



FOOD AND DRUG ADMINISTRATION
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

MEMORANDUM

Mid-Cycle CMC Review

To: File (BN110059) & Kelly Sonday

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Through: Timothy Lee, Ph.D., Acting Chief, LH/DH/OBRR

Subject: Mid-cycle Review of CMC information in HEMERUS LEUKOSEP® HWB-600-XL Leukocyte Reduction Filtration System for Whole Blood with CPD Anticoagulant and SOLX® Additive
Quality of plasma prepared from WB -----(b)(4)-----

Sponsor: Hemerus Medical, LLC

Mid-cycle due date: March 29, 2012

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1. Executive Summary

The subject of this review is the plasma quality characterization study submitted by Hemerus Medical, LLC in support of the use of their new LEUKOSEP® HWB-600-XL Leukocyte Reduction Filtration System for Whole Blood with CPD Anticoagulant and SOLX® Additive. The filter is designed for room temperature (RT) operation and whole blood is held at RT before separation into red blood cells and plasma.

Passage of plasma through large surface area leuko-reduction filter at room temperature may lead to the following events that may negatively affect the quality of plasma:

- 1) Adsorption of coagulation proteins to the filter

- 2) Degradation of coagulation proteins due to exposure to blood cells at room temperature
- 3) Denaturation of coagulation proteins by shearing action through the filter
- 4) Activation of coagulation by the surface of the filter
- 5) Activation of coagulation by blood cells at room temperature

These reactions may lead to degradation or denaturation of pro- and anti-coagulant proteins and activation of coagulation.

The results of the characterization study demonstrated that the quality of the plasma is not negatively affected during the passage of whole blood through the LEUKOSEP® HWB-600-XL filter at room temperature. In particular,

- 1) The quality of plasma *before* and *after* leukoreduction are comparable (paired study);
- 2) The quality of plasma after filtration through the Hemerus filter (*Test, FFP*) and through the licensed (b)(4) filter (*Control, FFP*) are comparable (un-paired study);
- 3) The quality of FFP and ----(b)(4)--- prepared with the Hemerus system are comparable within a 20% margin of error (un-paired study). -----

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Conclusion:

 ----- (b)(4) -----

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2. Introduction: ----- (b)(4) -----

Currently, licensed human Fresh Frozen Plasma (FFP) can be obtained either from apheresis or from whole blood and must be frozen within 8 hours after collection in accordance with the CFR.

The strict 8-hour time limit between collection and freezing of the plasma imposes certain constraints on the logistics of manufacture. For example, mobile collection units without freezer capacity are limited to a small area around the freezer bank because they should travel to collect plasma and then bring it back for freezing within 8 hours.

However, the quality of plasma is known to change when stored in liquid form: some coagulation proteins are known to deteriorate and/or be activated. The rate of deterioration may be negligible for some plasma proteins over a 24-hour period. -----
 ----- (b)(4) -----

1. **Biocompatibility study** was designed to assess the effects of the collection system components (tubing, bags, solutions, and filters) on blood and plasma.

Biocompatibility and physicochemical testing was conducted according to 21 CFR Part 58 – Good Laboratory Practice for Non-Clinical Laboratory Studies. Microscopic Particle Count Testing was conducted according to 21 CFR Part 820 Good Manufacturing Practices.

Reviewer note: at the request of the Chairperson, Dr. Xuan Chi, I reviewed three plasma related biocompatibility studies: the Prothrombin Time Assay study (Appendix 4-8) and sister studies Complement Activation Assay (Appendix 4-9) and Unactivated PTT Assay (Appendix 4-10). I found these studies unsatisfactory (see below).

2. Main **plasma and blood product quality study** was designed to *assess the quality of plasma product*, -----(b)(4)-----

The study included comparison of plasma characteristics under various conditions:

- plasma obtained before and after passage through leukoreducing filter (*effect of leukoreduction*)
- plasma obtained from WB held at room temperature for 2, 6 ----(b)(4)----- (*effect of hold time*)
- plasma obtained with a currently licensed leukoreduction system (-(b)(4)--, frozen within 8 hrs after collection) -----(b)(4)-----

- plasma stored frozen for 3, 6 and 12 months (*stability program*). NOTE: Only 3-month data are presented in full, 6-month data are presented partially and 12-month data are not presented. Collection of additional stability data for frozen plasma stored up to 12 months is on-going and will be completed and submitted to FDA during the NDA review.

Below is the design of the plasma quality study:

Investigational and Control Products:

- The investigational product was the Hemerus LEUKOSEP® HWB-600-XL Leukocyte Reduction Filtration System for Whole Blood with CPD Anticoagulant and SOLX® Additive (also called SOLX® System).
- The control product was the CPD/AS-1 whole blood collection set with integral --(b)(4)--- whole blood leukoreduction filter -----(b)(4)-----.

Three lots of Hemerus LEUKOSEP® HWB-600-XL leukocyte reduction filters were used during the clinical trial; lots -----(b)(4)-----

For added traceability purposes, each filter in each lot was also identified with a unique serial ID number.

Frozen Plasma Quality Study groups:

Three processing groups of 60 whole blood units each were studied using the SOLX[®] System. Sixty units of control whole blood units were also tested under the processing conditions of Group 2. Processing groups are summarized below:

- Group 1 (FFP8hrRT-WB2hr): Up to two hour room temperature hold prior to whole blood filtration and processing at room temperature (60 test units). RBCs refrigerated and plasma frozen within 8 hours.
- Group 2 (FFP8hrRT-WB6hr): Greater than six hour room temperature hold prior to whole blood filtration and processing at room temperature within eight hours (60 test and 60 control units). RBCs refrigerated and plasma frozen within 8 hours.
- -----(b)(4)-----.
- CONTROL for Group 2 (CONTROL-FFP8hrRT-WB6hr): Greater than six hour room temperature hold prior to whole blood filtration and processing at room temperature within eight hours (60 test and 60 control units). RBCs refrigerated and plasma frozen within 8 hours.

