Blood Products Advisory Committee
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The Hilton Washington DC North/Gaithersburg
620 Perry Parkway
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ISSUE SUMMARY

Topic: Evaluation of Potential New Plasma Products Manufactured Following Storage at Room Temperature for up to 24 Hours

Issue: FDA has been asked to consider whether plasma for transfusion manufactured either from Whole Blood or by apheresis could qualify for licensure when freezing does not take place for as long as 24 hours following storage at room temperature. These products are respectively identified as WB-PF24RT24 and A-PF24RT24, and are collectively termed PF24RT24. FDA seeks the advice of the Committee whether *in vitro* results comparing protein levels in Fresh Frozen Plasma (FFP) versus PF24RT24 plasma products raise potential safety concerns for the PF24RT24 products either generally or in certain clinical settings.

Background:

Licensed human Fresh Frozen Plasma (FFP) is manufactured by apheresis or from Whole Blood and placed in a freezer within 8 hours after collection in accordance with the Code of Federal Regulations (21CFR 640.34(b)). FDA has also licensed a plasma component derived from units of Whole Blood (WB) that have been held at room temperature for up to 8 hours and then moved to the refrigerator for an additional period provided that the plasma is frozen within 24 hours of collection. This plasma component, which is currently on the market, will be referred to in this summary as WB-PF24. Since 2007, medical device Sponsors have approached FDA with requests to license new plasma products (referred to in this document as PF24RT24) prepared either from Whole Blood that is held at room temperature (RT), 20 ^{o}C to 24 ^{o}C, for up to 24 hours, before plasma separation and freezing, or from apheresis plasma held at RT for up to 24 hours before freezing, for the replacement of non-labile coagulation factors and other plasma proteins. The reported purpose of this request is to address the 8-hour time limit between collection and freezing of the plasma for manufacturing of FFP, and the 8-hour limit between collection and refrigeration for manufacturing of WB-PF24 which impose constraints on the logistics of manufacturing. Currently, mobile collection sites, without freezers or refrigerators, are limited to a geographical distance which allows them to deliver the collected whole blood or plasma back to facilities for processing and freezing or refrigeration within 8 hours of collection. Storage at RT for 24 hours would allow a greater distance covered for blood and plasma collection on mobile units, and also allow platelet components to be prepared from the collections of whole blood. Preparation of platelets from such units would require submissions and approval from the FDA, and will not be discussed at this meeting.

According to the current AABB Circular of Information, the indications for FFP are:

(1) Management of preoperative or bleeding patients who require replacement of multiple plasma coagulation factors (e.g., liver disease, DIC);
(2) Patients undergoing massive transfusion who have clinically significant coagulation deficiencies;
(3) Patients taking warfarin who are bleeding or need to undergo an invasive procedure before vitamin K could reverse the warfarin effect or who need only transient reversal of warfarin effect;
(4) For transfusion or plasma exchange in patients with thrombotic thrombocytopenic purpura (TTP);
(5) Management of patients with selected coagulation factor deficiencies, congenital or acquired, for which no specific coagulation concentrates are available;
(6) Management of patients with rare specific plasma protein deficiencies, such as C1 inhibitor, when recombinant products are unavailable.

According to the AABB Circular of Information, WB-PF24 is used to replace the non-labile coagulation factors. However, in practice, FFP and WB-PF24 are used interchangeably by clinicians[1].

Plasma quality is known to change when stored in liquid form with deterioration of some coagulation proteins and activation of others over a 24-hour period at ambient temperature.[2,3] Several labile blood coagulation factors including Factors V,VIII, XI, and Protein S are especially prone to loss of activity.[4] The literature also suggests that anticoagulant, plasma collection and processing (filtration) procedures, and freezing rate and temperatures, might influence the changes in coagulation protein activities.[5,6] This Issue Summary is focused on the effect of temperature and time of storage on labile coagulation proteins. Recognizing certain fundamental differences in manufacturing methods, the following terminology is used in this Issue Summary to describe two categories of plasma products frozen within 24 hours of collection:

- Plasma units collected by apheresis held at room temperature for up to 24 hours post collection before freezing (A-PF24RT24).
- Plasma units from a Whole Blood collection held at room temperature up to 24 hours post collection before plasma separation and freezing (WB-PF24RT24).

