



Department of Health and Human Services
Public Health Services
Food and Drug Administration
Center for Biologics Evaluation and Research

Pharmacology/Toxicology Review
Division of Hematology
Office of Blood Research & Review

Product: HEMERUS LEUKOSEP® HWB-600-XL Leukocyte Reduction Filtration
System for Whole Blood with CPD Anticoagulant and SOLX® additive
Purpose: To evaluate pre-clinical test results used to support licensure of the SOLX®
system
Sponsor: Hemerus, NDA BN110059 and NDA BN110059/001 (x-ref IND 14199)
Date received: October, 28 2011
Reviewer: M. Keith Wyatt, Ph. D., Pharmacologist, CBER\OBRR\DH
Through: Jaroslav Vostal, M. D., Ph. D., Supervisory Medical Officer,
CBER\OBRR\DH\LCH

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A. Introduction

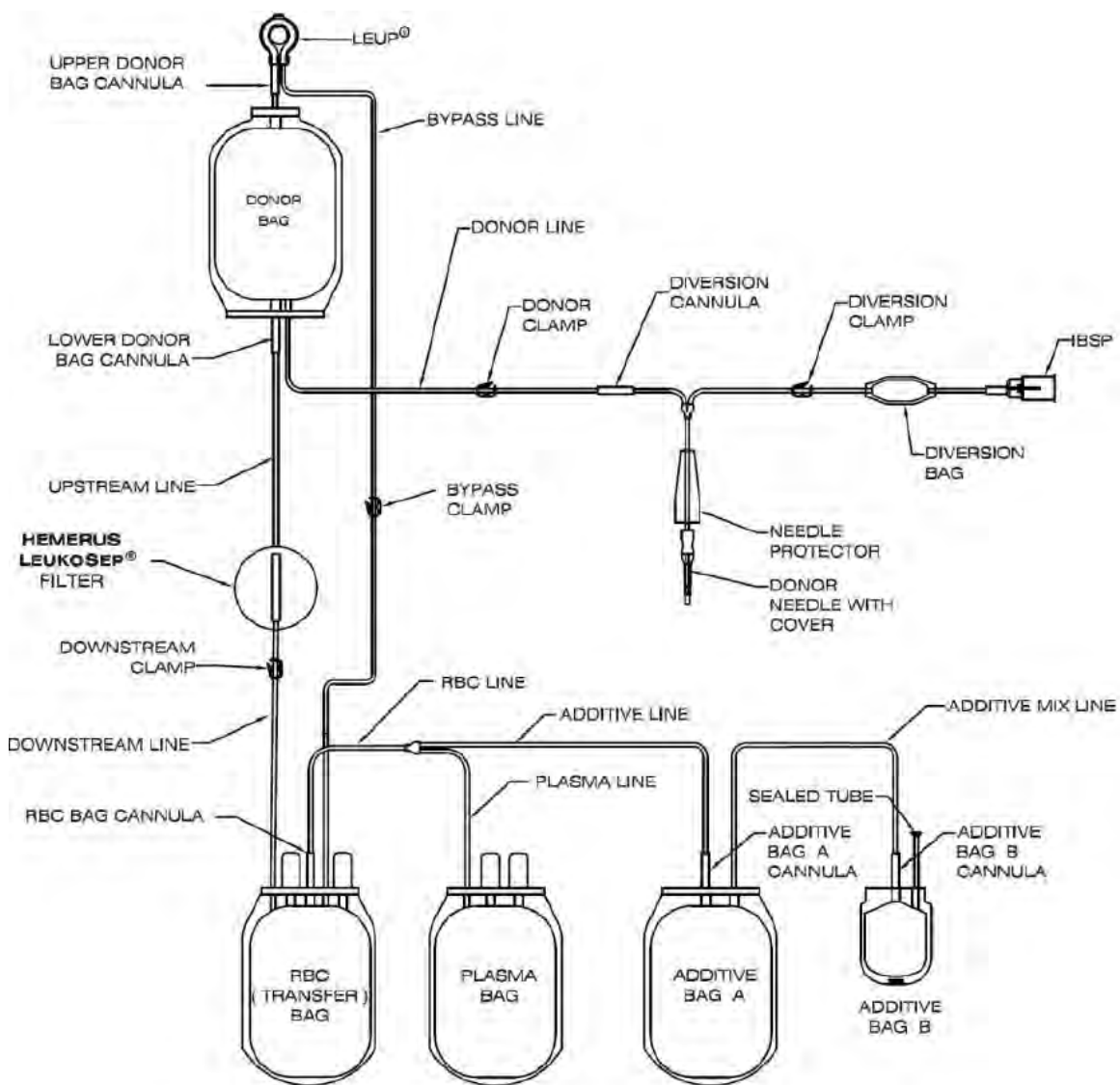
Hemerus (the Sponsor) has submitted a New Drug Application (NDA) for the licensure of LEUKOSEP® HWB-600-XL Leukocyte Reduction Filtration System for Whole Blood with CPD Anticoagulant and SOLX® additive (hereafter called Solx). Solx is a combination product designed for blood storage and/or the preparation of fresh frozen plasma (FFP) -----(b)(4)-----
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The Solx system is comprised of an elaborate arrangement of blood collection bags, various blood additive solutions and long-term storage containers all interconnected by PVC tubing. In addition, a donor needle has also been integrated into the Solx system. Some of the device components will be manufactured by the Sponsor in St. Paul, MN. Additional manufacturing and assembly of the Solx component parts and preparation of