Plasma processing:

Each whole blood unit was leukoreduced with the filter integral to the system (Test or Control) after the designated room temperature hold time. Leukoreduced whole blood units were processed using a room temperature, hard-spin method. After centrifugation, units were processed into SOLX[®] RBC or AS-1 RBC and platelet poor plasma (PPP). Plasma was frozen at -18°C or below. Samples for evaluation were taken at various testing time points according to the study protocol.

Laboratory testing of plasma products:

An extensive panel of plasma parameters was evaluated during the SOLX[®] System clinical study. Hoxworth Blood Center coordinated handling and shipping of all frozen plasma samples to the centralized testing laboratory, -----(b)(4)----- . If possible, plasma testing was performed by (b)(4). In some instances, assays not offered by (b)(4) were forwarded by (b)(4) to specialized testing facilities. All -----(b)(4)----- maintain CLIA certification.

Table 5-4 lists plasma assays performed for the study including general methodologies and assay regulatory status. *Note: only a subset of these tests was performed on certain groups of the samples, see note below.*

Table 5-4 Plasma Assay Methodology and Regulatory Status

Assay Name	General Methodology	Assay Status D* (Diagnostic) or RUO**
Total Protein	----- (b)(4) -----	D
IgG	----- (b)(4) -----	D
PT with INR	----- (b)(4) -----	D

Assay Name	General Methodology	Assay Status D* (Diagnostic) or RUO**
aPTT	----- (b)(4) -----	D
Fibrinogen	---- (b)(4) -----	D
Factor V Activity	--- (b)(4) -----	D
Factor VII Activity	--- (b)(4) -----	D
Factor VIII Activity	--- (b)(4) -----	D
Factor IX Activity	--- (b)(4) -----	D
Factor X Activity	--- (b)(4) -----	D
Factor XI Activity	--- (b)(4) -----	D
Factor XII Activity	--- (b)(4) -----	D
D-Dimer	---- (b)(4) -----	D
Protein C Activity	--- (b)(4) -----	D
Protein S Activity	--- (b)(4) -----	D
Antithrombin III (ATIII)	---- (b)(4) -----	D
ADAMTS 13	--- (b)(4) -----	RUO
vonWillebrand Factor Activity (vWF/Rco)	-(b)(4)-	D
Thrombin/Antithrombin Complex	-(b)(4)-	D
PF1.2	(b)(4)	D
Fibrin Monomer	---- (b)(4) -----	D
Fibrinopeptide A	-(b)(4)-	RUO
Factor VIIa	-(b)(4)-	RUO
Complement C3a	(b)(4)	RUO
Complement C5a	(b)(4)	RUO

* “Diagnostic” means an assay cleared or approved by FDA for diagnostic purposes.

** Research Use Only (RUO) means assays that have not been evaluated by FDA for performance claims or diagnostic purposes.

These coagulation tests can be considered as four functionally separate groups:

1. Extrinsic and intrinsic coagulation assays (seconds)
 - Prothrombin Time (PT)
 - Activated Partial Thromboplastin Time (aPTT)
2. Coagulation factors (activity, % of normal level or international units, 100%=1IU/mL):
 - Factor V (FV, ---- (b)(4) -----)
 - Factor VII (FVII, ---- (b)(4) -----)
 - Factor VIII (FVIII, ---- (b)(4) -----)
 - Factor IX (FIX, ---- (b)(4) -----)
 - Factor X (FX, ---- (b)(4) -----)
 - Factor XI (FXI, ---- (b)(4) -----)
 - Factor XII (FXII, ---- (b)(4) -----)
 - von Willebrand Factor (--- (b)(4) -----)

- Fibrinogen
- ADAMTS 13 (---(b)(4)-----)
- 3. Coagulation inhibitors (---(b)(4)-)
 - Protein C (PC, ---(b)(4)-----)
 - Protein S (PS, ---(b)(4)-----)
 - Antithrombin III (ATIII, ---(b)(4)-----)
- 4. Markers of activation
 - Activated Factor VII (FVIIa, --(b)(4)-)
 - Thrombin-Antithrombin complex (TAT, --(b)(4)-)
 - Prothrombin fragment 1.2 (--(b)(4)-)
 - Fibrinopeptide A
 - Fibrin monomer

Note: Full panel of coagulation tests was performed on some of the samples. However, some samples were tested with a severely abbreviated panel, e.g., pre-filtration Groups 2 and 3; see Table 5-8 below.