In November 2007 and February 2008, FDA discussed with manufacturers a possible approach to studies that might validate safety and efficacy of new plasma products produced from apheresis or Whole Blood and frozen up to 24 hours after collection. Based on the consideration that the main indication for FFP administration is correction of coagulopathy in various clinical settings, FDA determined that the activity of coagulation proteins in plasma would be a reasonable predictor of the therapeutic effectiveness of plasma. For this reason, FDA asked candidate product Sponsors to examine the activity of coagulation proteins in plasma prepared by procedures involving different hold times at room temperature using a common study design. The Sponsors stated at the meetings that they were not seeking to demonstrate equivalence between the new plasma product and the FFP. In response FDA recommended that a study that would characterize clotting factor levels in each product be conducted. The medical indications would be defined based on the characterization of the products and each label would be unique with the Sponsor's own testing data presented.

In these studies (see below), the Control plasma would be FFP frozen within 8 hours of collection. FDA expected that minimal differences in factor levels would be found and that this information would be provided to the end user in the Indications for Use. However, the data generated in these studies, which are discussed below, suggested a potential safety issue, namely statistically significant, though not necessarily clinically significant, reductions in the functional levels of Factors V, VIII, XI, and Protein S in PF24RT24 products.

The purpose of this BPAC discussion, therefore, is to provide a comprehensive review of the changes in coagulation proteins found in the Sponsors' studies, and to focus closely on the potential safety concerns of the reduction in Protein S seen with A-PF24RT24 and WB-PF24RT24 plasma products. At times in this document, these products are collectively referred to as PF24RT24. The following table defines current and potential plasma products.

Plasma Products described in the discussion	
FFP	FFP was the Control plasma unit that was held at room temperature up to 8 hours post collection before freezing. FFP may be collected through apheresis or separated from a Whole Blood unit.
A-PF24RT24	A-PF24RT24 was the Test plasma unit collected from an apheresis system that was held at room temperature up to 24 hours post collection before freezing.
WB-PF24RT24	WB-PF24RT24 was the Test plasma unit collected from the whole blood unit, which was held at room temperature up to 24 hours, before the plasma was separated and frozen.
WB-PF24	WB-PF24 is a plasma product approved by the FDA and currently on the market. The plasma is separated from the Whole Blood which is held at room temperature for up to 8 hours, and then moved to the refrigerator for an additional period so that the plasma is frozen within 24 hours of collection. This product is included here for comparison only.

Study Design:

The overall study design for characterization of PF24RT24 products was based on discussions at two meetings which were initiated by interested Sponsors and held in November 2007 and February 2008 with the FDA. The meetings concluded as follows:

> "The purpose of this study is to characterize coagulation factor activity levels in plasma collected by apheresis and stored at room temperature (RT) for up to 24 hours (PF24) by assessing the percent change in those factors compared to plasma collected by apheresis and stored at room temperature (RT) for up to 8 hours (FFP). The actual coagulation factor levels will be measured after freezing, storage, and thawing."

In general, the sponsors would conduct a paired study, to account for natural biological variation of plasma proteins and coagulation factors. Each donor would provide a Control unit (FFP) and a Test unit. Samples from Control and Test units would be evaluated for coagulation factor levels. Greater than 20% differences in a particular

assay would indicate a statistical failure. This design was partly based on a previously published study[3].

For A-PF24RT24:

For Sponsors A (n = 52) and B, (n = 54) large volumes of plasma (400 - 535 mL) were collected from normal (healthy) donors using an apheresis system. Half of the volume from each collected plasma unit was transferred to a second plasma storage bag. Paired plasma products (Control FFP and A-PF24RT24) were processed in accordance with the following and then were placed in a freezer at - 20°C ± 2°C:

a. The Control units (FFP) were held at room temperature for 7-8 hours post collection.
b. The Test units (A-PF24RT24) were held at room temperature for 23-24 hours post collection.

