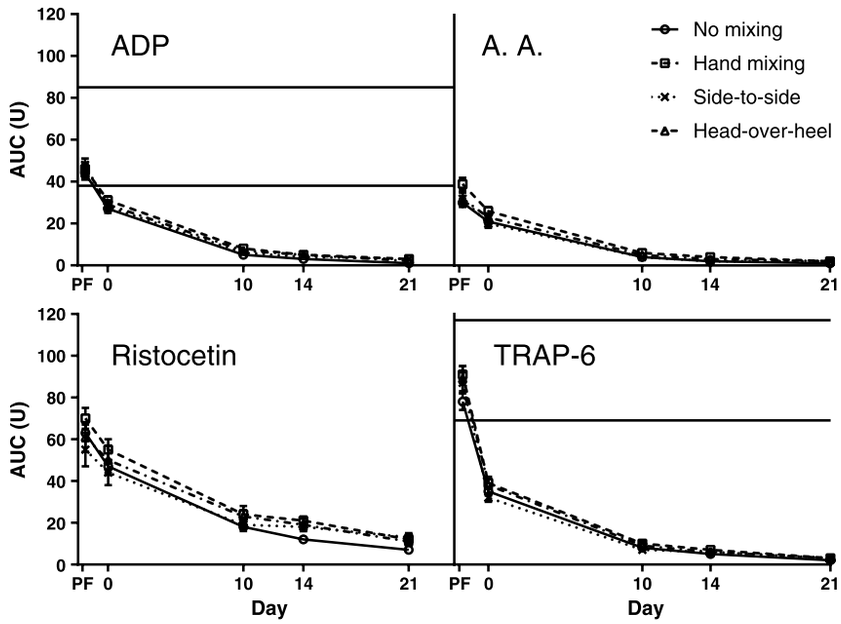

Figure 3. Multiplate (Roche Diagnostics GmbH, Mannheim, Germany) impedance aggregometry response in whole blood leukoreduced with the Imuflex WB-SP filter (Terumo BCT) and stored at 4°C. *PF* indicates response before LR. *Error bars* show standard error of the mean. *Horizontal lines* indicate reference 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, $p = 0.787$; AA, $p = 0.563$; ristocetin, $p = 0.484$; TRAP-6, $p = 0.206$).

$p < 0.001$), and ristocetin (73 ± 18 vs 61 ± 20; $p = 0.009$). The difference for TRAP-6 was not statistically significant (93 ± 15 vs 87 ± 17; $p = 0.131$).

Forced Filtration

Gravity filtration took 35 ± 3 minutes and produced a consistently leukoreduced product (<0.1×10^6 per unit). Filtration at 150 mm Hg reduced filtration time to 14 ± 1 minutes ($p < 0.001$) but resulted in 1 of 10 units with a residual WBC greater than 5.0×10^6 per unit. Increasing pressure to 300 mm Hg further reduced filtration time to 9 ± 1 minutes ($p < 0.001$) but caused 4 of 10 units to exceed 5.0×10^6 per unit. The highest residual WBC observed was 19.5×10^6 per unit at 150 mm Hg and 25.6×10^6 per unit at 300 mm Hg (Fig. 2).

Filtration yielded a platelet recovery of 90% ± 9% at gravity, 84% ± 9% at 150 mm Hg, and 83% ± 15% at 300 mm Hg. One bag filtered at 300 mm Hg had a platelet recovery of 39% but no abnormal prefiltration measurements. The reduction in PLT from filtration was not statistically significantly different between gravity and 150 mm Hg or gravity and 300 mm Hg (-0×10^9/L ± 15 vs -10×10^9/L ± 17; $p = 0.193$ and -11×10^9/L ± 22; $p = 0.203$, respectively). No change in hemolysis, HCT, mean corpuscular volume (MCV), potassium, international normalized ratio, APTT, fibrinogen, or factor VIII was seen at any pressure.