Table 5-8 Plasma Testing Study PC387580

Post-Processing Platelet Poor Plasma All Units	Pre-filtration Groups 2 and 3	Postfiltration- Post Processing Groups 2 and 3	3 / 6 /12 Months Frozen Stability Groups 2 and 3
Presample Gross Weight	PT	Total Protein	Total Protein
Residual WBC Count	aPTT	IgG	IgG
CBC	Factor VIII Activity	Albumin	Albumin
Plasma Hemoglobin	2 aliquots (1mL each) frozen for possible later testing.	PT with INR	PT with INR
Time Plasma Frozen		aPTT	aPTT
		Fibrinogen	Fibrinogen
		Factor V Activity	Factor V Activity
		Factor VII Activity	Factor VII Activity
		Factor VIII Activity	Factor VIII Activity
		Factor IX Activity	Factor IX Activity
		Factor X Activity	Factor X Activity
		Factor XI Activity	Factor XI Activity
		Factor XII Activity	Factor XII Activity
		D-Dimer	D-Dimer
		Protein C Activity	Protein C Activity
		Protein S Activity	Protein S Activity
		Antithrombin III (ATIII)	Antithrombin III (ATIII)
		ADAMTS 13	ADAMTS 13
		von Willebrand Factor Activity (vWF/Rco)	von Willebrand Factor activity (vWF/Rco)
		Thrombin/Antithrombin complex PF1.2	Thrombin/Antithrombin complex PF1.2
		Fibrin Monomer	Fibrin Monomer
		Fibrinopeptide A	Fibrinopeptide A
		Factor VIIa	Factor VIIa
		Complement C3a and C5a	

Investigational sites:

The study, sponsored by Hemerus Medical, was conducted at three United States investigational sites under Investigational New Drug Application 14199. Site name, principal investigator and Institutional Review Board (IRB) are summarized in Table 5-5.

Site Name	Principal Investigator	IRB Approving Study
Hoxworth Blood Center University of Cincinnati	Jose Cancelas-Perez, M.D., Ph.D. Research Division Director	University of Cincinnati Medical Institutional Review Board Institutional Review Board Office University Hall Suite 300 51 Goodman Drive Cincinnati, Ohio 45221
Dartmouth-Hitchcock Medical Center	Larry Dumont, Ph.D. Director, Dartmouth-Hitchcock Medical Center Cell Labeling Laboratory	Dartmouth-Hitchcock Medical Center Committee for the Protection of Human Subjects 11 Rope Ferry Road Hanover, NH 03755-1404
American Red Cross Mid- Atlantic Research Facility	Lou Ann Young Maes, M.D., M.S. Medical Director ARC Mid- Atlantic Blood Services	----- ----- ----- (b)(4) ----- ----- -----

The LEUKOSEP® HWB-600-XL System clinical study, conducted under protocol PC387580, was initiated in March 2010 and the last subject was enrolled into the trial on March 23, 2011. **For frozen plasma, three-month and six-month data are presented.** Collection of additional stability data for frozen plasma stored up to 12 months is on-going and will be completed and forwarded to FDA during the NDA review process.

(b)(4)