For WB-PF24RT24:

I. For Sponsor C, a paired study (n = 66 pairs) was conducted in which every subject donated two units of Whole Blood, 8 weeks apart. One unit was randomly assigned to the Test, and the other one was to the Control.

   a. The Control plasma (FFP) units (n = 66) were derived from Whole Blood (WB) units which were held at room temperature up to 8 hours for the control (note: the mean time from collection to filtration was 1.51 hours), WB was then leukocyte reduced through a FDA approved Whole Blood filter, and then separated plasma was placed in a freezer at – 20 °C ± 2 °C.

   b. The Test plasma (WB-PF24RT24) units (n = 66) were derived from Whole Blood units which were held at 20 °C to 24 °C up to 24 hours, WB was then leukocyte reduced through an investigational Whole Blood filter, and then separated plasma was placed in a freezer at – 20 °C ± 2 °C.

II. For Sponsor D, a non-paired study was conducted in which every subject donated one unit of Whole Blood. Three study groups (n = 60 units/group, total 180 units) were studied as follows:

   a. The Control plasma (FFP) units (n = 60) were derived from Whole Blood (WB) units which were held at room temperature up to 8 hours, WB was then leukocyte reduced through an FDA approved Whole Blood filter, and then the separated plasma was placed in a freezer at < –18°C.

   b. The Test plasma (Test-FFP) units were derived from Whole Blood units which were held at room temperature up to 8 hours, WB was then leukocyte reduced through an investigational Whole Blood filter, and then the separated plasma was placed in a freezer at < –18°C.

   c. The Test plasma (WB-PF24RT24) units were derived from Whole Blood units which were held at room temperature up to 24 hours, WB was then leukocyte

reduced through an investigational Whole Blood filter, and then the separated plasma was placed in a freezer at < –18°C.

All plasma units (studies for A-PF24RT24 and WB-PF24RT24) were stored frozen for at least 1 month. Sponsors pre-qualified the participating test sites according to the protocol to ensure that the sites followed the same standard operating procedures thereby minimizing site-specific effects. The procedures were carried out according to the instructions and precautions described in the Operator's Manual and instructions for use. For each Sponsor, all plasma units from the different sites were shipped to a central testing laboratory (different central laboratories were used by different Sponsors) after collection, for processing and freezing. Subsequently, Test plasma and FFP (Control) units were thawed at the same time at the central laboratory using a water-bath set to 30-37 °C. The expectation was that the Sponsor would be able to label the product with the content of clotting factors according to the outcome of this characterization study.

Objective

The purpose of these studies was to characterize the new plasma products compared to the Control (FFP) regarding the coagulation proteins (listed under the Primary Outcome Measures / Coagulation Assays section).

Primary Outcome Measures / Coagulation Assays

The primary outcome measured was the post-thaw assay results for Test and Control units. The following four groups of assays were performed:

Extrinsic and intrinsic coagulation pathways
- Prothrombin Time (PT)
- Activated Partial Thromboplastin Time (aPTT)

Coagulation factors
- Factor V (FV)
- Factor VIII (FVIII)
- Factor XI (FXI)
- Von Willebrand Factor Ristocetin Cofactor Activity (VWF/RCo)

Coagulation inhibitors
- Protein C
- Protein S
- Antithrombin III (AT III)

Markers of activation
- Activated Factor VII (FVIIa)
- Fibrinopeptide A (FPA) or other indicators of coagulation factor activation
- Thrombin-Antithrombin Complex (TAT)
- Prothrombin fragment 1.2 (f1.2)
- Fibrinogen

Results:

FDA conducted an analysis of the data provided by all four Sponsors for the plasma products.

