Plasma treated with amotosalen and ultraviolet A light retains activity for hemostasis after 5 days post-thaw storage at 1 to 6°C

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BACKGROUND: Plasma thawed and stored at 1 to 6°C for up to 5 days (thawed plasma [TP]) provides rapid availability in emergencies and reduces plasma waste, but it carries risks of coagulation factor loss or activation, bacterial outgrowth, and viral contamination. We characterized changes in amotosalen/ultraviolet A (UVA) light pathogen-reduced, fresh-frozen plasma (FFP) and plasma frozen within 24 hours (PF24) with post-thaw storage.

STUDY DESIGN AND METHODS: Amotosalen/UVA light-treated FFP and PF24 were thawed after approximately 3 to more than 12 months of frozen storage and held at 1 to 6°C for 5 days. Global assessments of coagulation and hemostatic, antithrombotic, and activation markers indicative of function were assessed.

RESULTS: Day 5, thawed amotosalen/UVA light-treated FFP and PF24 contained levels of Factors II, V, VIII, IX, X, von Willebrand factor ristocetin cofactor (vWF:RCo), fibrinogen, antithrombin III (ATIII), protein C, and protein S similar to the levels measured in Day 5 TP, as described in the *Circular of Information*. Thrombin generation was robust on Day 5 (amotosalen/UVA: FFP = 1866 ± 402 nM/minute; PF24 = 1800 ± 277 nM/minute). Most factor activities on Day 5, including von Willebrand factor-cleaving protease (ADAMTS-13), were more than 90% of Day 0 values, except for known labile Factors V and VIII and protein S. All units contained greater than 0.4 IU/mL protein S and α2 plasmin inhibitor on Day 5. Global functional indices, including thrombin-antithrombin complexes, nonactivated thromboplastin time, and thrombin-generation peak height, did not indicate activation of the coagulation cascade, although isolated units showed raised levels of Factor VIIa and Complement 3a.

CONCLUSION: Amotosalen/UVA light-treated FFP and PF24 demonstrated retention of procoagulant and antithrombotic activity after 5 days post-thaw storage at 1 to 6°C.

Over the last decade, the application of evidence-based medical practices and the need to conserve resources has resulted in an approximately 25% decrease in the overall use of plasma for transfusion and an increasing proportional use of thawed plasma (TP) that represented the majority of plasma transfusions in the United States in 2013.[1] Most types of plasma are used interchangeably by clinicians, and a wide range of products is now available, as categorized by the method of manufacture in the *Circular of Information for the use of human blood and blood components-2013* (COI).[2] The COI is a document prepared jointly by the AABB, the American Red Cross, America's Blood Centers, and the Armed Services Blood Program and it is recognized by the US Food and Drug Administration (FDA) as an acceptable extension of blood component labeling under Title 21 CFR 606.122. Differences between various plasma types are generally poorly characterized with few universally accepted standards for each type.

ABBREVIATIONS: ETP = endogenous thrombin potential; PF24 = plasma frozen within 24 hours; TP = thawed plasma; RT24PF24 = plasma stored at room temperature for up to 24 hours and frozen within 24 hours; vWF:RCo = von Willebrand factor ristocetin cofactor.

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The COI defines TP as derived from plasma frozen within 8 hours (FFP), from plasma frozen within 24 hours (PF24), or from plasma stored at room temperature for up to 24 hours and frozen within 24 hours (RT24PF24); it is prepared using aseptic techniques in a functionally closed system. It is thawed at 30 to 37°C and maintained at 1 to 6°C for up to 4 days after the initial 24-hour post-thaw period has elapsed. TP is described as containing stable coagulation factors, such as Factor II and fibrinogen, in concentrations clinically similar to those of FFP but variably reduced amounts of other factors. It serves as a source of nonlabile plasma proteins.[2] The levels and activation state of coagulation proteins in TP are described as being variable and subject to change over time.

There are no published, predetermined acceptability criteria for TP, and studies have evaluated only a limited number of coagulation and antithrombotic factors after thaw and storage out to 5 days.[3-6] Discussions with the FDA suggested minimum criteria for protein S and α2-plasmin inhibitor of greater than 0.4 IU/mL, based on prior experience with solvent detergent pathogen-reduced plasma that linked low levels of these factors to thrombotic events (FDA written response to Cerus Corporation dated March 8, 2013).[7,8]

In this setting, we sought to characterize the in vitro function and quality of TP derived from FFP or PF24 treated with pathogen-reduction technology using amotosalen and ultraviolet A (UVA) light prior to freezing (INTERCEPT Blood System; Cerus Corporation immediately post thaw and over 5 days of storage at 1 to 6°C. The objective was to provide assurance that thawed amotosalen/UVA-treated plasma contains "stable coagulation factors, such as Factor II and fibrinogen, in concentrations *clinically similar* to FFP,"[2] in order to allow conversion to TP with extended storage out to 5 days. We also sought to determine the stability of pathogen-reduced plasma in terms of activation of coagulation with storage post thaw.

The amotosalen/UVA light treatment process (INTERCEPT Blood System) for plasma was approved by the FDA in December 2014 and is intended for the ex vivo pathogen reduction of apheresis and whole blood (WB)-derived plasma.[9] The system is designed to reduce the risk of transfusion-transmitted infections by inactivating a broad range of pathogens, including viruses, bacteria, and protozoan parasites as well as leukocytes that can contaminate plasma intended for transfusion. Amotosalen/UVA-treated plasma has a shelf-life of 12 months when stored at 18°C or less and should be transfused as soon as possible after thaw. With post-thaw storage at 1 to 6°C, it is discarded if not used within 24 hours. In this study, the comparability of the hemostatic capacity for thawed amotosalen/UVA-treated FFP and PF24 to that of conventional TP derived from FFP and PF24, respectively, was determined by comparing the following:

- Absolute values of factors on Day 5 post thaw compared with reference Day 5 levels described in the COI, with reference to Scott and colleagues[3];
- Day 0 (immediately post thaw) and Day 5 (approximately 120 hours post thaw) coagulation factor and plasma protein levels to define changes during storage at 1 to 6°C post thaw;
- Global integrated assays of hemostatic capacity, such as thrombin generation, endogenous thrombin potential (ETP), prothrombin time (PT), and activated partial thromboplastin time (aPTT);
- FDA-recommended minimal values for select factors (e.g., protein S and α2-plasmin inhibitor); and
- Normal clinical reference range values for factors not described for TP in the COI.