CMC review (Mikhail Ovanesov): Hemerus' -----(b)(4)----- 3/27/12

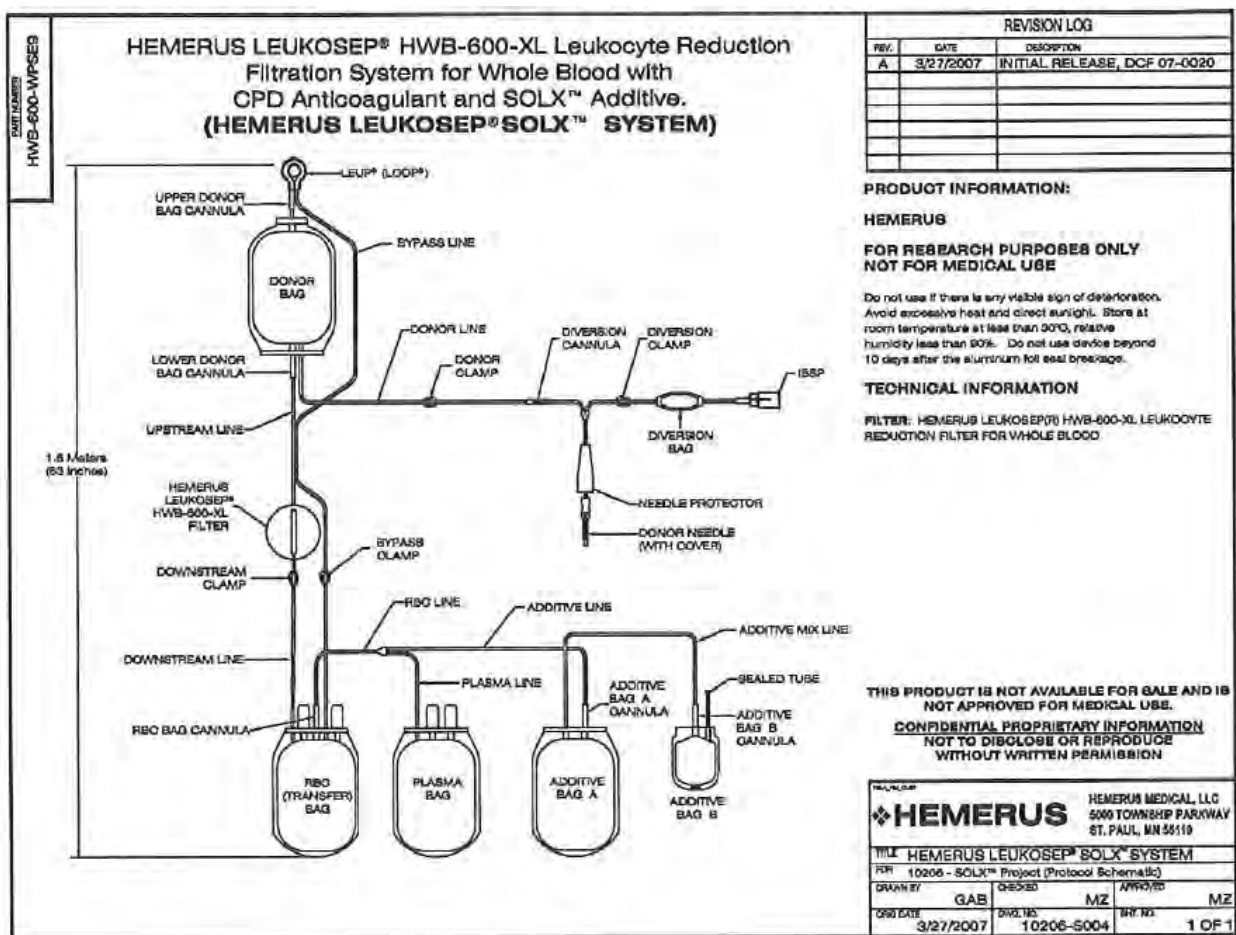


Table 4-2 below summarizes biocompatibility and physicochemical testing performed for the SOLX® System. --(b)(4)---, the contract testing facility, maintains a Quality System compliant with GLP and GMP regulations and is accredited to ISO/IEC 17025 General Requirements for the Competence of Testing and Calibration Laboratories.

Table 4-2 Biocompatibility Testing Summary for SOLX® System

Test	Method	Purpose	Results	Report Number Appendix
Cytotoxicity	ISO 10993-5	Determine the biological reactivity of a mammalian cell culture (b)(4) in response to the test article.	Pass	Report: 09-3504-G1 Appendix 4-1
Kligman Maximization Test	ISO 10993-10	To evaluate the allergenic potential or sensitizing capacity of a test article using a guinea pig model.	Pass	Report: 09-3504-G7 Appendix 4-2
Intracutaneous Injection Test -ISO	ISO 10993-10	A procedure for screening test article extracts for potential irritation effects as a result of an intracutaneous injection in (b)(4)	Pass	Report: 09-3504-G8 Appendix 4-3
Systemic Toxicity - ISO	ISO 10993-11	To screen test article extracts for potential toxic effects as a result of a single-dose systemic injection in mice.	Pass	Report: 09-3504-G9 Appendix 4-4
14-Day Repeat Dose Intravenous Toxicity Study	ISO 10993-11	To evaluate the potential of the test article extract to produce systemic toxicity after daily intravenous administration for 14 consecutive days in albino mice.	Pass	Report: 09-5442-G1 Appendix 4-5
Rabbit Pyrogen Test - ISO	ISO 10993-11	To determine the presence of chemical pyrogens in extracts of solid materials.	Pass	Report: 09-3504-G10 Appendix 4-6
Hemolysis Human Blood – ISO	ISO 10993-4	To determine the hemolytic activity of a test article in direct or indirect contact with human blood.	Pass	Report: 09-3504-G11 Appendix 4-7
Prothrombin Time Assay ISO	ISO 10993-4	To measure the effect of a test article on human blood coagulation time. The assay does not isolate and measure prothrombin but has become a suitable clinical means of determining the presence and functional ability of prothrombin in the process of coagulation.	Pass	Report: 09-3504-G5 Appendix 4-8
Complement Activation Assay - ISO	ISO 10993-4	To measure complement activation in human plasma as a result of exposure of the plasma to the test article.	Pass	Report: 09-3504-G4 Appendix 4-9
Unactivated Partial Thromboplastin Time Assay –ISO	ISO 10993-4	To measure the affect of a test article on the clotting time of human plasma.	Pass	Report: 09-3504-G6 Appendix 4-10
Lee and White Coagulation Test - ISO	ISO 10993-4	To determine if the clotting time of whole human blood is affected by the presence of a test article or a test article extract.	Pass	Report: 09-3504-G3 Appendix 4-11

Test	Method	Purpose	Results	Report Number Appendix
<i>In Vitro</i> Hemocompatibility	ISO 10993-4	To show that a test article does not adversely affect selected hematological parameters of blood including complete blood count, platelets, hematocrit, erythrocyte indices and plasma free hemoglobin.	Pass	Report: 09-3504-G2 Appendix 4-12
<i>S. Typhimurium</i> and <i>E. Coli</i> Reverse Mutation Assay	ISO 10993-3	To evaluate the potential of the test article to induce reverse mutations in histidine (his ⁻ to his ⁺) and tryptophan (trp ⁻ to trp ⁺) genes in <i>S. Typhimurium</i> and <i>E. Coli</i> respectively.	Pass	Report 09-5442-G2 Appendix 4-13
Mouse Lymphoma Mutagenesis Assay	ISO 10993-3	To evaluate the ability of the test article to induce an increase in the formation of homozygous thymidine kinase mutants (TK ⁻) over the background rate in a mouse lymphoma cell line.	Pass	Report: 09-5442-G3 Appendix 4-14
Rodent Bone Marrow Micronucleus Assay (50 Animals)	ISO 10993-3	To evaluate the ability of the test article and/or its metabolites to induce micronuclei in maturing erythrocytes of mice. The procedure is designed to detect damage to the chromosomes or mitotic apparatus caused by the test article.	Pass	Report 09-5442-G4 Appendix 4-15
Physiochemical Test for Plastics	USP 32 <661>	To determine the physical and chemical properties of a plastic test article including: nonvolatile residue, residue on ignition, heavy metals and buffering capacity.	Pass	Report 10-1868-G1 Appendix 4-16
Analysis of Extract for ISO 3826*	ISO 3826-1:2003 Annex A.3	To determine the physical and chemical properties of a plastic test article including: nonvolatile residue, residue on ignition, heavy metals and buffering capacity.	Pass	Test Certificate 06-5803-N2 Appendix 4-17
Microscopic Particle Count Test	USP 32 <788>	To extract and enumerate particulate matter in or on the test article by membrane filtration technique, utilizing a Bright Illumination Microscope for visual counting.	Pass	Report 09-5436-M1 Appendix 4-18

*Testing performed previously on identical PVC container material. Material passed all requirements of ISO 3826-1:2003 Part 6.3.