Filtration resulted in reduced Multiplate platelet aggregation response below reference ranges. Thromboelastography showed increased time to initial clot formation (*R*), decreased speed of clot formation (α), and reduced maximum clot strength (MA). A reduction in clot strength was also seen on thromboelastometry (ROTEM MCF). At 300-mm Hg pressure, an increased platelet-inhibited (fib-tem) clot formation speed (α) and decreased time to initial clotting (CT) was observed. Fibrinolysis in the form of a clinically relevant increase in TEG LY30 or ROTEM LI30 was not seen. Table 1 shows detailed data.

Delayed Filtration

A small decrease in PLT from Day 0 to Day 7 was observed. After filtration, 45×10^9/L ± 11×10^9/L remained ($p = 0.003$). Multiplate platelet function was reduced, but remaining. Thromboelastography//thromboelastometry showed decreased hemostatic function in the form of increased clotting time, slower clot generation, and reduced clot strength (Table 2). Filtration on Day 7 was unsuccessful in all units, with less than half of the volume leukoreduced before clogging of the filter.

Cold Storage Conditions

Hemolysis did not exceed a mean of 0.2% on day 21. Platelet loss was greatest during the first ten days, followed by a leveling out. There was no clinically relevant change in Hgb, RBC or HCT over the 21 days. There were no statistically significant differences between the groups. Mean corpuscular volume 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.

International normalized ratio increased from Day 1 to Day 10, and then remained stable until Day 21. Activated partial thromboplastin time increased until Day 10, followed by a decline until Day 21. The increase in APTT from Day 0 to Day 21 was greater in the no-mixing group compared to the head-over-heel group ($p = 0.027$). Fibrinogen levels remained

TABLE 3. Storage at 4°C of Whole Blood Leukoreduced Using the Imuflex WB-SP Filter