Table 1 presents the proportion and percentage of paired samples in which coagulation protein levels in PF24RT24 are greater or less than those of FFP by more than 20% and the associated Clopper Pearson 95% confidence intervals. The left side of the table shows the number of pairs with protein values in PF24RT24 stored plasma that are greater than FFP by more than 20%. Whereas the right side of the table shows the number of pairs with protein values in PF24RT24 stored plasma that are less than FFP by more than 20%. The results from Sponsor D are not included in Table 1 because a non-paired design and hence a paired analysis was not performed.

Table 1. Proportion and percentage of pairs exceeding more than 20% differences and Clopper Pearson 95% CI. The values in parentheses are upper and lower 95% confidence limits.

Factor	PF24RT24 > FFP by more than 20%			PF24RT24 < FFP by more than 20%		
	Sponsor A A-PF24RT24 (n=52)	**Sponsor B A-PF24RT24 (n=54)**	**Sponsor C WB-PF24RT24 (n=66+)**	**Sponsor A A-PF24RT24 (n=52)**	**Sponsor B A-PF24RT24 (n=54)**	**Sponsor C WB-PF24RT24 (n=65-66+)**
PT (sec)	0	0	0	0	0	1/66 (1.5%) (0, 8.2)
aPTT (sec)	0	0	14/66 (21.2%) (12.1, 33.0)	0	0	1/66 (1.5%) (0, 8.2)
FV (IU/dL)	1/52 (1.9%) (0, 10.3)	1/54 (1.9%) (0, 9.9)	7/65 (10.8%) (4.4, 20.9)	0	0	10/65 (15.4%) (7.6, 26.5)
FVIII (IU/dL)	0	0	6/66 (9.1%) (3.4, 18.7)	4/52 (7.7%) (2.1, 18.5)	8/54 (14.8%) (6.6, 27.1)	23/66 (34.8%) (23.5, 47.6)
FXI (IU/dL)	0	0	0	0	0	48/66 (72.7%) (60.4, 83.0)
vWF (IU/dL)	0	4/54 (7.4%) (2.1, 17.9)	19/66 (28.8%) (18.3, 41.3)	1/52 (1.9%) (0, 10.3)	3/54 (5.6%) (1.2, 15.4)	14/66 (21.2%) (12.1, 33.0)
Protein C (IU/dL)	0	0	7/66 (10.6%) (4.4, 20.6)	0	0	2/66 (3.0%) (0.4, 10.5)
Protein S (IU/dL)	0	1/54 (1.9%) (0, 9.9)	1/66 (1.5%) (0, 8.2)	**4/52 (7.7%) (2.1, 18.5)**	**7/54 (13.0%) (5.4, 24.9)**	**30/66 (45.5%) (33.1, 58.2)**
AT III (IU/dL)	0	1/54 (1.9%) (0, 9.9)	7/66 (10.6%) (4.4, 20.6)	0	0	0
FVIIa (IU/dL)*	17/52 (32.7%) (20.3, 47.1)	2/54 (3.7%) (0, 12.8)	43/66 (65.2%) (52.4, 76.5)	17/52 (32.7%) (20.3, 47.1)	8/54 (14.8%) (6.6, 27.1)	9/66 (13.6%) (6.4, 24.3)
FPA (nM)	27/52 (51.9%) (37.6, 66.0)	NA	NA	15/52 (28.9)% (17.1, 43.1)	NA	NA
TAT (ng/mL)	NA	1/54 (1.9%) (0, 9.9)	22/66 (33.3%) (22.2, 46.0)	NA	3/54 (5.6%) (1.2, 15.4)	12/66 (18.2%) (9.8, 28.6)
Fibrinogen (g/L)	NA	NA	7/66 (10.6%) (4.4, 20.6)	NA	NA	5/66 (7.6%) (2.5, 16.8)

+ An apparent outlier was excluded from the analysis for one plasma unit: factor V value of 2. This was deemed a laboratory error

*For Sponsor B, the FVIIa levels were expressed as %.

Table 2 provides the descriptive statistics of the Test (A-PF24RT24, WB-PF24RT24) and Control (FFP) products for the four factors (V, VIII, XI, and Protein S).