Reviewer notes:

Although I am not qualified to review the entire biocompatibility study, I have comments regarding the Prothrombin Time Assay study (Appendix 4-8) and sister studies Complement Activation Assay (Appendix 4-9) and Unactivated PTT Assay (Appendix 4-10).

I believe these assays are invalid due to a serious deviation from the study protocol. According to the study reports, Hemerus wanted the tested article to be filled with plasma in a particular order (divert bag, then filter, then RBC bag and so on) without mixing the plasma with fluids (citrate in donor bag, additive in bag a, etc.). However, the testing laboratory --(b)(4)-- allowed a deviation which resulted in plasma passing through donor bag (this bag is filled with citrate). This means that citrated plasma was mixed with additional citrate resulting in a double amount of citrate in the test sample. Since high amounts of citrate are known to change coagulation tests PT and NaPTT and since citrate dilutes plasma, all three tests are compromised and need to be repeated. In addition, I think that n=3 is not sufficient for coagulation tests.

A side note on the Complement Activation Assay (Appendix 4-9): the study reports negative concentrations of SC5b-9 protein. This means that the assay was not properly validated. Either these results were below the lower limit of detection, or the effect of plasma on --(b)(4)- was not accounted for.

Given the above problems with --(b)(4)- data, I would not be surprised if other --(b)(4)- studies (including animal studies, e.g., 14-Day Repeat Dose Intravenous Toxicity Study) were done in such a way that citrate or additives were mistakenly added to extracting fluids. The effects of fluids should have been investigated separately because citrate is known to have biological effects. We need to ask Hemerus how exactly the extraction was performed in each case.

6. Results of the frozen plasma quality study

a. Characterization of whole blood units used for plasma collection: donors and hold times

Test and control whole blood units were collected from volunteer subjects meeting all donation and study criteria. **Test units** were drawn into the investigational product, the Hemerus LEUKOSEP® HWB-600-XL Leukocyte Reduction Filtration System for Whole Blood with CPD Anticoagulant and SOLX® Additive. **Control units** were drawn into a CPD/AS-1 whole blood collection set with integral --(b)(4)- whole blood leukoreduction filter -----(b)(4)-----.

Table 5-12 and 5-13 summarize unit collection volume, collection time period and blood type as well as hold times prior to filtration for units evaluated for study endpoints. These tables show that comparable volumes of blood were collected for all study groups.

Table 5-12 Whole Blood Collection Summary – Subjects Completing Study

	Group 1 Test	Group 2 Test	Group 2 Control	(b)(4)
Unit Volume (mL)				
Mean	572.4	574.0	570.4	(b)(4)
SD	13.3	7.0	12.0	(b)(4)
Min	514.9	559.4	502.0	(b)(4)
Max	601.3	589.4	590.4	(b)(4)
n	60	60	60	(b)(4)
Collection Time (min:sec)				(b)(4)
Mean	07:48	08:11	08:36	(b)(4)
SD	02:26	02:10	02:23	(b)(4)
Min	04:00	04:00	05:00	(b)(4)
Max	15:00	15:25	15:00	(b)(4)
n	60	60	60	(b)(4)
Blood Type (# units)				(b)(4)
O+	27	24	19	(b)(4)
O-	2	3	3	(b)(4)
A+	19	21	25	(b)(4)
A-	3	3	3	(b)(4)
B+	7	6	7	(b)(4)
B-	2	2	1	(b)(4)
AB+	0	1	2	(b)(4)
AB-	0	0	0	(b)(4)

Table 5-13 Post-Collection Hold Times Prior to Filtration

Post Collection Hold Time Prior to Filtration	Group 1 Test	Group 2 Test	Group 2 Control	(b)(4)
Mean (hr:min:sec)	00:56:24	06:10:11	06:09:07	--(b)(4)---
SD	00:56:13	00:09:58	00:13:11	--(b)(4)---
min	00:14:55	06:02:10	05:16:37	--(b)(4)---
max	4:25:45	07:07:28	07:09:24	--(b)(4)---
n	60	60	60	b(4)

b. Plasma Processing Summary

Leukoreduced whole blood units were processed by a room temperature “hard spin” method to produce RBC in additive solution and platelet poor plasma (PPP). Group 2 units were to be placed at -18° C or below within 8 hours of collection. ----- (b) (4) -----
----- . Group 1 units were not tested, as noted above. Units were frozen into three aliquots for subsequent analysis at 3, 6 and 12 months of frozen storage.

Table 5-30 summarizes plasma volumes and times (post-collection) that plasma units were placed in frozen storage.