	No Agitation					Hand Mixing				
	PF	**Day 0**	**Day 10**	**Day 14**	**Day 21**	**PF**	**Day 0**	**Day 10**	**Day 14**	**Day 21**
General										
PLT, 10^9/L**	160 ± 19	158 ± 23	129 ± 22	127 ± 22	132 ± 30	195 ± 27	189 ± 21	156 ± 21	160 ± 13	158 ± 16
HGB, g/dL	12.9 ± 1	12.8 ± 0.9	12.9 ± 0.9	13 ± 0.9	12.9 ± 0.9	12.8 ± 0.7	12.7 ± 0.7	12.8 ± 0.7	12.7 ± 0.8	12.8 ± 0.7
RBC, 10^{12}/L	4 ± 0.3	3.9 ± 0.3	4 ± 0.3	4 ± 0.4	3.9 ± 0.3	4.1 ± 0.3	4 ± 0.3	4 ± 0.3	4.1 ± 0.3	4 ± 0.3
HCT, %**	0.38 ± 0.03	96 ± 3	0.38 ± 0.02	0.38 ± 0.03	0.39 ± 0.03	0.39 ± 0.02	94 ± 3	0.38 ± 0.02	0.39 ± 0.03	0.39 ± 0.02
MCV, fL**‡	96 ± 3	0.38 ± 0.02	96 ± 3	97 ± 3	98 ± 3	94 ± 3	0.38 ± 0.02	95 ± 3	96 ± 3	97 ± 3
Hemolysis, %**	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.2 ± 0.1	0.1 ± 0	0 ± 0.1	0.1 ± 0	0.1 ± 0	0.2 ± 0.1
K, mmol/L**	3.3 ± 0.2	3.3 ± 0.2	16.1 ± 2.6	18 ± 2.6	22.1 ± 3.2	3.2 ± 0.1	3.2 ± 0.1	17.4 ± 2.9	19.6 ± 2.7	23.2 ± 2.8
Coagulation										
INR**	1 ± 0.1	1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
APTT, s**†	34 ± 3	34 ± 4	42 ± 4	41 ± 4	40 ± 4	36 ± 2	36 ± 2	42 ± 3	41 ± 3	41 ± 3
Fibrinogen, g/dL	2.8 ± 0.4	2.8 ± 0.4	2.7 ± 0.5	2.7 ± 0.4	2.7 ± 0.4	2.6 ± 0.3	2.6 ± 0.4	2.6 ± 0.3	2.6 ± 0.3	2.5 ± 0.3
FVIII, %**	122 ± 32	123 ± 31	45 ± 18	44 ± 20	48 ± 18	131 ± 22	132 ± 20	49 ± 10	46 ± 11	48 ± 13
TEG										
R, min	6.1 ± 1.1	7.6 ± 1.2	7.4 ± 0.6	7.3 ± 1	7.2 ± 0.7	6.2 ± 1.1	8.1 ± 1.6	7.3 ± 1.1	7.9 ± 0.8	8.1 ± 1
K, min**	1.5 ± 0.2	1.8 ± 0.2	3 ± 0.3	3.3 ± 0.5	3.9 ± 0.9	1.5 ± 0.3	1.9 ± 0.4	3.1 ± 0.6	3.3 ± 0.7	4.4 ± 1.2
Angle, degree**	67.3 ± 3.6	61.9 ± 3.2	51.6 ± 2.7	48.6 ± 4.6	47.6 ± 4.9	68.6 ± 2.7	59.2 ± 6.7	50 ± 6.7	46.3 ± 5.4	41.3 ± 7.4
MA, mm**	61 ± 5.9	59.1 ± 4.3	54.2 ± 5	54 ± 4.5	50.5 ± 4.4	64.2 ± 5.5	57.4 ± 4.8	54.6 ± 5.4	52.3 ± 5.1	51.1 ± 4.9
LY30, %*	0.3 ± 0.4	0.8 ± 0.9	0 ± 0	0 ± 0	0 ± 0	1.6 ± 1.3	1.5 ± 2	0.9 ± 2.8	0.4 ± 1.3	0.6 ± 1.7