the citrate phosphate dextrose (CPD) anticoagulant and other Solx solutions will be performed by JMS Singapore PRT located in Singapore.

A brief overview of the Solx system is as follows: Following blood donation, leukoreduction will be achieved by applying whole blood to the LeukoSep filter. The filter has been incorporated into the Solx system circuitry to expedite the reduction process. The partially purified leukoreduced blood product may then be stored in Solx bags for up to 42 days at 6° C. The leukoreduced blood may also be further processed to FFP or -(b)(4)--, which may then be stored in Solx bags for up to one year at – 18° C. The schematic diagram of the entire Solx system is provided below:

Diagram of the Solx system



The chemical formulations of the additive solutions used in the Solx system are provided in the table below. Solutions based on these formulations have been used clinically for many years and are considered safe.

Solution (Dosage)	Chemical	g/100 ml Solution
Citrate Phosphate Dextrose (CPD) Anticoagulant (70 mL)	(b)(4)	(b)(4)
SOLX [®] Additive Solution A (80 mL)		
SOLX [®] Additive Solution B (30 mL)		

B. Recommendation

The results from biocompatibility studies appear adequate to support approval of NDA BN110059 for Solx. However, the reduction in platelet counts observed in mice following repeated administration of Solx extracts and several potential deviations that may have occurred during the toxicity evaluation are still a concern.

C. Letter-ready (IR) comments

1. Regarding extraction procedures used on Leukosep filters and Solx circuits

- a. The Sponsor should confirm the LeukoSep filter was included in the Solx circuit when the saline or ---(b)(4)----- extractions were performed.
- b. The Sponsor should justify why an extraction of the LeukoSep filtration unit using an appropriate solvents followed by analysis of the extracts by GC/MS and HPLC/MS was not performed.
- c. Based on the total surface area of the Solx circuit and an established extraction ratio of --(b)(4)-, the Sponsor should confirm that extract volumes of 500 ml used in the biocompatibility studies was the appropriate amount. Using the same -----(b)(4) ratio, the Sponsor should confirm that the extract volume of -(b)(4)- used in the metal analyses (Study 06-5803-N2) was also the appropriate amount.