Table 5-30 Plasma Processing Summary

	Group 1 Test	Group 2 Test	Group 2 Control	--(b)(4)---
Plasma Unit Volumes				
Mean (mL)	305.3	301.2	292.0	(b)(4)
SD	18.5	16.8	17.1	(b)(4)
Min	262.0	258.5	250.0	(b)(4)
Max	343.9	337.3	329.2	(b)(4)
n	60	59	60	(b)(4)

	Group 1 Test	Group 2 Test	Group 2 Control	---(b)(4)---
Time Placed in Frozen Storage Post-Collection				
Mean (hr:min:sec)	2:38:31	7:20:55	07:16:16	--(b)(4)---
SD	1:22:54	0:42:01	0:32:35	--(b)(4)---
Min	1:24:45	4:12:00	04:49:00	--(b)(4)---
Max	6:59:00	9:14:00	08:36:00	--(b)(4)---
n	60	60	60	--(b)(4)---

c. Effect of LEUKOSEP® HWB-600-XL Leukocyte Reduction Filtration System on plasma quality.

Due to sampling volume constraints, only PT, aPTT and Factor VIII activity were evaluated at the pre-filtration time point.

Table 5-32 Pre-Filtration Plasma Parameters vs. Post-Filtration (NR = Normal Range)

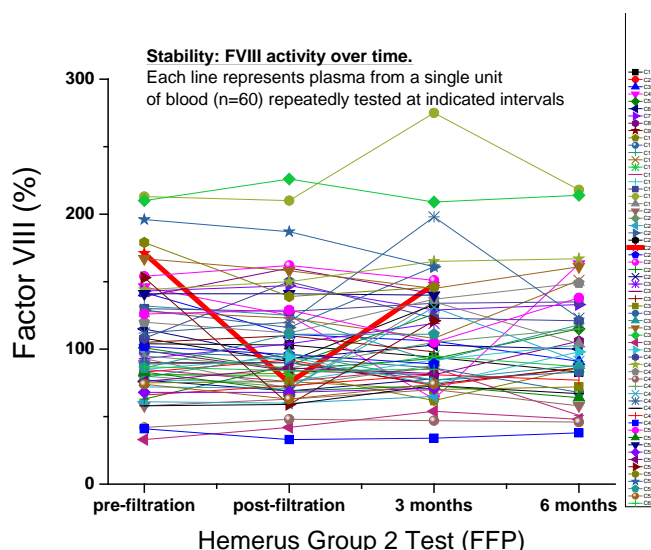
Parameter	Lab Reference Range	<8 Hour Room Temp Hold				---(b)(4)---	
		Group 2 Test Pre-Filter Mean \pm SD	Group 2 Test Post-Filter Mean \pm SD	Group 2 Control Pre-Filter Mean \pm	Group 2 Control Post-Filter Mean \pm	-(b)(4)-	(b)(4)
		% in NR	% in NR (#/Total)	% in NR	% in NR (#/Total)	--(b)(4)-	--(b)(4)-
PT (sec)	9.0 – 11.5	11.8 \pm 0.7	11.9 \pm 0.7	12.0 \pm 1.2	11.9 \pm 0.7	-(b)(4)-	-(b)(4)-
		45% (27/60)	35% (21/60)	38% (23/60)	35% (21/60)	(b)(4)	(b)(4)
aPTT	22-34	29 \pm 3	29 \pm 2	29 \pm 5	29 \pm 2	-(b)(4)-	-(b)(4)-

Parameter	Lab Reference Range	<8 Hour Room Temp Hold				---(b)(4)---	
		Group 2 Test Pre- Filter Mean ± SD	Group 2 Test Post- Filter Mean ± SD	Group 2 Control Pre- Filter Mean ±	Group 2 Control Post- Filter Mean ±	-(b)(4)-	(b)(4)
		% in NR	% in NR (#/Total	% in NR	% in NR (#/Total	--(b)(4)-	--(b)(4)-
		98% (59/60)	95% (57/60)	98% (59/60)	100% (60/60)	(b)(4)-	(b)(4)-
(sec)	50-180	106 ± 41	101 ± 39	105 ± 39	101 ± 34	-(b)(4)-	-(b)(4)-
		90% (54/60)	87% (52/60)	95% (57/60)	87% (52/60)	(b)(4)	(b)(4)-

 -----(b)(4)-----
 -----. Evaluation of PT, aPTT and
 Factor VIII activity demonstrated that the number of units in normal range was similar for
 each group prior to filtration.
 -----(b)(4)-----
 -----:

[(b)(4)]

- (b)(4)-----:
- 1) -----(b)(4)-----
 -----.
 - 2) -----
 -----(b)(4)-----



d. Comparison of Hemerus (Test) and --(b)(4)-- (Control) filtration.

Paired study design (i.e., blood from a single donor collected using Test and Control filtration systems) was not used by Hemerus. This limits the ability to detect differences between Test and Control systems because significant variability in coagulation system components is observed between normal donors. It can be expected that variability between donor populations for Test and Control groups may “mask” the difference in effects of Test and Control systems on plasma. Therefore, analysis of means is the only way of comparing quality of plasma obtained with Hemerus and --(b)(4)-- leukoreduction collection systems. This analysis indicated that Test FFP is comparable or better than Control FFP (see below).

Table 5-33 presents plasma protein and coagulation factor data for Group 2 Test and Group 2 Control units (FFP) and -----(b)(4)----- . Group 2 plasma units were frozen within 8 hours of collection. -----(b)(4)----- . The mean and SD for each parameter are listed along with the percent of units in the normal range and the total units in normal range divided by the total samples tested.

The data were analyzed for differences greater than 20% between the SOLX[®] System FFP and FFP prepared with the approved control system. The differences were calculated from the ratio of the mean values, at the corresponding time points, as follows:

$$\frac{(\text{Test Group 2 Mean} / \text{Control Group 2 Mean}) \times 100}{100}$$

Ratios less than 80% or greater than 125% were identified. Parameters resulting in greater than 20% differences, as compared to control FFP, were noted in the table as blue shaded cells.