ROTEM in-tem										
CT, min**	168 ± 15	180 ± 11	211 ± 20	214 ± 19	234 ± 19	167 ± 11	181 ± 11	213 ± 17	210 ± 12	238 ± 21
CFT, min**	81 ± 8	86 ± 13	186 ± 35	226 ± 33	307 ± 60	79 ± 12	83 ± 13	185 ± 46	212 ± 52	299 ± 72
Angle, degree**	74 ± 2	73 ± 2	59 ± 6	57 ± 6	52 ± 8	74 ± 2	74 ± 2	60 ± 6	57 ± 5	48 ± 7
MCF, mm**	58 ± 3	56 ± 3	54 ± 4	51 ± 3	48 ± 4	58 ± 2	56 ± 3	54 ± 3	52 ± 4	48 ± 4
LI30, %**	100 ± 1	99 ± 1	100 ± 0	100 ± 0	100 ± 0	99 ± 1	99 ± 1	100 ± 0	100 ± 0	100 ± 0
ROTEM ex-tem										
CT, min**	64 ± 3	58 ± 5	70 ± 6	73 ± 8	77 ± 9	62 ± 6	64 ± 5	69 ± 7	74 ± 8	77 ± 5
CFT, min**	90 ± 14	95 ± 18	228 ± 42	281 ± 52	395 ± 94	88 ± 11	93 ± 13	232 ± 56	290 ± 72	401 ± 98
Angle, degree**	72 ± 3	71 ± 4	57 ± 10	56 ± 9	52 ± 15	72 ± 2	71 ± 2	55 ± 7	52 ± 7	46 ± 8
MCF, mm**	60 ± 3	58 ± 4	52 ± 4	49 ± 4	45 ± 4	59 ± 2	58 ± 2	52 ± 4	49 ± 4	45 ± 4
LI30, %**	100 ± 1	100 ± 1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
ROTEM fib-tem										
CT, min	57 ± 2	57 ± 4	69 ± 5	69 ± 8	78 ± 7	63 ± 7	61 ± 6	66 ± 8	67 ± 8	108 ± 113
Angle, degree*	65 ± 6	64 ± 5	57 ± 14	60 ± 13	61 ± 7	61 ± 4	62 ± 4	56 ± 6	59 ± 3	54 ± 5
MCF, mm	12 ± 3	12 ± 3	12 ± 3	12 ± 3	12 ± 3	11 ± 2	11 ± 2	11 ± 1	11 ± 2	11 ± 1
LI30, %	100 ± 1	100 ± 1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 1	100 ± 0
Multiplate										
ADP, U**	44 ± 9	27 ± 7	5 ± 2	3 ± 1	1 ± 1	46 ± 10	31 ± 5	8 ± 3	5 ± 2	3 ± 2
AA, U**	30 ± 8	21 ± 7	4 ± 2	2 ± 1	1 ± 1	39 ± 9	26 ± 6	6 ± 2	4 ± 2	2 ± 1
Ristocetin, U**	63 ± 12	47 ± 10	18 ± 7	12 ± 4	7 ± 4	70 ± 17	55 ± 16	24 ± 8	21 ± 8	12 ± 5
TRAP-6, U**	78 ± 13	35 ± 6	8 ± 2	5 ± 2	2 ± 1	91 ± 11	39 ± 7	10 ± 4	7 ± 4	3 ± 2
Cytometry										
CD62P, %**	23.9 ± 12.3	19.2 ± 11.2	72.9 ± 7.7	84 ± 5.1	86.4 ± 4.8	19 ± 5.9	14.9 ± 5.2	76.2 ± 10.6	86.4 ± 4.5	84.6 ± 7.9
CD42b, %**	98 ± 0.8	98.4 ± 0.7	61.7 ± 10	66.5 ± 11	64.8 ± 7.5	99.1 ± 0.7	99.4 ± 0.3	72.1 ± 16.1	70.4 ± 16.2	55.9 ± 12.9

