

Coagulation Factor Activity in Photochemically Treated Plasma Prepared from Previously Frozen Plasma

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Introduction & Objective

Photochemical treatment (PCT) using amotosalen HCl and long wavelength UVA light inactivates a broad spectrum of blood-borne pathogens in platelets and plasma. A single process treats 385-635 mL of apheresis or whole blood-derived plasma and produces up to three 200 mL doses of INTERCEPT™ Plasma (I-FFP). Two jumbo units can be illuminated simultaneously. Previous studies have evaluated coagulation factor activity in I-FFP prepared from fresh plasma.

Study Aim

The purpose of this study was to evaluate coagulation function in photochemically treated plasma prepared from previously frozen plasma [I-FFP(PF)], as the use of frozen plasma as the starting material for INTERCEPT Plasma would increase operational flexibility for blood centers.

Figure 1: Helinx™ Technology

The INTERCEPT Blood System™ for plasma uses Helinx technology to inactivate viruses, bacteria, and parasites that may contaminate plasma intended for transfusion. Amotosalen, a psoralen molecule used in Helinx technology, penetrates into cells and nuclear membranes and intercalates into the helical regions of DNA and RNA. Covalent crosslinks to the nucleic acid base pairs form upon exposure to UVA light and block DNA and RNA replication.

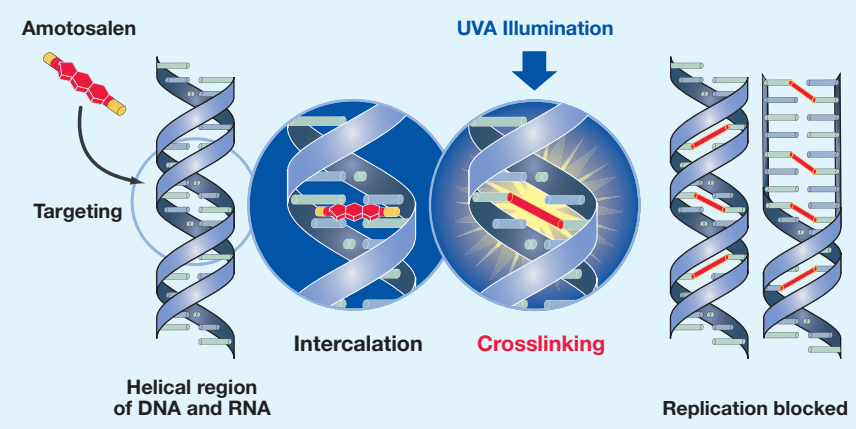
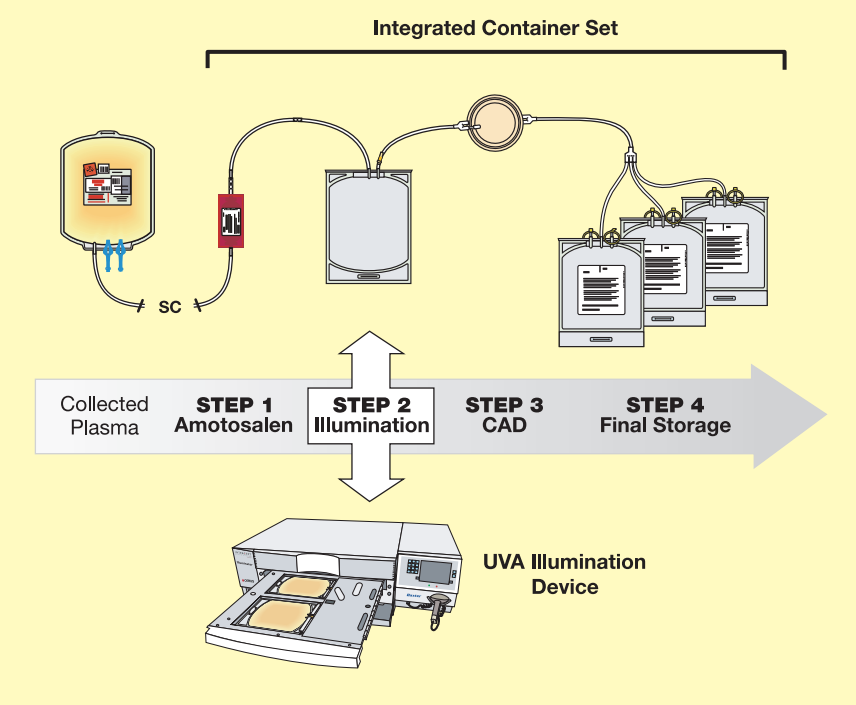


Figure 2: The INTERCEPT Treatment of Plasma

The collected plasma is sterile connected to the PCT kit. Amotosalen (1) is added by gravity flow and the plasma is illuminated with UVA light (2). Residual amotosalen and its photoproducts are reduced to low levels using a flow-through compound adsorption device (CAD) (3) during transfer to the storage containers (4).