MATERIALS AND METHODS

FFP was prepared from apheresis plasma obtained from healthy donors using the Trima Accel blood-collection system (Terumo BCT). Plasma was collected and diluted into acid citrate dextrose (ACD-A) anticoagulant using Trima Accel MultiPlasma (catalog no. 80700; Terumo BCT) collection sets. Amotosalen/UVA light treatment was performed according to the Cerus Corporation's instructions for use. Volumes of 636 ± 16 mL (586-650 mL) were treated, split into three components of 180 mL or more and frozen within 8 hours of donation. Amotosalen/UVA FFP was stored in 400 mL PL 269 plastic (EVA) containers at -18°C or less for 101 ± 5 days (approximately 3 months) or 385 ± 7 days (>12 months) before thawing (Table 1).

PF24 was prepared from small pools ($\leq$ 765 mL) of WB-derived plasma diluted in citrate-phosphate-dextrose (CPD) anticoagulant during routine WB collection (approximately 14 mL CPD per 100 mL WB) from three ABO-matched, healthy blood donors.[10] Volumes of 620 ± 26 mL (588-650 mL) were amotosalen/UVA treated according to the Cerus Corporation's instructions for use, split into three components of 180 mL or more, and frozen within 24 hours of donation (Table 1). Amotosalen/UVA PF24 was stored in 400 mL PL 269 plastic (EVA) containers at 18°C or less for 82 ± 8 days (approximately 3 months) or 384 ± 1 days (>12 months) before thawing.

The INTERCEPT Blood System for plasma consists of a plastic disposable set (INT31), comprising an amotosalen container, an illumination container, a compound adsorption device, three 400-mL storage containers, and an illumination device that delivers a controlled dose of UVA light. Amotosalen solution is mixed with plasma to a final nominal concentration of 150 μM. The plasma/amotosalen mixture is illuminated with a 3.0 Joules/cm^2 (J/cm^2) UVA light treatment using a microprocessor-controlled light source with integral reciprocal shaking during illumination. After UVA illumination, treated

TABLE 1. Characteristics of the amotosalen/ultraviolet A light-treated plasma

Characteristic	FFP		PF24	
Duration of storage at ≤−18°C	Approximately 3 Months	>12 Months	Approximately 3 Months	>12 Months
Collection/anticoagulant	Apheresis/ACD-A	Apheresis/ACD-A	Whole blood/CPD	Whole blood/CPD
No. of donors in each blood group				
A	2	3	1	3
O	2	2	3	2
B or AB	2	1	2	1
Donor sex	1 Woman, 5 men	2 Women, 4 men	Pools of both sexes	Pools of both sexes
Process input volume: Mean ± SD (range), mL	629 ± 21 (586-644)	643 ± 5 (637-650)	611 ± 23 (589-650)	629 ± 29 (588-649)
Time in storage at ≤ −18° C: Mean ±SD (range), days	101 ± 5 (93-106)	385 ± 7 (378-392)	82 ± 8 (73-95)	384 ± 1 (382-385)

plasma is passed by gravity flow through a compound absorption device (CAD) into final storage containers. The addition of 15 mL of amotosalen solution to an approximately 600-mL plasma component results in a dilution of approximately 2.5%.

The levels of global coagulation functional parameters, coagulation factors and proteins of the hemostatic system, anticoagulant proteins, and coagulation and complement activation markers were assessed according to a predetermined protocol.

Thawing, sampling, and storage of thawed plasma components

Amotosalen/UVA-treated FFP and PF24 components were thawed following AABB guidelines, using a QuickThaw Plasma Thawing System (Helmer, Inc.). For both amotosalen/UVA-treated FFP and PF24, six products were thawed after approximately 3 months of storage, and another six were thawed after more than 12 months of storage. After thawing, the units were placed into storage at 1 to 6°C, and samples were taken on Days 0 and 5 post thaw. Plasma components were only removed from 1 to 6°C storage for sampling. Luer adaptors were connected to the storage container of all plasma components using a sterile connection device. Before sampling, plasma components were gently mixed end-over-end, a sample of approximately 30 mL was withdrawn from the plasma components and was divided evenly among twenty 2-mL polypropylene, screw-top tubes labeled with replicate numbers and time points. The samples were frozen and maintained at or below −65°C until analysis.

Sample analysis

Analyses of samples for plasma characteristics and coagulation function, as listed in Table S1 (available as supporting information in the online version of this paper), were conducted by Machaon Diagnostics, a Clinical Laboratory Improvement Amendments-certified laboratory, following standard operating procedures established by the testing facility. Sample boxes were transported on dry ice to Machaon Diagnostics, where they were stored frozen until immediately before testing. Samples from all time points were analyzed on the same day. Thrombin generation was performed using a commercial Calibrated Automated Thrombogram (CAT) assay (Diagnostica Stago). For each plasma type, data from the approximately 3-month (six products) and more than 12-month (six products) frozen products were combined to provide data for a mix of frozen storage periods.

Statistical analysis

The stability of thawed amotosalen/UVA-treated FFP and PF24 on Days 0 and 5 of storage was descriptively summarized. The mean treatment difference and 95% confidence interval (CI) between the Day 0 and Day 5 means were compared using two-tailed t tests for paired samples. For protein S and α2-plasmin inhibitors, the proportions of components with values 0.4 IU/mL or more (40 IU/dL) were summarized, and the lower bound of a one-sided 95% Clopper-Pearson CI was calculated. A p value less than 0.05 was considered significant.