The changes in mean values during storage (*$p < 0.05$, **$p < 0.001$) and between the different study groups (†$p < 0.05$, ‡$p < 0.001$) were compared using repeated-measures ANOVA. PF indicates measurements before LR.

stable throughout in all groups. Platelet-inhibited clot strength (fib-tem MCF) was stable during the whole storage period. International normalized ratio, APTT, and fibrinogen remained within reference ranges throughout the storage period, whereas factor VIII levels went down until Day 10 and then stabilized at approximately 50%.

All groups showed a significant loss of platelet aggregation response by Day 10 (Fig. 3). The greatest change in CD62P and CD42b expressions occurred during the first 10 days. More details are found in Table 3.

Thromboelastography and ROTEM showed a reduction of clot formation speed (α) and maximum clot strength

Side-to-side					Head-over-heel				
PF	Day 0	Day 10	Day 14	Day 21	PF	Day 0	Day 10	Day 14	Day 21
170 ± 26	168 ± 29	135 ± 24	134 ± 21	130 ± 19	163 ± 23	164 ± 29	130 ± 38	135 ± 33	131 ± 32
12.8 ± 0.7	12.7 ± 0.7	12.8 ± 0.8	12.6 ± 0.8	12.7 ± 0.7	13.1 ± 0.6	13.1 ± 0.7	13.2 ± 0.7	13.3 ± 0.7	13.1 ± 0.7
4.1 ± 0.2	4 ± 0.2	4.1 ± 0.3	4.1 ± 0.3	4.1 ± 0.3	4.2 ± 0.2	4.1 ± 0.3	4.2 ± 0.2	4.2 ± 0.2	4.2 ± 0.2
0.39 ± 0.02	95 ± 4	0.38 ± 0.02	0.39 ± 0.02	0.39 ± 0.02	0.39 ± 0.02	95 ± 3	0.39 ± 0.02	0.4 ± 0.02	0.4 ± 0.02
95 ± 4	0.38 ± 0.02	94 ± 4	94 ± 4	95 ± 5	95 ± 3	0.39 ± 0.02	94 ± 3	95 ± 4	96 ± 4
0.1 ± 0	0.1 ± 0	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0
3.3 ± 0.2	3.3 ± 0.2	17.8 ± 3.6	20.3 ± 3.3	24 ± 3.5	3.4 ± 0.3	3.4 ± 0.3	17 ± 2.7	19.5 ± 2.7	23.5 ± 2.9
1 ± 0.1	1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1 ± 0.1	1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
34 ± 2	34 ± 2	40 ± 2	40 ± 3	39 ± 3	34 ± 3	34 ± 3	40 ± 5	38 ± 5	37 ± 6
2.7 ± 0.4	2.7 ± 0.4	2.7 ± 0.3	2.7 ± 0.4	2.7 ± 0.3	2.8 ± 0.4	2.9 ± 0.4	2.9 ± 0.4	2.9 ± 0.4	2.9 ± 0.5
141 ± 6	143 ± 13	52 ± 6	50 ± 6	64 ± 14	126 ± 27	126 ± 25	47 ± 16	49 ± 16	54 ± 22
6.2 ± 1	7.9 ± 0.9	7.2 ± 0.9	7.5 ± 1	7.7 ± 1.3	6.3 ± 0.8	7.1 ± 1.3	7.6 ± 1	7.5 ± 0.8	8.2 ± 1.3
1.5 ± 0.3	1.8 ± 0.2	2.8 ± 0.4	3.4 ± 0.4	4.3 ± 1	1.5 ± 0.2	1.8 ± 0.3	3.6 ± 1.5	3.5 ± 1	4.7 ± 1.9
67.3 ± 3.6	62.8 ± 2.6	51.2 ± 6.5	49.9 ± 4.3	44.6 ± 6.1	67.8 ± 3.4	64.2 ± 2.9	48 ± 9	47.6 ± 7.2	42.1 ± 7.1
61.3 ± 3.7	56 ± 3.9	53.1 ± 2.3	52.5 ± 4	49.5 ± 4	61.7 ± 5.2	59 ± 5.4	55.2 ± 7.4	54.6 ± 7.2	49.7 ± 7.8
0.7 ± 0.6	2.3 ± 2.8	0.2 ± 0.4	0 ± 0	0 ± 0	1.3 ± 1.2	1.3 ± 1.9	0 ± 0	0 ± 0	0 ± 0
170 ± 10	181 ± 8	204 ± 20	214 ± 18	224 ± 15	172 ± 13	181 ± 13	209 ± 16	213 ± 19	247 ± 52
78 ± 6	83 ± 9	186 ± 22	232 ± 41	303 ± 60	77 ± 9	84 ± 15	239 ± 161	270 ± 153	435 ± 317
74 ± 1	74 ± 2	62 ± 6	57 ± 6	52 ± 7	75 ± 2	74 ± 3	58 ± 12	55 ± 12	46 ± 17
59 ± 2	57 ± 3	53 ± 3	51 ± 4	48 ± 5	60 ± 2	57 ± 2	52 ± 8	49 ± 8	46 ± 10
100 ± 0	100 ± 1	100 ± 0	100 ± 0	100 ± 0	99 ± 1	99 ± 2	100 ± 0	100 ± 0	100 ± 0
62 ± 3	65 ± 4	73 ± 12	72 ± 6	79 ± 10	62 ± 5	65 ± 5	70 ± 6	69 ± 9	78 ± 10
88 ± 8	91 ± 10	225 ± 25	296 ± 41	407 ± 73	85 ± 12	90 ± 15	278 ± 201	304 ± 156	471 ± 257
72 ± 2	72 ± 2	60 ± 6	56 ± 8	51 ± 12	73 ± 2	72 ± 3	58 ± 14	58 ± 13	50 ± 20
60 ± 2	58 ± 2	52 ± 2	49 ± 3	44 ± 4	61 ± 2	59 ± 3	51 ± 9	49 ± 8	45 ± 9
100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	99 ± 1	100 ± 0	100 ± 0	100 ± 0
61 ± 5	62 ± 5	68 ± 10	70 ± 10	72 ± 6	59 ± 6	59 ± 3	66 ± 11	70 ± 8	77 ± 10
66 ± 6	66 ± 5	63 ± 10	62 ± 8	62 ± 7	67 ± 7	63 ± 8	68 ± 5	61 ± 10	62 ± 4
12 ± 2	12 ± 2	13 ± 3	12 ± 2	12 ± 2	13 ± 3	13 ± 2	13 ± 3	13 ± 4	12 ± 4
100 ± 1	100 ± 0	100 ± 0	100 ± 0	100 ± 0	99 ± 2	99 ± 1	100 ± 0	100 ± 0	100 ± 0
48 ± 11	28 ± 6	6 ± 2	4 ± 2	3 ± 2	44 ± 8	29 ± 8	7 ± 4	5 ± 2	2 ± 2
32 ± 8	20 ± 7	4 ± 2	2 ± 2	2 ± 1	32 ± 8	23 ± 7	5 ± 3	3 ± 2	2 ± 2
55 ± 26	44 ± 18	19 ± 6	18 ± 6	13 ± 6	61 ± 22	50 ± 22	23 ± 16	19 ± 11	11 ± 8
87 ± 14	32 ± 6	7 ± 3	6 ± 4	3 ± 2	89 ± 17	38 ± 12	9 ± 6	6 ± 4	3 ± 2
24.8 ± 9.1	20.8 ± 8.2	80.8 ± 8.6	85.5 ± 7.9	85.6 ± 5.3	13.1 ± 4.5	9.3 ± 3.3	76.3 ± 13.7	81.6 ± 5.8	85.1 ± 6.3
99.1 ± 0.2	99.3 ± 0.2	64 ± 11.8	67.4 ± 10.8	60.2 ± 9.8	97.5 ± 1.4	97.7 ± 1.6	70.8 ± 12.8	66.4 ± 11.8	65.3 ± 11.2