2. Regarding (b)(4) MEM elution assay, 09-3504-G1

- a. A (b)(4) amount of Solx extract was applied to (b)(4) cells, but the total volume of media used during incubation of the cells was not indicated. The Sponsor should provide the Solx extract dilution factor and final Solx extract concentrations used during these experiments.

3. Regarding Chemical and physicochemical characterization of extracts, 10-1868-G1

- a. The Sponsor should justify why total organic carbon, GC/MS and HPLC/MS analyses were not conducted to further identify and quantify chemical components, in addition to metals and non-volatiles, in Solx extracts.

4. Regarding the Repeat-dose toxicity study, 09-5442-G1

- a. Platelet counts in male mice administered Solx extract following repeat dosing were reduced to 1075 ± 147 K/ μ l compared with 1374 ± 45 K/ μ l in saline-treated male mice. Platelets were also reduced to 489 ± 348 in female mice administered Solx extracts compared with 983 ± 306 in female mice administered saline controls. Although sample clotting was observed during this study, the Sponsor should still provide an explanation for decreased platelet counts and perform histopathology on splenic tissues to rule out any potential immunotoxicity.
- b. The potential of DEHP and other plasticizers present in Solx extracts to disrupt endocrine function is a safety concern. However, results from the histological examination of rat testes tissues were not reported. If available, the Sponsor should provide these data.
- c. The Sponsor should explain why a recovery period, to monitor the possible occurrence of delayed toxicity, was not included in the experimental design.

5. Regarding Verification tests of Solx blood bags with a focus on blood bag labels, TP/119/PED/2009

- a. The Sponsor should submit a Master File to FDA describing the composition of the ink used on Solx blood bag labels and indicate where the printing process will be performed.
- b. The Sponsor should submit a Master File describing the composition of the adhesives used on Solx blood bag label stocks.
- c. The Sponsor should perform a MEM elution, USP <87>, or comparable assay on printed labels to rule out any cytotoxicity associated with ink used on Solx blood bag labels.

D. List of biocompatibility assays performed with Solx

Test	Method	Study #
Cytotoxicity	ISO 10993-5	09-3504-G1
Klingman maximization test	ISO 10993-10	09-3504-G7
Intracutaneous injection test	ISO 10993-10	09-3504-G8
Systemic toxicity	ISO 10993-11	09-3504-G9
14-day repeat dose IV toxicity study	ISO 10993-11	09-5442-G1
Rabbit pyrogen test	ISO 10993-11	09-3504-G10
Hemolysis human blood	ISO 10993-4	09-3504-G11
Prothrombin time assay	ISO 10993-4	09-3504-G5
Complement activation assay	ISO 10993-4	09-3504-G4
Unactivated partial thromboplastin time	ISO 10993-4	09-3504-G6
Lee and White coagulation test	ISO 10993-4	09-3504-G3
<i>In vitro</i> hemocompatibility	ISO 10993-4	09-3504-G2
<i>S. typ</i> and <i>E. coli</i> mutation assay	ISO 10993-3	09-5442-G2
Mouse lymphoma mutagenesis assay	ISO 10993-3	09-5442-G3
Bone marrow nuclease assay	ISO 10993-3	09-5442-G4
Physicochemical test for plastic	USP 32 <661>	10-1868-G1
Analysis of extract based on ISO 3826	ISO 3826-1	06-5803-N2
Microscopic particle count	USP 32 <788>	09-5436-M1

E. Indication/IND 14199

Feasibility studies have been conducted by the U.S. Army Blood Research Detachment in patients infused with leukoreduced blood, FFP or -(b)(4) prepared using Solx. Hemolysis, ATP levels and cell morphology data generated during these studies suggest Solx is appropriate for the preparation and storage of leukoreduced blood, FFP and -----(b)(4) for clinical use.

To confirm results from the feasibility studies, a larger more complete clinical trial using blood products prepared with Solx has been initiated under IND 14199. The larger trial will include 180 patients infused with either whole blood, leukoreduced blood, FFP or (b)(4) processed using Solx and stored in Solx bags for 42 days at 6 °C. The trial is currently underway.