Methods

Plasma

Fresh frozen jumbo apheresis plasma units were obtained from Interstate Blood Bank (Memphis, TN). Units were shipped to Cerus on dry ice and stored at -20°C for up to 2 weeks. Immediately prior to photochemical treatment, units were thawed in a 37°C plasma thawer.

Photochemical Treatment

Plasma units were processed as is described in Figure 2. The treatment volume (plasma with amotosalen) was 600 mL.

Sample Collection

Plasma samples were collected before starting PCT (baseline) and after CAD treatment. Samples were frozen in 1.5 mL aliquots at -80°C for up to 3 weeks. Immediately prior to analysis, samples were thawed in a 37°C waterbath.

Coagulation Testing Methods

Fibrinogen was quantitated by the Clauss method. Factors II, V, VII, VIII, IX, X, and XI were measured using PT- or APTT-based one-stage clotting assays. Factor XIII was measured using a chromogenic assay manufactured by DadeBehring (Marburg, Germany). Antithrombin (AT), protein C (PC), protein S (PS), and α -2 antiplasmin (AP) were measured using assays from Diagnostica Stago (Asnieres-sur-Seine, France). α -2 antiplasmin was analyzed by Esoterix, Inc. (Aurora, CO). All other assays were conducted by Cerus Corporation.

Results

The coagulation factor activity levels in plasma units before and after photochemical treatment are shown in Figures 3 and 4, respectively. Retention of coagulation factor activity relative to baseline is shown in Figure 5. Data is summarized in Table 1.