(MA/MCF) during storage, falling below reference ranges by Day 10. Thromboelastometry additionally showed an increase in time to first clot formation (Fig. 4). There was no clinically relevant level of fibrinolysis (LY30/LI30). Measurements were not statistically significantly different between the groups.

Storage at or Above Room Temperature

Storage at 22°C did not cause hemolysis beyond 0.1%. There was no clinically relevant change in PLT, Hgb, or RBC over the 3-day period. Hematocrit and mean corpuscular volume showed a small increase not seen with cold storage. Potassium increased at a slower rate than for cold storage, and peaked

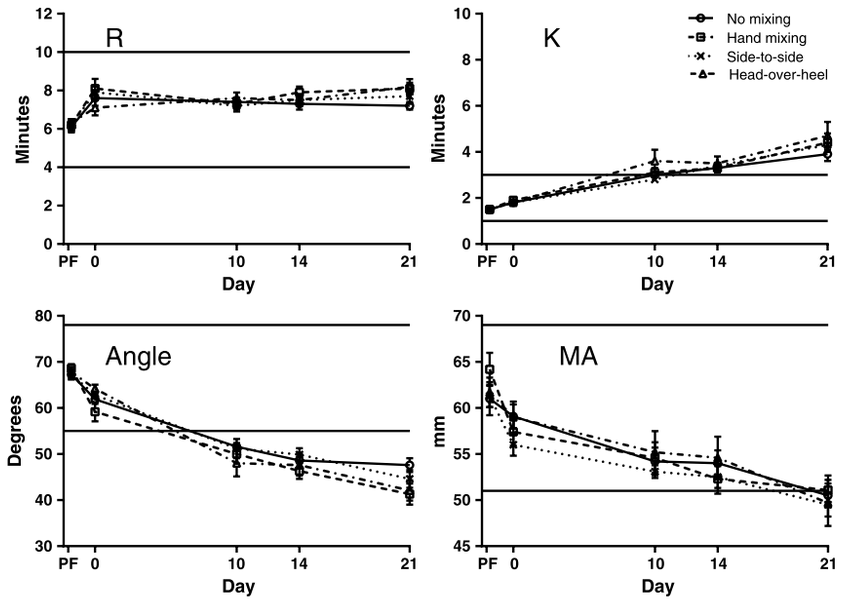

Figure 4. Thromboelastography (TEG; Haemonetics Corporation; Braintree, MA, USA) response in whole blood leukoreduced with the Imuflex WB-SP filter (Terumo BCT) and stored at 4°C. *PF* indicates response before LR. *Error bars* show standard error of the mean. *Horizontal lines* indicate reference range. 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, $p = 0.755$).

at 6.9 ± 0.8 mmol/L on Day 3 compared to 8.2 ± 1.1 mmol/L in the cold storage group ($p = 0.011$). Results of bacterial tests were negative for all units.

International normalized ratio increased marginally from Day 0 to Day 3, while APTT and fibrinogen showed no statistically significant change. 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 less in the 22°C group compared to the 4°C group (125% ± 25% to 76% ± 19% vs 131% ± 24% to 48% ± 15%; $p < 0.001$).