F. Summary of biocompatibility studies

(b)(4) MEM elution test, --(b)(4)---, GLP-report 09-3504-G1, Sept 15, 2009

Purpose: To determine biologic reactivity of mammalian cells (b)(4) following exposure to Solx extract.

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

Result: The results indicate Solx extracts were not cytotoxic. Cells exposed to Solx extracts received a grade of 0, on a scale of 4, indicating the material was not biologically active. Cells exposed to polypropylene negative and natural rubber positive control extracts yielded grades of 0 and 4, respectively. Additional reactivity results are presented in the table below:

[(b)(4)]

Klingman maximization test, -(b)(4)--, GLP-09-3504-G7, Oct 9, 2009

Purpose: To determine the allergenic potential or sensitizing capacity of Solx extracts in guinea pigs.

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

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

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

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-(b)(4)-

Results: Solx extracts did not cause skin irritation or any significant reactions. The sensitization rate following induction was not considered significant. Extracts used in the study met ISO 10993-10 standards. The potential of Solx extracts comprised of -----
----(b)(4)----- to induce an allergic response was considered weak. No weight loss or decrease in weight gain was observed.

Reviewer concerns:

Lower than expected allergenic scores were obtained in male guinea pigs administered positive controls. The Sponsor should confirm that the immune systems of male guinea pigs used in the study were not compromised.

[(b)(4)]

Intracutenous injection test, (b)(4)--, 09-3504-G8, Sept 21, 2009

Purpose: To evaluate the potential of Solx extracts to induce irritation after subcutaneous injection.

-(b)(4)-

Result: The absence of significant erythema and edema following injection indicates that both the -----(b)(4)----- Solx extracts were not capable of inducing skin reactions or skin irritations. No significant change in weight gain or loss was reported in rabbits administered the Solx extracts.

Systemic injection test, (b)(4)--, report GLP 09-3504-G9, Sept 21, 2009

Purpose: To evaluate the potential of Solx extracts to produce systemic toxicity.

-(b)(4)-

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

Results: Solx extracts did not induce systemic toxicity in mice. No significant change in weight gain or loss was reported in mice administered Solx extract compared with changes in control mice.

14-day repeat dose intravenous toxicity study, (b)(4), GLP 09-5442-G1, March 12, 2010

Purpose: To evaluate the potential of Solx extracts to produce systemic toxicity following 14 daily injections.

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

----- (b)(4) -----
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Results: No differences in animal weights or changes in signs and symptoms were reported over the 14 day duration. Organ weights were unchanged in mice administered either extract or control extracts. Histology of liver and lung tissue from mice administered either extract were not remarkable compared with tissues from control treated mice.

Contrastingly, monocyte levels in male mice administered Solx extract were slightly higher than in male mice administered control extracts. Platelet counts were decreased in both male and female mice administered Solx extract compared with control treated mice. Differences in platelet and monocyte counts and some unexpected blood clotting that occurred during collection were not considered biologically significant by the Sponsor. Additional results are presented in the table below:

[(b)(4)]

[(b)(4)]

Reviewer concerns:

Results from female mice administered Solx extracts, even when data from samples that clotted are excluded, suggests extracts may lower platelet counts. Although no significant changes in spleen weight were reported, potential immunotoxicity attributable to the bag extract cannot be ruled out in the absence of a histopathologic evaluation of the splenic tissue.

This study was also reviewed by Mikhail Ovenesov, Ph.D., OBRR\DH\LH. Dr. Ovenesov believes that the PVC extraction conducted by (b)(4)-- was not performed according procedures specified by Hemerus. As a result, the concentration of CPD in the dose administered during the mouse repeat-dose study was potentially twice the expected amount. This additional amount may have impacted results from the toxicity study. This reviewer agrees and suspects that the lower than expected PLT levels observed during this study could be related to the additional CPD. The Sponsor's reply regarding whether or not the extraction procedure was properly conducted will determine if an additional repeat dose study in mice is needed. In any event, the decreased PLT counts and clotting reported during the repeat-dose study are still a concern.