Figure 3: Coagulation Factor Activity Before PCT

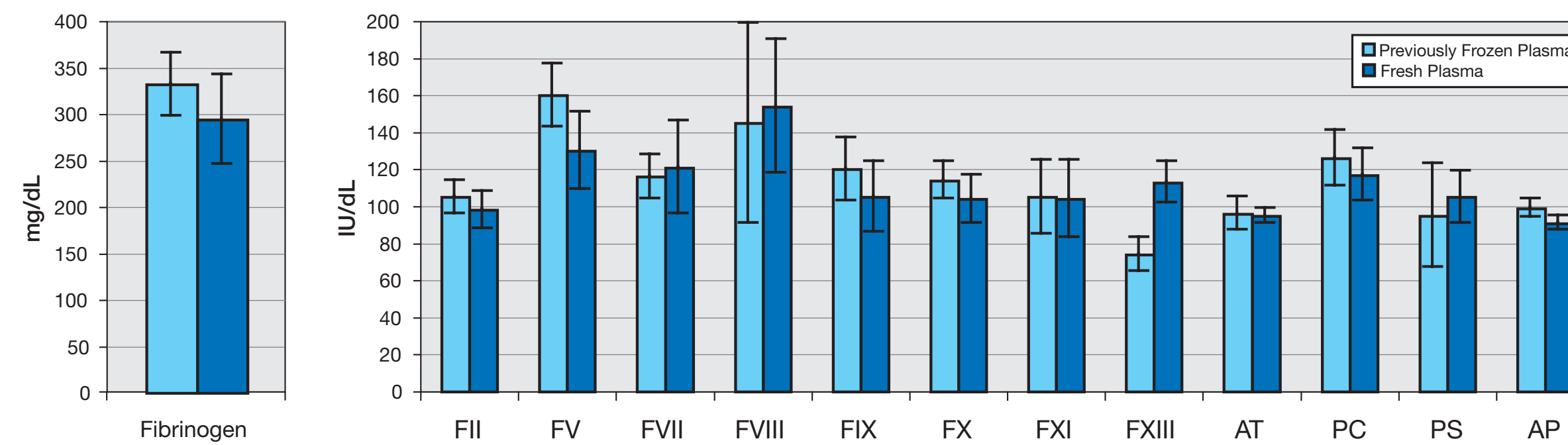


Figure 4: Coagulation Factor Activity After PCT

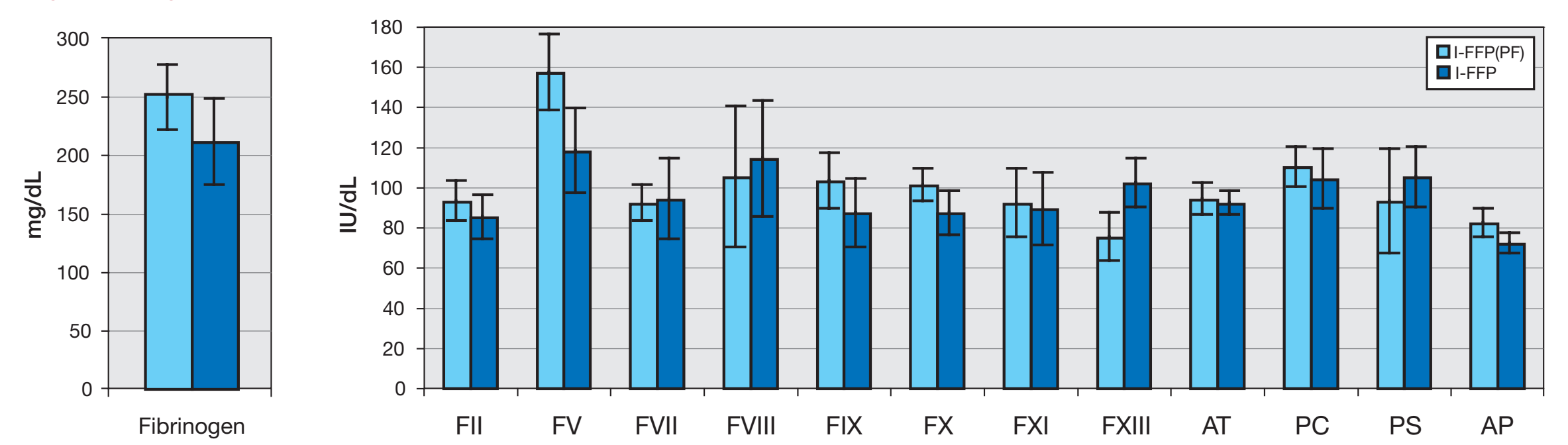
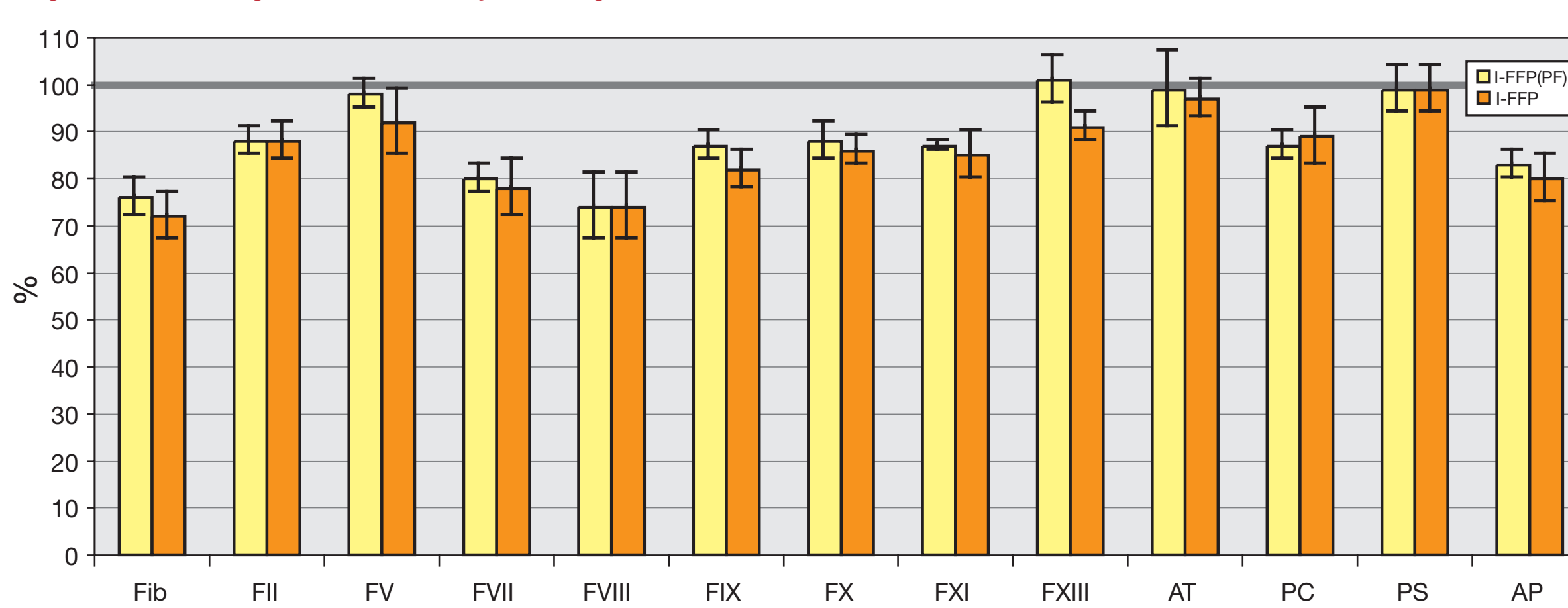