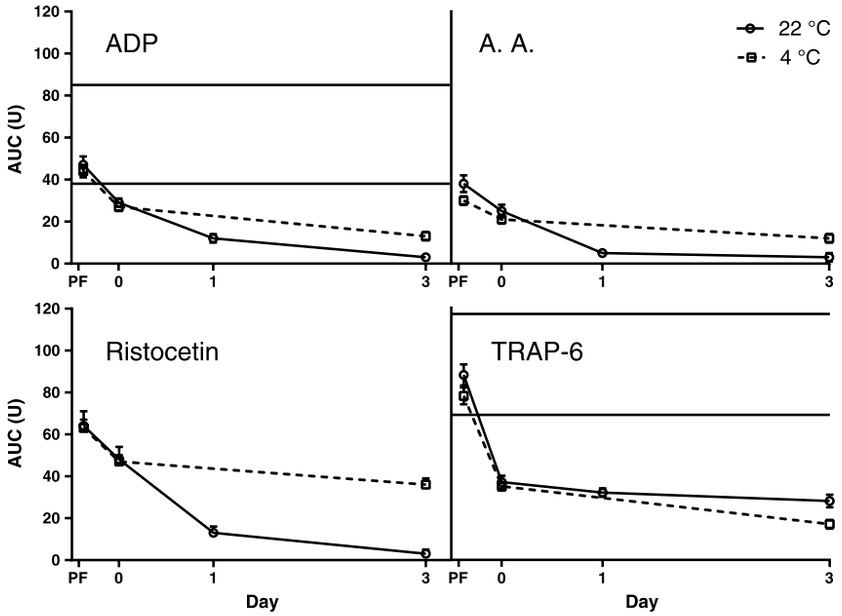

Figure 5. Multiplate (Roche Diagnostics GmbH) impedance aggregometry response in whole blood leukoreduced with the Imuflex WB-SP filter (Terumo BCT) and stored at 22°C. *PF* indicates response before LR. *Error bars* show standard error of the mean. *Horizontal lines* indicate reference range for citrated 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 ($p = 0.001$). Thrombin receptor activating peptide–induced aggregation was better preserved at 22°C ($p = 0.002$).

Most aggregation responses to ADP, AA, and ristocetin were lost on day 3 (Fig. 5). The loss was greater than in the cold storage group, with the biggest difference observed for ristocetin. Thrombin receptor activating peptide response remained relatively stable and was higher than the cold group on Day 3. Storage at 22°C (Fig. 6) 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 at 22°C but not at 4°C. Fibrinolysis (LY30/LI30) was clinically insignificant. Table 4 shows detailed data.

At 32°C, a minor decrease in aggregation response to ADP and AA along with a minor increase for TRAP-6 was observed from after filtration to Hour 2 of storage. A greater reduction was seen for ristocetin. A slight increase in time to initial clot formation was seen on TEG and ROTEM in-tem. The number of platelets expressing CD62P was halved at Hour 2. No other parameters were affected. Table 5 shows detailed data.

DISCUSSION

The effects of LR on WB have been researched previously,[6–8] but no studies on forced LR are published. This is easily explained by the fact that collection and production of blood products usually is 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 while maintaining a platelet recovery within US requirements (>80%) and not causing increased hemolysis. Increasing filtration pressure resulted in minor LR failure at 150-mm Hg pressure and major at 300 mm Hg (Fig. 2), but 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 mm Hg 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.

The clinical relevance of the changes seen on viscoelastic tests and aggregometry, if the product is transfused immediately after filtration, is uncertain. Platelet function measured by Multiplate aggregometry seems to be affected negatively by filtration to an extent that does not seem to correlate with TEG/ROTEM, CD62P-expression, or light transmission aggregometry.[8] 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 to around the lower reference ranges. The rate of reduction mirrors that previously shown.[9] Storage mixing conditions demonstrated no benefit to manipulation or mixing of WB bags through the 21-day cold storage period, neither for platelet content or function, hemolysis, or Hgb