RESULTS

Twelve components each of amotosalen/UVA-treated FFP and PF24 were prepared from apheresis and pooled WB plasma, respectively. One-half of the components were either thawed after approximately 3 months or more than 12 months of frozen storage respectively, and the data from all 12 components of each type were combined, in order to mimic routine and worst case use. The study was not powered to examine the effects of frozen storage time on factor levels. The plasma components comprised a mixture of ABO types and donor genders, and were anticoagulated with ACD-A or CPD according to standard collection practices (Table 1), in order to test the diversity of plasma sources that may be converted to TP. These differences in manufacturing limit the direct comparisons of the amotosalen/UVA-treated FFP and PF24 results; therefore, we restricted the analyses to the factor content of

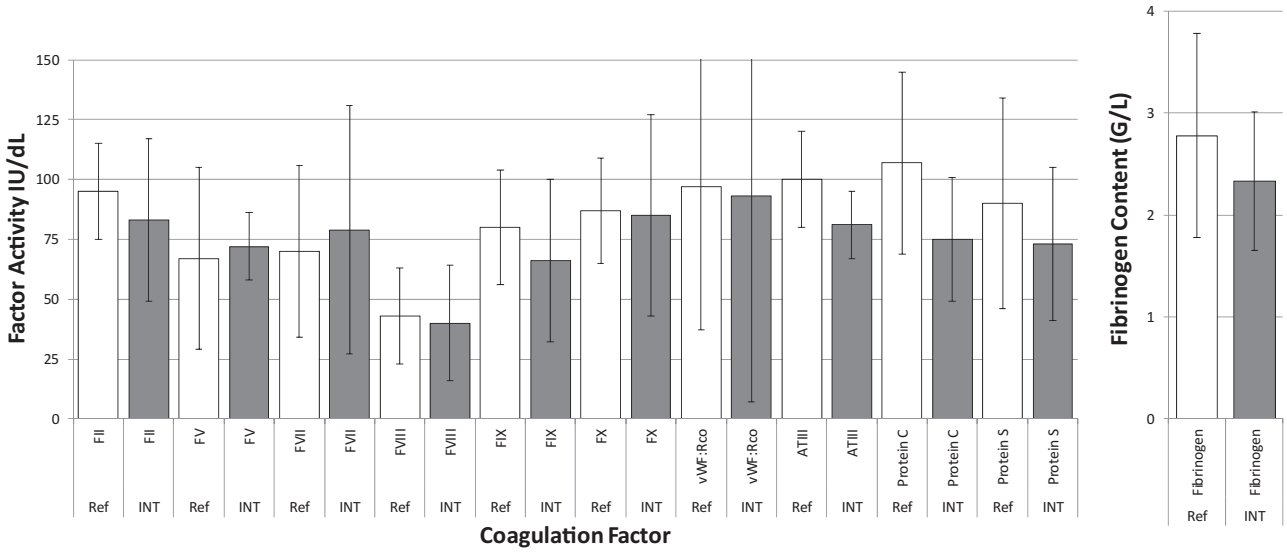

Fig. 1. Comparison of amotosalen/UVA light-treated fresh-frozen plasma (FFP) (INT): Day 5 mean results and 95% confidence intervals with Day 5 thawed FFP results (Ref) quoted in the *Circular of Information*.[3]

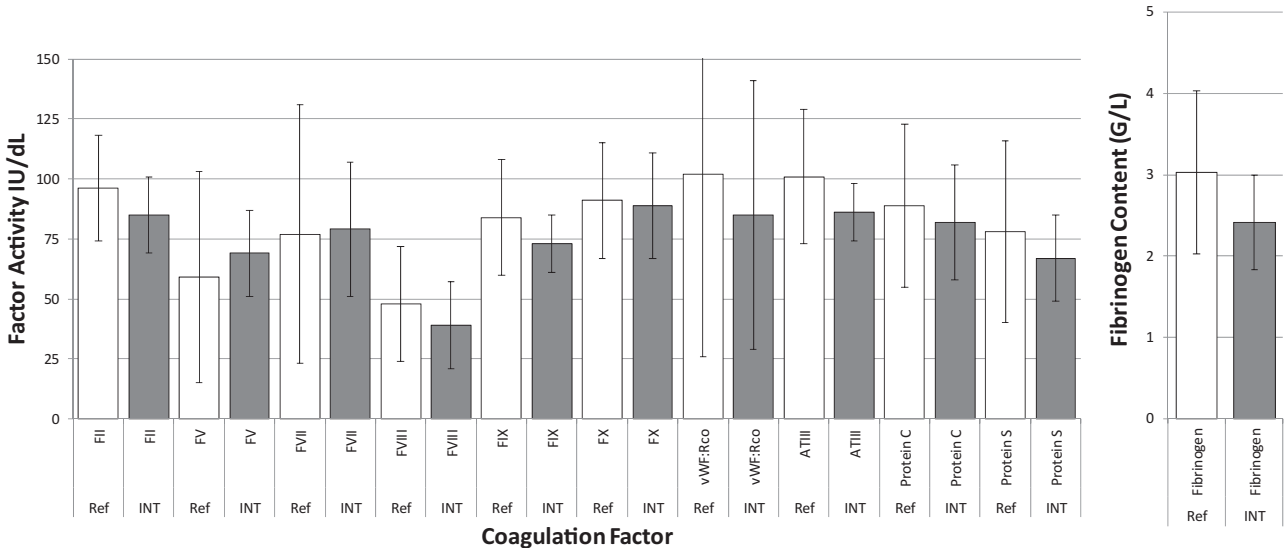

Fig. 2. Comparison of amotosalen/UVA light-treated plasma frozen within 24 hours (PF24) (INT): Day 5 mean results and 95% confidence intervals (INT) with Day 5 thawed PF24 results (Ref) quoted in the *Circular of Information*.[3]

each plasma type on Days 0 and 5 of storage. For Factor VIII and von Willebrand factor ristocetin cofactor (vWF:RCo), as expected, Group O donors tended to have lower levels than non-Group O donors (data not shown), and the study was not powered to allow a separate analysis of Group O versus non-Group O components.