Rabbit pyrogen test, (b)(4), GLP-09-3504-G10, Sept 18, 2009

Purpose: To determine the presence of chemical pyrogens in Solx extracts.

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----- (b)(4) -----
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Prothrombin time assay-ISO direct contact, (b)(4), GLP-09-3504-G5, October 20, 2009

Purpose: To determine the potential Solx extract have on prothrombin time (PT).

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

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

Results: PT values were not affected by Solx extracts. PT values in plasma treated with either Solx extract, negative or positive controls were -----

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

[(b)(4)]

Reviewer concerns:

The PT of 14.4 sec observed in one Solx treated plasma sample is a concern.

Complement activation assay, (b)(4), GLP-09-3504-G4, October 22, 2009

Purpose: To determine the potential of Solx extract to activate complement C3 and C5 in plasma.

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

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

Results: The protein concentration results presented in the tables below suggest Solx extract did not activate or increase the expression of C3 and C5 compared with expression in positive and negative control extract plasma.

[(b)(4)]

[(b)(4)]

[(b)(4)]

[(b)(4)]

Dr. Ovenesov has also reviewed the results from the SC5b-9 assay. He has indicated that negative values reported for the untreated plasma and negative control are unacceptable. (b)(4) explained these data in the following quote excerpted from the final study report “The calculated negative val[u]es are due to a lower background in the plasma used for the test compared to the serum in the standard sample. This effect has no impact on the sensitivity of the test as the positive control is clearly higher than the negative control”. The reviewer has no experience with this assay and will await (b)(4) reply to Dr. Ovenesov’s request for more information.

Unactivated partial thromboplastin time (UPTT) assay, (b)(4), GLP 09-3504-G6, October 20, 2009

Purpose: To determine the potential of Solx extract to affect UPTT.

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

Lee and White coagulation test, (b)(4), GLP-09-3504-G3, Sept 21, 2009

Purpose: To determine the potential of Solx extract to affect blood clotting times.

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

Result: Solx extracts did not affect clotting. -----

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

[(b)(4)]

***In vitro* hemocompatibility assay, (b)(4), GLP 09-3504-G2, September 22, 2009**

Purpose: To determine the potential Solx extracts have on hematologic parameters in human whole blood.

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

Results: No adverse effects or significant changes in CBC, hematocrit, erythrocyte indices and platelet counts were reported in blood exposed to Solx extracts compared with blood exposed to negative control extracts and untreated blood. Additional results are presented in the tables below.

[(b)(4)]

[(b)(4)]

Reviewer concerns:

Platelet counts were not decreased during this study, but this result contrasts with reductions observed in mice repeatedly administered Solx extracts during study 09-5442-G1. The Sponsor should provide an explanation for the differences.

***Salmonella typhimurium* and *E. coli* reverse mutation assay, (b)(4), GLP-09-5442-G2, Jan 18, 2010**

Purpose: To determine the mutagenic potential of Solx extracts.

Results: No statistically significant increase in the number of revertant *Salmonella* or *E. coli* colonies was observed in either Solx extract compared with negative control extracts. Based on the results, both Solx extracts were not considered mutagenic in either assay.

[(b)(4)]

Metabolic activation did not appear to increase the number of revertant bacterial colonies (data not shown).

Mouse lymphoma mutagenesis assay, (b)(4), GLP-09-5442-G3, February 11, 2010

Purpose: To determine differences in the frequency of homozygous mutations (TK^{+/+} to TK^{-/-}) that occurred in mouse lymphoma cells exposed to Solx and control extracts.

[(b)(4)]

[(b)(4)]

Reviewer concerns:

In general, the test article yielded ~10% more mutations than the negative control in the assay which is a concern. However, according assay descriptions in ASTM, E 1280-97, the statistically insignificant differences reported during this study suggest the extracts were probably not mutagenic.