All three-month data points are completed, 6-month partial data is completed and 12-month stability data is on-going.

Table 5-33 Plasma Protein Summary (NR=Normal Range; NT=Not Tested)

Parameter	Lab Reference Range	Group 2 Test (≤ 8 Hour RT Hold)			Group 2 Control (≤ 8 Hour RT Hold)			(b)(4)
		Post-Filter	3 Month	6 Month	Post-Filter	3 Month	6 Month	(b)(4)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
		% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	
Total Protein (g/dL)	6.2-8.3	5.6 ± 0.5 7% (4/60)	5.7 ± 0.3 8% (5/60)	5.8 ± 0.3 8% (3/39)	5.6 ± 0.6 13% (8/60)	5.8 ± 0.3 13% (8/60)	5.9 ± 0.3 25% (9/36)	(b)(4)
Albumin (g/dL)	3.6-5.1	3.5 ± 0.4 42% (25/60)	3.6 ± 0.2 53% (32/60)	3.6 ± 0.2 56% (22/39)	3.5 ± 0.3 50% (30/60)	3.7 ± 0.2 65% (39/60)	3.7 ± 0.2 67% (24/36)	
IgG (mg/dL)	694-1618	854 ± 196 85% (51/60)	857 ± 191 87% (52/60)	845 ± 206 82% (32/39)	836 ± 194 78% (47/60)	867 ± 204 75% (45/60)	859 ± 198 83% (30/36)	
PT (sec)	9.0-11.5	11.9 ± 0.7 35% (21/60)	11.9 ± 0.7 32% (19/60)	12.0 ± 0.6 23% (9/39)	11.9 ± 0.7 35% (21/60)	11.9 ± 0.7 29% (17/60)	12.0 ± 0.6 25% (9/36)	
INR	0.9-1.1	1.2 ± 0.1 57% (34/60)	1.1 ± 0.1 63% (38/60)	1.1 ± 0.1 64% (25/39)	1.2 ± 0.1 58% (35/60)	1.1 ± 0.1 58% (34/60)	1.1 ± 0.1 64% (23/36)	
aPTT (sec)	22-34	29 ± 2 95% (57/60)	30 ± 2 93% (56/60)	30 ± 2 95% (37/39)	29 ± 2 100% (60/60)	29 ± 2 100% (60/60)	30 ± 1 100% (36/36)	
Fibrinogen (mg/dL)	175-425	264 ± 58 98% (59/60)	263 ± 54 98% (59/60)	257 ± 51 97% (38/39)	268 ± 46 98% (59/60)	272 ± 46 100% (60/60)	269 ± 49 100% (36/36)	
Parameter	Lab Reference Range	Group 2 Test (≤ 8 Hour RT Hold)			Group 2 Control (≤ 8 Hour RT Hold)			(b)(4)
		Post-Filter	3 Month	6 Month	Post-Filter	3 Month	6 Month	(b)(4)
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
		% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	
Factor V (%)	65-150	85 ± 17 90% (54/60)	85 ± 15 93% (56/60)	85 ± 14 97% (38/39)	87 ± 18 92% (55/60)	89 ± 15 97% (58/60)	88 ± 14 97% (35/36)	(b)(4)
Factor VII (%)	60-150	95 ± 23 98% (59/60)	95 ± 19 97% (58/60)	91 ± 17 92% (36/39)	94 ± 21 90% (54/60)	95 ± 20 95% (57/60)	95 ± 19 97% (35/35)	
Factor VIII (%)	50-180	101 ± 39 87% (52/60)	107 ± 42 92% (55/60)	105 ± 42 85% (33/39)	101 ± 34 87% (52/60)	106 ± 33 98% (59/60)	107 ± 29 97% (35/35)	
Factor IX (%)	60-160	109 ± 24 100% (60/60)	113 ± 22 100% (60/60)	113 ± 23 100% (39/39)	105 ± 25 100% (60/60)	112 ± 24 100% (60/60)	109 ± 23 100% (36/36)	
Factor X (%)	70-150	95 ± 16 100% (60/60)	94 ± 16 98% (59/60)	95 ± 14 100% (39/39)	92 ± 17 97% (58/60)	92 ± 14 97% (58/60)	87 ± 13 94% (34/36)	
Factor XI (%)	65-150	96 ± 25 95% (57/60)	100 ± 23 93% (56/60)	97 ± 23 95% (37/39)	99 ± 21 100% (60/60)	99 ± 18 97% (58/60)	99 ± 17 97% (35/36)	
Factor XII (%)	50-150	114 ± 37 93% (56/60)	121 ± 43 97% (58/60)	117 ± 39 85% (33/39)	118 ± 40 95% (57/60)	118 ± 36 100% (60/60)	122 ± 38 64% (23/36)	
D-Dimer (μg/mL)	<0.50	0.66 ± 2.69 92% (55/60)	0.34 ± 0.45 90% (54/60)	0.35 ± 0.5 92% (36/39)	0.29 ± 0.15 93% (56/60)	0.31 ± 0.16 92% (55/60)	0.30 ± 0.15 89% (34/38)	