Hemostatic capacity, coagulation factors, and proteins of the hemostatic system

Comparisons of Day 5 thawed, amotosalen/UVA-treated FFP and PF24 with conventional thawed FFP and PF24 were conducted to characterize the impact of pathogen-reduction treatment (Figs. 1 and 2). Conventional values are derived from Scott and colleagues.[3] Factors II, V, VII, VIII, IX, and X; vWF:RCo; antithrombin III (ATIII); protein C; and protein S levels were not substantially different between amotosalen/UVA-treated versus conventional FFP or PF24, and all factors had broadly overlapping 95% CIs. Most factors described by Scott and colleagues,[3] as well as Factor XI, α2 plasmin inhibitor, and von Willebrand factor-cleaving protease (ADAMTS-13) antigen and activity, were more than 90% of Day 0 levels when measured on Day 5 (Tables 2 and 3). In contrast, comparison of Day 0

TABLE 2. Thawed amotosalen/ultraviolet A light-treated fresh-frozen plasma after 5 days of storage at 1 to 6°C

Marker	Day 0 value: Mean ± SD (range), N = 12*	Day 5 value: Mean ± SD, (range) N = 12*	Mean difference between Day 0 and Day 5 results [95% CI]	Day 5 percentage of Day 0 value: Mean ± SD (range), %	Thawed FFP COI with Day-1 as the reference: Mean ± SD (range)†	Laboratory clinical reference range, 95% CI‡
Factor						
Factor II, IU/mL	0.80 ± 0.17 (0.61-1.16)	0.83 ± 0.17 (0.62-1.17)	0.02 [0.00, 0.05]§	103 ± 5 (93-108)	0.97 ± 0.10 (0.83-1.25)	0.72-1.20
Factor V, IU/mL	0.83 ± 0.12 (0.66-1.05)	0.72 ± 0.07 (0.63-0.84)	−0.10 [−0.15, −0.05]§	88 ± 8 (71-95)	0.85 ± 0.13 (0.63-1.04)	0.66-1.02
Factor VII, IU/mL	0.72 ± 0.24 (0.43-1.32)	0.79 ± 0.26 (0.46-1.44)	0.07 [− 0.02, 0.16]	112 ± 26 (96-194)	1.05 ± 0.25 (0.50-1.63)	0.45-1.33
Factor VIII, IU/mL	0.65 ± 0.17 (0.32-0.87)	0.40 ± 0.12 (0.19-0.57)	−0.25 [−0.31, −0.18]§	62 ± 11 (42-80)	0.81 ± 0.19 (0.47-1.17)	0.50-1.49
Factor IX, IU/mL	0.72 ± 0.18 (0.51-1.07)	0.66 ± 0.17 (0.47-1.10)	−0.06 [−0.10, −0.02]§	92 ± 7 (80-103)	0.82 ± 0.13 (0.62-1.08)	0.68-1.36
Factor X, IU/mL	0.83 ± 0.20 (0.40-1.18)	0.85 ± 0.21 (0.41-1.28)	0.02 [−0.02, 0.06]	103 ± 8 (91-122)	0.94 ± 0.10 (0.71-1.12)	0.68-1.44
vWF:RCo activity, IU/ml	1.01 ± 0.34 (0.53-1.76)	0.93 ± 0.43 (0.38-1.94)	−0.08 [−0.20, 0.04]	90 ± 19 (40-110)	1.01 ± 0.26 (0.61-1.52)	0.50-1.50
Fibrinogen, g/L	2.38 ± 0.35 (1.71-2.85)	2.33 ± 0.34 (1.66-2.76)	−0.05 [−0.15, 0.04]	98 ± 7 (86-112)	2.80 ± 0.52 (2.23-4.55)	1.70-4.10
Antithrombin III, IU/mL	0.83 ± 0.08 (0.67-0.96)	0.81 ± 0.07 (0.65-0.90)	−0.02 [−0.03, −0.01]§	98 ± 2 (94-100)	0.97 ± 0.09 (0.85-1.18)	0.86-1.16
Protein C, IU/mL	0.77 ± 0.13 (0.55-1.03)	0.75 ± 0.13 (0.59-1.05)	−0.02 [− 0.07, 0.02]	98 ± 9 (74-107)	1.07 ± 0.20 (0.74-1.48)	0.68-1.21
Protein S, IU/mL	0.86 ± 0.12 (0.71-1.04)	0.73 ± 0.16 (0.41-0.95)	−0.13 [−0.19, −0.07]§	85 ± 0.12 (56-105)	0.97 ± 0.18 (0.61-1.23)	0.55-1.43
Factors not listed in COI Table 3a						
α2-Plasmin inhibitor, IU/mL	0.83 ± 0.16 (0.57-1.20)	0.83 ± 0.16 (0.57-1.14)	0.00 [−0.03, 0.03]	100 ± 0.6 (86-109)	Not applicable	0.68-1.36
Factor XI, IU/mL	0.73 ± 0.17 (0.49-1.15)	0.69 ± 0.19 (0.46-1.18)	−0.03 [−0.05, −0.015]§	95 ± 4 (90-103)	Not applicable	0.60-1.52
ADAMTS-13 antigen, μg/mL	1.29 ± 0.44 (0.80-2.25)	1.32 ± 0.43 (0.71-2.22)	0.03 [−0.07, 0.17]	103 ± 16 (83-134)	Not applicable	0.60-1.60
ADAMTS-13 activity, %	92 ± 19 (48-126)	91 ± 19 (56-118)	−1.3 [−9.6, 7.0]	100 ± 14 (78-118)	Not applicable	40-130
Markers of global coagulation						
Prothrombin time, s	15.6 ± 1.8 (12.7-17.8)	16.0 ± 2.0 (13.0-18.9)	0.5 [−0.1, 1.0]	103 ± 5 (86-106)	Not applicable	10.4-12.3
Activated partial thromboplastin time, s	30.8 ± 3.1 (23.8-36.1)	34.1 ± 3.2 (27.2-40.0)	3.3 [3.0, 3.7]	111 ± 2 (107-114)	Not applicable	19.7-30.8
Thrombin generation, endogenous thrombin potential, nM/min; 5 pM TF	2005 ± 395 (1426-2782)	1866 ± 402 (1432-2668)	−138.4 [−267.3, −9.5]§	93 ± 9 (70-106)	Not applicable	1336-2195
Markers of activation						
Thrombin-generation peak height, nM; 5 pM TF	232 ± 67 (149-368)	179 ± 85 (95-368)	−52.8 [−80.1, −25.6]	75 ± 19 (49-119)	Not applicable	127-328
Thrombin-antithrombin complexes, μg/L	2.2 ± 0.3 (<2.0-3.0)	2.3 ± 0.9 (<2.0-5.3)	0.15 [−0.3, 0.6]	105 ± 23 (84-177)	Not applicable	<4.3
Nonactivated partial thromboplastin time, s	115.1 ± 14.7 (96.8-145.1)	133.0 ± 29.2 (60.2-167.6)	18.0 [2.7, 33.2]§	116 ± 23 (62-155)	Not applicable	79.8-139.0
Factor VIIa, ng/mL	4.1 ± 1.1 (<3.6-6.9)	17.6 ± 44.9 (<3.6-159.9)	13.5 [−14.4, 41.4]	303 ± 638 (100-2328)	Not applicable	~5.0
Complement C3a, ng/mL	64.0 ± 45.4 (17.4-186.2)	346.2 ± 463.4 (40.0-1730.8)	282.3 [−0.8, 565.3]	508 ± 512 (179-2006)	Not applicable	33.8-268.1