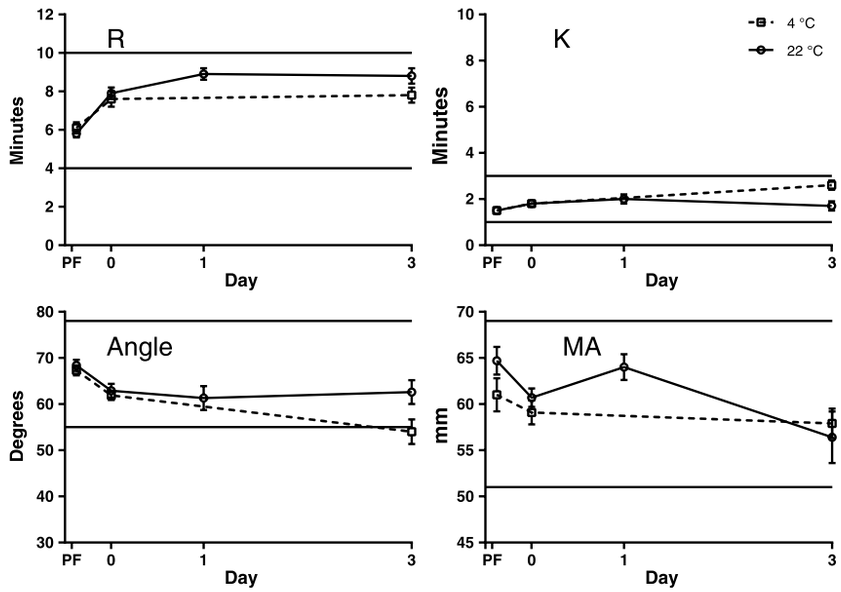

Figure 6. Thromboelastography (TEG) response in whole blood leukoreduced with the Imuflex WB-SP filter and stored at 22°C. *PF* indicates response prior to LR. *Error bars* show standard error of the mean. *Horizontal lines* indicate reference range. Repeated-measures ANOVA showed an increase in *K* ($p = 0.012$) 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 4. Storage at 22°C of Whole Blood Leukoreduced Using the Imuflex WB-SP Filter

	Prefiltration	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
FVIII, %	122 ± 26	125 ± 25	95 ± 27	76 ± 19
TEG				
R, min	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, degree	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, degree	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, degree	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, degree	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

Measurements on Day 1 and Day 3 were compared to Day 0 using a paired sample *t*-test (*$p < 0.05$, **$p < 0.001$).

content. Additionally, Stubbs et al.[10] have previously shown that orbital agitation does not improve the loss of collagen-induced aggregation. While the starting point at storage on Day 0 is lower, the function of leukoreduced WB does not seem to degrade faster than what previously has been shown in nonleukoreduced blood.[11] 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.[12] 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.

TABLE 5. Storage at 32°C of Whole Blood Leukoreduced Using the Imuflex WB-SP Filter

	Prefiltration	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
FVIII, %	124 ± 24	124 ± 22	123 ± 23	125 ± 23
TEG				
R, min	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, degree	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, degree	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, degree	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, degree	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

Measurements at Hour 1 and Hour 2 were compared to Hour 0 using a paired sample *t*-test (*$p < 0.05$, **$p < 0.001$).

Storing the leukoreduced blood at 22°C seems to cause a complete loss of aggregation response to ADP, AA, and ristocetin after the first day. However, ambient temperature storage preserved TRAP-induced aggregation and hemostatic function better. Two-hour storage at 32°C did not induce any notable reduction in quality.

Any clinical significance of these in vitro findings can only be resolved by clinical studies.

AUTHORSHIP

P.C.S., W.D., and E.K.K. designed the study. J.S., H.B., and T.H.F.L. contributed to study design and performed the research. J.S., T.O.A., and E.K.K. analyzed the data. J.S. wrote the article with contributions, critical revision, and approval of the manuscript from all authors.

ACKNOWLEDGMENTS

Office of the Secretary of Defense Comparative Technology Office/Air Force Foreign Comparative Testing, Air Force Research Laboratory, United States Air Force School of Aerospace Medicine/Aeromedical Research Department, University of Cincinnati, Terumo BCT and BloodSolutions, LLC. We also acknowledge the personnel at Department of Immunology and Transfusion Medicine, Haukeland University Hospital for their valuable contribution to this study.

DISCLOSURE

The authors declare no conflicts of interest. Funding by USAFSAM was funneled by the University of Cincinnati.

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