Figure 5: Percent Coagulation Factor Activity Remaining After PCT



Conclusions

- Levels of hemostatic factors in INTERCEPT Plasma prepared from previously frozen apheresis plasma were comparable to levels in I-FFP prepared from fresh apheresis plasma
- I-FFP prepared from both fresh and previously frozen plasma contained therapeutic levels of coagulation factor activity
- The use of previously frozen plasma as a starting material for I-FFP would allow blood centers to provide a pathogen-reduced plasma product with increased operational flexibility

Table 1: Coagulation Activity and Retention in I-FFP (mean \pm SD)

	Activity ^a		% Retention	
	I-FFP (PF) ^b	I-FFP ^c	I-FFP (PF) ^b	I-FFP ^c
Fibrinogen (FI)	249 \pm 28	211 \pm 37	76 \pm 4	72 \pm 5
Factor II (FII)	93 \pm 10	85 \pm 11	88 \pm 3	88 \pm 4
Factor V (FV)	157 \pm 19	118 \pm 21	98 \pm 3	92 \pm 7
Factor VII (FVII)	92 \pm 9	94 \pm 20	80 \pm 3	78 \pm 6
Factor VIII (FVIII)	105 \pm 35	114 \pm 29	74 \pm 7	74 \pm 7
Factor IX (FIX)	103 \pm 14	87 \pm 17	87 \pm 3	82 \pm 4
Factor X (FX)	101 \pm 8	87 \pm 11	88 \pm 4	86 \pm 3
Factor XI (FXI)	92 \pm 17	89 \pm 18	87 \pm 1	85 \pm 5
Factor XIII (FXIII)	75 \pm 12	102 \pm 12	101 \pm 5	91 \pm 3
Antithrombin (AT)	97 \pm 4	92 \pm 6	99 \pm 8	97 \pm 4
Protein C (PC)	89 \pm 6	104 \pm 15	87 \pm 3	89 \pm 6
Protein S (PS)	99 \pm 5	105 \pm 15	99 \pm 5	99 \pm 5
Antiplasmin (AP)	80 \pm 5	72 \pm 5	83 \pm 3	80 \pm 5

a. FI mg/dL, AP %, others IU/dL

b. N=6

c. FII, FX: N=45; FXIII, PC, PS, AT, AP: N=12; others N=77

Table 2: Reference Ranges

Reference ranges represent the mean \pm 2SD from \geq 355 units of fresh plasma (apheresis and whole blood-derived) prior to photochemical treatment.

Fibrinogen	171–383 mg/dL	Factor XI	62–142 IU/dL
Factor II	71–127 IU/dL	Factor XIII ^a	85–135 IU/dL
Factor V	77–161 IU/dL	Antithrombin ^a	85–105 IU/dL
Factor VII	59–171 IU/dL	Protein C ^a	80–140 IU/dL
Factor VIII	72–236 IU/dL	Protein S ^a	85–135 IU/dL
Factor IX	65–145 IU/dL	Antiplasmin ^b	80–150 %
Factor X	68–132 IU/dL		

a. Derived from analysis of 25 units of fresh plasma

b. Range established by Esoterix, Inc.