*Means ± standard deviations (ranges) are shown for combined results from samples that were assayed after approximately 3 months and >12 months of frozen storage.
†The COI Table 3a Day 0 value was used as a reference when available.
‡Reference ranges were established by Machaon Laboratory on fresh-frozen, normal-donor plasma samples.
§$P < 0.05$ (two-tailed t tests on paired samples) when comparing the mean activity at thaw (Day 0) with the mean activity after storage for 120 hours at 1 to 6°C.

TABLE 3. Thawed amotosalen/ultraviolet A light-treated plasma frozen within 24 hours after 5 days of storage at 1 to 6°C

Marker	Day 0 value: Mean ± SD, (range), N = 12*	Day 5 value: Mean ± SD (range), N = 12*	Mean difference between Day 0 and Day 5 results [95% CI]	Day 5 percentage of Day 0 value: Mean ± SD (range), %	Thawed PF24 COI with Day 0 as the reference: Mean ± SD (range†)	Laboratory clinical reference range, 95% CI‡
Factor						
Factor II, IU/mL	0.83 ± 0.09 (0.65-0.95)	0.85 ± 0.08 (0.68-0.97)	0.02 [−0.01, 0.04]	103 ± 5 (94-109)	0.97 ± 0.08 (0.80-1.13)	0.72-1.20
Factor V, IU/mL	0.8 ± 0.11 (0.67-1.02)	0.69 ± 0.09 (0.56-0.83)	−0.18 [0.22, −0.13]§	80 ± 8 (67-97)	0.86 ± 0.16 (0.54-1.24)	0.66-1.02
Factor VII, IU/mL	0.72 ± 0.08 (0.61-0.86)	0.79 ± 0.14 (0.64-1.20)	0.06 [−0.04, 0.17]	110 ± 27 (95-197)	0.89 ± 0.22 (0.54-1.45)	0.45-1.33
Factor VIII, IU/mL	0.58 ± 0.12 (0.42-0.78)	0.39 ± 0.09 (0.24-0.58)	−0.19 [−0.25, −0.13]§	68 ± 13 (38-84)	0.66 ± 0.17 (0.30-1.00)	0.50-1.49
Factor IX, IU/mL	0.77 ± 0.09 (0.62-0.95)	0.73 ± 0.06 (0.59-0.81)	−0.03 [−0.07, 0.01]	96 ± 8 (78-109)	0.88 ± 0.13 (0.70-1.05)	0.68-1.36
Factor X, IU/mL	0.88 ± 0.08 (0.78-1.03)	0.89 ± 0.11 (0.74-1.12)	0.01 [−0.02, 0.04]	101 ± 5 (95-113)	0.94 ± 0.11 (0.72-1.12)	0.68-1.44
vWF:RCo activity, IU/ml	0.88 ± 0.22 (0.52-1.06)	0.85 ± 0.28 (0.42-1.36)	−0.03 [−0.14, 0.08]	96 ± 22 (57-142)	1.23 ± 0.47 (0.58-2.38)	0.50-1.50
Fibrinogen, g/L	2.44 ± 0.32 (1.80-2.87)	2.41 ± 0.29 (1.71-2.75)	−0.03 [−0.17, 0.11]	99 ± 10 (85-122)	3.09 ± 0.70 (2.11-5.00)	1.70-4.10
Antithrombin III, IU/mL	0.89 ± 0.07 (0.77-0.99)	0.86 ± 0.06 (0.78-0.95)	−0.03 [−0.05, −0.00]§	97 ± 4 (88-101)	0.97 ± 0.11 (0.77-1.10)	0.86-1.16
Protein C, IU/mL	0.87 ± 0.12 (0.68-1.02)	0.82 ± 0.12 (0.61-0.97)	−0.05 [−0.11, 0.02]	95 ± 12 (80-117)	0.88 ± 0.16 (0.65-1.20)	0.68-1.21
Protein S, IU/mL	0.82 ± 0.08 (0.72-1.00)	0.67 ± 0.09 (0.52-0.84)	−0.15 [−0.18, −0.12]§	81 ± 6 (68-94)	0.92 ± 0.18 (0.54-1.21)	0.55-1.43
Factors not listed in the COI						
α-2-Plasmin inhibitor, IU/mL	0.88 ± 0.09 (0.75-1.03)	0.88 ± 0.09 (0.70-1.01)	0.00 [−0.05, 0.05]	100 ± 10 (86-124)	Not applicable	0.68-1.36
Factor XI, IU/mL	0.77 ± 0.11 (0.56-0.94)	0.76 ± 0.12 (0.55-0.95)	−0.01 [−0.04, 0.01]	98 ± 4 (92-106)	Not applicable	0.60-1.52
ADAMTS-13 antigen; μg/mL	1.36 ± 0.38 (0.66-2.12)	1.26 ± 0.35 (0.64-1.82)	−0.10 [−0.19, −0.02] §	93 ± 9 (78-110)	Not applicable	0.60-1.60
ADAMTS-13 activity; %	93 ± 11 (73-112)	90 ± 12 (71-108)	−3 [−7.3, 1.5]	97 ± 7 (78-107)	Not applicable	40-130
Markers of global coagulation						
Prothrombin time, s	15.4 ± 1.1 (14.0-17.0)	16.5 ± 1.0 (15.4-18.8)	1.1 [0.5-1.6]§	107 ± 5 (91-111)	Not applicable	10.4-12.3
Activated partial thromboplastin time, s	30.1 ± 3.4 (25.7-37.2)	32.5 ± 3.3 (27.3-39.4)	2.4 [1.9-2.9]§	108 ± 3 (103-112)	Not applicable	19.7-30.8
Thrombin generation, endogenous thrombin potential, nM/min; 5 pM TF	1918 ± 289 (1414-2356)	1800 ± 277 (1355-2206)	−118 [−181, −56]§	94 ± 5 (87-106)	Not applicable	1336-2195
Markers of activation						
Thrombin-generation peak height, nM; 5-pM TF	228 ± 47 (150-336)	170 ± 63 (98-318)	−57.8 [−83, −32]§	74 ± 17 (58-125)	Not applicable	127-328
Thrombin-antithrombin complexes, μg/L	2.3 ± 0.6 (<2.0-3.9)	2.4 ± 0.8 (<2.0-4.7)	0.1 [−0.3, 0.5]	96 ± 7 (87-100)	Not applicable	<4.3
Nonactivated partial thromboplastin time, s	96.4 ± 10.7 (75.8-111.8)	121.0 ± 24.0 (59.9-144.9)	24.6 [7.8, 41.4]§	127 ± 28 (57-159)	Not applicable	79.8-139.0
Factor VIIa, ng/mL	5.3 ± 3.0 (3.1-12.7)	20.2 ± 43.2 (<3.6-156.6)	14.9 [−12.4, 42.2]	372 ± 742 (67-2709)	Not applicable	~5.0
Complement C3a, ng/mL	56.5 ± 28.2 (18.7-110.7)	232.2 ± 249.4 (64.6-962.1)	175.8 [28.2, 323.3]§	389 ± 258 (179-975)	Not applicable	33.8-268.1