Parameter	Lab Reference Range	Group 2 Test (≤ 8 Hour RT Hold)			Group 2 Control (≤ 8 Hour RT Hold)			(b)(4)
		Post-Filter	3 Month	6 Month	Post-Filter	3 Month	6 Month	
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
		% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	
Antithrombin III (%)	80-120	89 ± 10	91 ± 9	93 ± 9	92 ± 10	97 ± 12	98 ± 11	(b)(4)
		90% (54/60)	90% (54/60)	100% (39/39)	92% (55/60)	93% (56/60)	92% (33/36)	
Protein C Activity (%)	70-180	96 ± 21	92 ± 20	86 ± 18	103 ± 26	99 ± 25	94 ± 27	
		92% (55/60)	90% (54/60)	90% (35/39)	92% (55/60)	92% (55/60)	78% (28/36)	
Protein S Activity (%)	60-150	84 ± 19	79 ± 15	79 ± 19	83 ± 18	81 ± 16	79 ± 16	
		95% (57/60)	95% (57/60)	95% (37/39)	87% (52/60)	88% (53/60)	89% (32/36)	
vWF/Rco Activity (%)	42-200	101 ± 37	96 ± 34	93 ± 33	103 ± 32	102 ± 28	95 ± 29	
		97% (58/60)	95% (57/60)	95% (37/39)	100% (60/60)	100% (60/60)	100% (36/36)	
TAT Complex (μg/L)	<4	5.2 ± 11.7	4.9 ± 10.8	2.5 ± 0.7	7.8 ± 14.7	7.5 ± 14.9	3.7 ± 4.9	
		88% (51/60)	88% (53/60)	95% (37/39)	78% (46/59)	78% (46/59)	78% (28/36)	
Prothrombin Fragment 1.2 (pmol/L)	41-372	175 ± 145	168 ± 119	132 ± 46	170 ± 127	163 ± 134	119 ± 33	
		95% (56/59)	100% (60/60)	100% (39/39)	93% (56/60)	93% (55/59)	100% (36/36)	
Fibrin Monomer	Negative	59 Neg 1 Pos	59 Neg 1 Pos	39 Neg 0 Pos	60 Neg 0 Pos	59 Neg 1 Borderline	36 Neg 0 Pos	
		98% (59/60)	98% (59/60)	100% (39/39)	100% (60/60)	98% (59/60)	100% (36/36)	
ADAMTS13 (%)	68-163%	112 ± 24	125 ± 25	126 ± 28	118 ± 21	129 ± 24	133 ± 23	
		97% (57/59)	95% (57/60)	87% (34/39)	95% (57/60)	95% (59/60)	94% (34/36)	

Parameter	Lab Reference Range	Group 2 Test (≤ 8 Hour RT Hold)			Group 2 Control (≤ 8 Hour RT Hold)			(b)(4)
		Post-Filter	3 Month	6 Month	Post-Filter	3 Month	6 Month	
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	
		% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	% in NR (#/Total)	
Fibrinogen-peptide A (ng/mL)	0.1-3.0	12 ± 19	8 ± 12	5 ± 7	16 ± 17	14 ± 19	7 ± 12	(b)(4)
		25% (15/60)	33% (19/58)	50% (19/38)	12% (7/60)	32% (19/60)	53% (19/36)	
Factor VIIa Chromo-genic mU/mL	30-170	75 ± 51	66 ± 43	69 ± 60	82 ± 59	77 ± 57	60 ± 53	
		78% (47/60)	81% (47/58)	68% (26/38)	80% (48/60)	75% (45/60)	72% (26/36)	
*C3a des Arg Fragment	<205 Ng/mL	640 ± 329 n=19	NT	NT	898 ± 282 n=13	NT	NT	
	55-486 ng/mL	408 ± 222 n=40	NT	NT	415 ± 265 n=47	NT	NT	
	Overall % in NR	69% (41/59)	NT	NT	52% (31/60)	NT	NT	
C5a (ng/mL)	4.7- 9.5	9 ± 3	NT	NT	14 ± 14	NT	NT	
		68% (41/60)	NT	NT	60% (36/60)	NT	NT	

*Two assays were used during the study for C3a evaluation. Mean and SD are given for each method. Overall percent in normal range is given for combined methods.

Conclusion:

Fresh frozen plasma (FFP) prepared from whole blood with the SOLX[®] System and placed at -18° C or below within 8 hours of collection demonstrated comparable levels of plasma proteins (within 20% as compared to control FFP) including: coagulation factors (V, VII, VIII, IX, X, XI and XII), fibrinogen, IgG, Protein C, Protein S, von Willebrand Factor (vWF/Rco) and ADAMTS 13.

For data collected to date using $\pm 20\%$ criteria, the SOLX[®] System FFP demonstrated lower Fibrinopeptide A (FPA) and TAT Complex at all testing time points and lower C3a and C5a post-filtration as compared to the control FFP.

Lower FPA and TAT levels indicate lower coagulation activation representing a potentially good trend in plasma quality.

e. Comparison of Hemerus' FFP (frozen within 8 hrs) and

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1. Regarding the Prothrombin Time Assay study (Appendix 4-8), Complement Activation Assay (Appendix 4-9) and Unactivated PTT Assay (Appendix 4-10), we note that a serious deviation from the study protocol occurred which resulted in the plasma passing through the donor bag and mixing with citrate. Since high amounts of citrate are known to change the outcome of coagulation tests PT and NaPTT (Unactivated PTT), and since citrate also dilutes the plasma, all three (3) studies were compromised. In addition, $n = 3$ is not sufficient for the above coagulation tests. Please repeat these 3 tests following the specifics of the protocol, especially those related to the preparation of the test plasma, using a sample size that is statistically sound.
2. Regarding the Complement Activation Assay (Appendix 4-9), please explain the negative concentrations of SC5b-9 protein described on Page 17 of the study report 09-3504-G4.