Rodent bone marrow micronucleus assay, (b)(4), GLP-09-5442-G4, February 3, 2010

Purpose: To determine the potential of Solx extract to induce clastogenic effects resulting in micronuclei formation in erythrocytes using a standard bone marrow assay.

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

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

-(b)(4)-

Results: Solx extracts did not induce clastogenic effects. Specifically, Solx extract did not induce a statistically significant increase in the number of micronucleated erythrocytes or an increase in the ratio of polychromatic versus normochromatic erythrocytes 24 (data not shown) and 48 hrs after dosing. Moreover, there was no difference in the number of micronucleated cells exposed to Solx extract compared with cells exposed to negative control extracts.

No significant changes in weight gain or loss was observed in mice administered Solx extract compared with mice administered positive or negative control extracts. Additional ratio data are presented in the table below:

[(b)(4)]

Physicochemical test for plastics, GLP 10-1868-G1, May 13, 2010

Purpose: To determine the amount of non-volatile compounds and heavy metals in Solx extract.

-(b)(4).

Results: The results indicate the non-volatile residues and heavy metal content of Solx extract were acceptable. -----

Analysis of extract using ISO 3826, 06-5803-N2, January 31, 2007

Purpose: To evaluate Solx extract for DEHP content and other metals.

-(b)(4)-

Results: Results for DEHP and metal content in Solx extracts were within acceptable limits. Additional data is provided in the tables that follow.

[(b)(4)]

[(b)(4)]

Reviewer concerns:

Although DEHP is virtually insoluble in water, extractions to determine leachable DEHP were performed with WFI which may not accurately represent blood lipophilicity.

Microscopic particle count test, GMP-09-5436-M1, December 31, 2009

Purpose: To determine the amount of particulate matter in Solx circuits.

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

Results: Counts for particles and fibers $\geq 5 \mu\text{m}$, $\geq 10 \mu\text{m}$, $\geq 25 \mu\text{m}$ and $\geq 100 \mu\text{m}$ size (considered fibers) were acceptable based on USP and the Sponsor's internal specifications.

Reviewer concerns:

DMPQ should also review the results from this study.

**G. Verification test of Solx blood bags with a focus on blood bag labels,
TP/119/PED/2009**

Purpose: To demonstrate labels affixed to Solx blood bags maintain their stability and integrity.

-(b)(4)

Results: Labels remained affixed to blood bags during a variety of storage conditions and were considered tamper proof based on the results from both studies.

Reviewer concerns:

The reviewer was not qualified to evaluate the label manufacturing process or the affect of proposed changes in the bag arrangement/configuration to prevent interior wall sticking during autoclaving described by the Sponsor. An additional reviewer is needed to make this assessment.

H. The reviewer is not expert or qualified to accurately assess the reports that follow. A CMC/DMPQ reviewer may be needed.

1. Bench top performance of Solx, Protocol PC400210, August 3, 2009,
2. Design verification testing of Solx, PC400210, Sept 23, 2009, performed by Hemerus
3. Design verification testing of Solx, FR400210, Sept 23, 2009, performed by Hemerus
4. Design verification testing of Solx, TR/105/PED/2007, June, 2007, performed by JMS Singapore
5. Design verification testing of Solx, TR/105/PED/2007, November 2007, performed by JMS Singapore
6. Transportation simulation testing, Report number 0706135 Rev. C, November 27, 2007, performed by ----(b)(4)-----
7. Transportation testing for Solx, Report TP/077/PED/2008, June 5, 2008
8. Transportation testing for Solx, Report TP/077/PED/2008, July 7, 2008
9. A partial review of the study entitled Verification Test on Solx blood bags with focus on Blood Bag labels and issue on the sticking of the interior walls of the blood bag, Report TP/119/PED/2009, Sept 28, 2009 was performed