*Means, standard deviations, and ranges of combined results are shown from samples that were assayed after approximately 3 months and >12 months of frozen storage.
†See COI Table 3a (the Day 0 value was used as reference when available).
‡Reference ranges were established by the Machaon Laboratory on fresh-frozen, normal-donor plasma samples.
§$P < 0.05$ (two-tailed *t* test on paired samples) when comparing the mean activity at thaw (Day 0) versus the mean activity after 120 hours of storage at 1 to 6°C.

and Day 5 post-thaw factor levels for Factors V and VIII and protein S displayed a greater than 10% decline after 5 days at 1 to 6°C, as previously described by Scott and colleagues.[3]

Factor VIII had the greatest decline in activity, with a mean value of 62% of Day 0 results for amotosalen/UVA-treated FFP and 68% for amotosalen/UVA-treated PF24. Analysis by ABO blood group revealed that Group O donors had the lowest absolute Factor VIII levels on Day 5 (amotosalen/UVAFF = 35.5 ± 13.8 IU/dL [n = 4]; amotosalen/UVA PF24 = 31.8 ± 6.6 IU/dL [n = 5]), although the relative decline from Day 0 was similar to that observed in other blood groups (data not shown).

Of note, α2-plasmin inhibitor levels were unchanged on Day 5 post thaw compared with Day 0 values, and all were above 0.4 IU/mL. Similarly, all protein S levels were greater than 0.4 IU/mL on Day 5 post thaw, despite a statistically significant ($p < 0.05$) mean decline of 15% and 19% in amotosalen/UVA-treated FFP and PF24, respectively.

Analyses using paired t tests revealed statistically significant differences between Day 0 and Day 5 results for both amotosalen/UVA-treated FFP and PF24 for Factors V and VIII, ATIII, and protein S ($p < 0.05$). Similarly, significant differences for amotosalen/UVA-treated FFP were demonstrated for Factors II and IX and for amotosalen/UVA-treated PF24 for ADAMTS-13 antigen.

Global coagulation parameters: thrombin generation, PT, and aPTT

Thrombin generation, ETP, as a measure of global hemostatic capacity, was maintained in thawed amotosalen/UVA-treated plasma over 5 days of storage at 1 to 6°C (Tables 2 and 3). Day 5 values for thawed amotosalen/UVA-treated FFP and PF24 were 1866 ± 402 nM/minute and 1800 ± 277 nM/minute, respectively, when stimulated with 5 pM tissue factor and 4 μM phospholipids, indicating robust thrombin generation activity, although it was statistically significantly decreased ($p < 0.05$) compared with the Day 0 results. The mean percentage activity for Day 5 compared with Day 0 was 93% in thawed, amotosalen/UVA-treated FFP and 94% in thawed, amotosalen/UVA-treated PF24 compared with Day 0 baseline values. All components had levels higher than the minimum laboratory clinical reference range for normal donor plasma at the time of initial thaw (Tables 2 and 3).

PT increased over 5 days of storage at 1 to 6°C in thawed amotosalen/UVA-treated FFP and PF24 (Tables 2 and 3). The Day 5 PT mean percentages for thawed amotosalen/UVA-treated FFP and PF24 were 103% and 107%, respectively, compared with freshly thawed plasma (Day 0), achieving statistical significance for amotosalen/UVA-treated PF24. Over 5 days of storage at 1 to 6°C, the aPTT was statistically significantly ($p < 0.05$) prolonged in thawed amotosalen/UVA-treated FFP and PF24 (Tables 2 and 3). The mean values for Day 5 aPTT in thawed amotosalen/UVA-treated FFP and PF24, compared with Day 0 values, were 111% and 108%, respectively.

Markers of activation of coagulation and complement

Excessive activation of the coagulation cascade may be demonstrated by increased thrombin-antithrombin complexes, increased peak thrombin generation, or shortened nonactivated partial thromboplastin time.[11,12] In contrast, on Day 5 compared with Day 0, thrombin-antithrombin complexes were unchanged. Thrombin-generation peak height was decreased (statistically significantly for amotosalen/UVA-treated PF24), and nonactivated partial thromboplastin time was statistically significantly increased for both amotosalen/UVA-treated FFP and PF24 ($p < 0.05$). These data indicate that there was no excessive activation of coagulation with post-thaw storage. Mean levels of Factor VIIa and complement (C3a) trended higher with post-thaw storage, achieving statistical significance for C3a in amotosalen/UVA-treated PF24. Examination of individual thawed units for Factor VIIa revealed isolated increases of Day 5 values for two units stored frozen for more than 12 months (Table 4: FFP, unit # 11 =159.9 ng/mL and PF24, unit # 8 =156.6 ng/mL), whereas all units showed moderately increased levels of complement C3a on Day 5 post thaw compared with Day 0 (Table 4), and the highest values were observed in the same two units that had elevated Factor VIIa levels. Despite these observations, these two units showed robust thrombin generation, ETP (2021 nM/minute and 2030 nM/minute, respectively).

DISCUSSION

Plasma transfusion was historically administered in the form of FFP and was indicated for replacement of multiple plasma coagulation factors (e.g., in consumptive coagulopathies and liver failure), warfarin anticoagulation reversal, single coagulation factor replacement (e.g., Factor XI deficiency), and as a replacement fluid in plasma exchange for specific diseases states (e.g., thrombotic thrombocytopenic purpura [TTP]).[10] The lack of a robust evidence basis for many indications led to the realization that plasma is often transfused inappropriately and, when indicated, tends to be under-dosed. With the introduction of evidence-based medical practices, the use of plasma transfusion has declined by approximately 25% over the last 6 years.[1] Simultaneously, the adoption of transfusion-related acute lung injury (TRALI) mitigation measures, which include the use of plasma from mostly male donors, led to the predominant use of plasma frozen within 24 hours (PF24), a form more easily manufactured and thus in increased supply, especially for ABO blood group AB plasma. There has also been a growing realization that early and aggressive transfusion of plasma may be lifesaving in the treatment of military and civilian trauma[13-16]

TABLE 4. Factor VIIa and complement C3a in individual units of amotosalen/ultraviolet A fresh-frozen plasma and plasma frozen within 24 hours on post-thaw Days 0 and 5

	Amotosalen/UVA FFP						Amotosalen/UVA PF24					
	Approximately 3 months			>12 months			Approximately 3 months			>12 months		
Marker	Unit	Day 0	Day 5	Unit	Day 0	Day 5	Unit	Day 0	Day 5	Unit	Day 0	Day 5
Factor VIIa, ng/mL	1	<3.6	<3.6	7	<3.6	6.6	1	5.0	<3.6	7	3.8	11.3
	2	<3.6	<3.6	8	<3.6	4.4	2	5.4	<3.6	8	5.8	156.6
	3	<3.6	<3.6	9	<3.6	5.0	3	<3.6	<3.6	9	3.1	10.9
	4	<3.6	<3.6	10	<3.6	<3.6	4	<3.6	<3.6	10	12.7	14.7
	5	<3.6	<3.6	11	6.9	159.9	5	<3.6	<3.6	11	4.0	11.8
	6	<3.6	<3.6	12	6.1	10.1	6	<3.6	<3.6	12	10.0	16.1
Complement C3a, ng/mL	1	46.8	84.0	7	37.9	182.3	1	66.2	126.8	7	65.4	227.0
	2	58.8	141.8	8	71.8	331.6	2	72.2	129.2	8	98.7	962.1
	3	39.4	99.8	9	23.9	214.3	3	31.0	79.8	9	110.7	293.6
	4	17.4	40.0	10	93.3	463.9	4	31.8	64.6	10	61.9	334.5
	5	34.2	68.2	11	86.3	1730.8	5	38.4	71.0	11	18.7	132.0
	6	186.2	538.8	12	71.5	259.0	6	34.0	75.6	12	48.4	290.6

and in patients who require massive transfusion. Retrospective observational studies support the ready availability of TP, or even liquid plasma, in trauma units and even on ambulances or helicopters for transfusion before admission for exsanguinating patients.[15] The randomized controlled PROPPR study[17] revealed that plasma given in a 1:1:1 ratio with platelets and red blood cell concentrates was more effective in achieving early hemostasis than a lower 1:1:2 ratio, although no difference was apparent in 24-hour or 30-day survival. Taken together, these findings led to the common use of massive transfusion protocols incorporating plasma (especially of Group A or AB type) that are rapidly available for massively bleeding patients. With these changes in practice, the majority of plasma for transfusion in the United States is now converted to TP, allowing for maintenance of a stock of emergency use plasma in or close to the trauma center, and simultaneously reducing waste because the 5-day post-thaw shelf-life permits plasma to be rotated to patient use near the outdate.[1] The use of TP is especially important for patients undergoing daily plasma exchange for TTP in which extra units thawed on one day may now be used on the next, because ADAMTS-13 activity is well conserved.[6]

Conventional TP, however, carries the risk of window-period and/or emerging pathogen transmission and of bacterial outgrowth during storage. Little is known about the risk of bacterial contamination of TP; however, plasma is known to be contaminated at similar levels as WB or apheresis platelet collections; and cryophilic bacteria, such as *Pseudomonas* spp., *Listeria* spp., and *Yersinia* spp., may proliferate despite storage at 1 to 6°C. FFP and PF24 prepared with pathogen-reduction treatment using amotosalen/UVA light was efficacious for factor replacement in patients with multiple and isolated coagulation deficiencies and for the treatment of TTP, in which patients may be exposed to more than 100 units of plasma in a short period of time during plasma exchange.[18-20] Amotosalen/UVA treatment has also been shown to offer robust pathogen inactivation of a wide range of bacteria, viruses, parasites, and leukocytes.[9,21]

The objective of this study was to evaluate whether the hemostatic capacity of thawed amotosalen/UVA plasma is functionally equivalent to the blood component TP, as defined by the COI. Our analysis of thawed amotosalen/UVA-treated plasma was limited by the lack of established standards for comparison. The COI quotes a single data source (Scott et al., 2009),[3] and that reference describes FFP and PF24 from WB-derived plasma only. No ranges are provided for apheresis plasma, for plasma derived from PF24RT24, or for the similar product "thawed plasma, cryoprecipitate reduced." Given these differences and because our measurements were performed by different laboratories using different methods, a statistical comparison with Scott and colleagues. is not valid. Nevertheless, the broadly overlapping 95% CIs suggest that there are no clinically significant differences between amotosalen/UVA and conventional TP (Figs. 1 and 2). Pathogen-reduced TP may be derived from either apheresis or WB plasma, which have different levels of dilution with anticoagulant (10.3 to 21.7% dilution with ACD-A for apheresis plasma (collected with an ACD-A:WB ratio from 1:8 to 1:14; calculated AC volume percentage = 100/[ratio + 1-hct*ratio], where ratio = WB/AC; AC = volume anticoagulant; hct = hematocrit L/L) [personal communication, L. J. Dumont, November 21, 2016]; and 17.0% to 25.7% dilution for WB-derived plasma collected with CPD (assuming 14 mL CPD per 100 mL WB, WB volumes of 450 to 550 mL, and a hematocrit of 0.38-0.55),[10] and from FFP or PF24 with inherently different concentrations of labile coagulation factors. There are no clear metrics for defining the acceptability of TP. Amotosalen/UVA treatment may affect the levels of coagulation factors directly and incorporates an approximately 2.5% dilution with addition of the amotosalen compound.

There are few reports regarding the activation of coagulation factors and complement during post-thaw storage.

We focused on the demonstration of comparability of hemostatic capacity for Day 5 post-thaw, amotosalen/UVA-treated FFP and PF24 with Day 0 values for a broad range of hemostatic and antithrombotic factors, and we included the absolute standards for protein S and α2 plasmin inhibitor levels greater than 0.4 IU/mL. We show that Day 5 thawed, amotosalen/UVA-treated FFP and PF24 contain essentially the same amount of fibrinogen and Factor II as on Day 0 (e.g., 98-103% of Day 0 values) and contain sufficient other factors to initiate robust thrombin generation as an index of hemostatic capacity (mean, 1800-1866 nM/minute on Day 5), measured as the Thrombin generation, ETP after stimulation with 5 pM tissue factor and phospholipids.[22] Thrombin-generation assays provide a global measure of hemostasis, measuring both the propagation phase (with positive feedback loops involving Factors V, VIII, and XI) and the termination phase (including down-regulation by anticoagulant pathways and plasma protease inhibitors) of coagulation. The resulting thrombin-generation curve reflects and integrates all procoagulant and anticoagulant reactions that regulate the formation and inhibition of thrombin.[23] Most individual hemostatic and anticoagulant factors had similar levels (>90%) on Day 5 versus Day 0, with the exception of the known labile factors (Factors V and VIII and protein S), in which levels were decreased more than 10% but were comparable with changes shown for TP in the COI. Similar to the findings of Scott and colleagues, we found that many factors were statistically significantly different on Day 5 relative to Day 0 post thaw; however, there is no indication that these differences amount to clinically significant disparities because, in every case, the individual factor levels were sufficient to support robust thrombin generation.[3] All thawed amotosalen/UVA-treated plasma components met the predetermined FDA acceptance criteria for protein S and α2-plasmin inhibitor (≥0.4 IU/mL) after 5 days of storage (Tables 2 and 3). ADAMTS-13 antigen and activity were retained (97-100% of Day 0 mean values) over 5 days of storage, indicating that thawed amotosalen/UVA-treated plasma may be useful for the treatment of TTP. NAPPT was increased, TAT was unchanged (mean, 95-105% of Day 0 values), and thrombin-generation peak levels were decreased, indicating an overall decrease rather than an increase in coagulation cascade activation. One marker of coagulation activation (Factor VIIa) was sporadically increased, indicating isolated activation of the factor, but these components retained thrombin-generation potential.

Complement C3a levels were sporadically increased on Day 5 after thawing; however, the highest levels measured were comparable to those demonstrated in fresh apheresis plasma (both nonleukoreduced, WB-derived plasma and autopheresis C plasma; Fresenius Kabi). In the study by Norda and colleagues, comparable levels of C3a were rapidly eliminated after transfusion and were not associated with adverse events when transfused into autologous donors.[24]

Our study was limited by the need to freeze and thaw the product and then to freeze and thaw the samples before assay. Scott and colleagues.[3] quote reports suggesting that this freeze/thaw process has little impact on coagulation Factors II, V, VII, IX, and X; fibrinogen; or protein C, protein S, and ATIII values. However, plasma activation was not studied, and it is possible that the isolated increases we detected in Factor VIIa and C3a were due to this limitation.[3] These samples were also 2 to 3 weeks past the outdate when assessed. Nevertheless, our findings suggest that measures of activation should be included in studies of plasma acceptability in the future. We did not assess the sterility of our plasma products or samples due to the robust pathogen-reduction capacity of amotosalen/UVA treatment and the rigorous use of sterile technique and frozen storage for product and samples other than during the post-thaw storage process. Although we did not detect any clinically relevant differences between products stored for approximately 3 months versus more than 12 months, with only 12 samples, our study was not powered to examine the effect of storage duration or small differences between Day 0 and Day 5 samples. Future studies may be designed to examine the impact of prolonged storage, especially if there is a desire to extend frozen storage shelf-life beyond 12 months.

In conclusion, amotosalen/UVA-treated, thawed plasma demonstrated robust thrombin generation and conservation of stable factors (Factor II and fibrinogen concentrations) after 5 days of storage at 1 to 6°C, despite the expected declines in labile Factors V and VIII and protein S. The hemostatic capacity and antithrombotic activity were equivalent to those of conventional TP with the added benefit of pathogen reduction to reduce the risks of bacterial, viral, protozoan, and leukocyte contamination. These data support the use of amotosalen/UVA plasma for up to 5 days with storage at 1 to 6°C post thaw but have not been submitted for FDA review. The INTERCEPT Blood System for plasma is not FDA approved for 5-day post-thaw storage at this time.

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CONFLICT OF INTEREST

AE, KW, TD, NH, SR, LC, NM, and RJB are employees of Cerus Corporation.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's website.

Table S1. Assay methods used by reference laboratory.