Table 2. Descriptive statistics of the Coagulation Factors for the Tests (A-PF24RT24, WB-PF24RT24) and Control (FFP)

	Sponsor A A-PF24RT24 (n=52)		Sponsor B A-PF24RT24 (n=54)		Sponsor C WB-PF24RT24 (n=65-66)+		Sponsor D WB-PF24RT24 (n=60)	
	Control	**Test**	**Control**	**Test**	**Control**	**Test**	**Control**	**Test**
Factor V (IU/dL)								
Mean	100.8	99.7	90.2	89.2	109.3	103.5	88.25	85.2
SD	17.5	16.6	19.1	18.2	22.2	19.8	16.9	17.6
Median	103	101	90	88	111	102	88	86.5
Min	52	52	35	35	67	67	53	46
Max	138	136	136	131	177	157	136	135
Factor VIII (IU/dL)								
Mean	80.5	73.2	99.3	86.1	73.8	63.9	102.3	86.7
SD	24.7	24	31.7	26.9	25.5	23.3	33.2	32.9
Median	75	68	96.5	87	73.5	60.5	97.5	86
Min	37	36	49	40	28	20	43	35
Max	163	157	193	156	141	114	196	189
Factor XI (IU/dL)								
Mean	73.8	74.1	103.8	104.0	87.1	60.4	99.4	103.1
SD	11.4	11.0	18.3	20.0	20.5	15.6	19.4	25.7
Median	72	71	101.5	98.5	85	61	98	94.5
Min	53	52	77	74	49	31	59	33
Max	109	103	150	158	168	101	146	176
Protein S (IU/dL)								
Mean	93.7	83.1	82.2	73.2	86.0	72.5	79.43	72.3
SD	19.9	19.4	17.8	14.1	13.7	14.5	17.7	17.8
Median	92	81	80.5	73.5	84	69	78	70
Min	53	48	29	47	60	48	44	28*
Max	161	145	124	109	122	111	119	116

+ An apparent outlier was excluded from the analysis for one plasma unit with the factor V value of 2. This was considered as a laboratory error. ***** This plasma donation was tested at 3 time-points with values of 28, 45, and 51, so that it is likely the lowest value, 28 was an outlier. The next lowest value in a different donor was 39.

Table 3 shows the mean differences between the Test and the Control for the four coagulation factors and the 95% Confidence Intervals (CIs). For Sponsor A, B, and C the mean differences are tested by paired t-test. For Sponsor D the mean difference is tested by 2-sample t-test. The statistically significant results in Table 3 are marked as * p = < 0.05; and ** p = < 0.0001. The values in parentheses are upper and lower 95% confidence limits.

Table 3 The mean difference: Test - Control (95%CI)
Coagulation Factors for the Tests (A-PF24RT24, WB-PF24RT24, Test-FFP) and Control (FFP)

Factor	**Sponsor A[#] A-PF24RT24 (n=52)**	**Sponsor B[#] A-PF24RT24 (n=54)**	**Sponsor C[#] WB-PF24RT24 (n=65-66)[+]**	**Sponsor D[#]**		
				Test-FFP vs. Control	**WB-PF24RT24 vs. Control**	**WB-PF24RT24 vs. Test-FFP**
FV	-1.08 (-2.09, -0.07)*****	-0.98 (-2.59, 0.62)	-5.83 (-10.18, -1.48)*****	-3.58 (-9.58, 2.42)	-3.03 (-9.03 , 2.97)	0.55 (-5.45 , 6.55)
FVIII	-7.29 (-9.43, -5.15)******	-13.22 (-15.96, -10.48)******	-9.89 (-14.41, -5.38)******	4.62 (-8.5, 17.73)	-15.6 (-28.76 , -2.53)*****	**-20.3 (-33.38, -7.15)***
Factor XI	0.31 (-0.39, 1.02)	0.17 (-1.28, 1.61)	-26.70 (-30.80, -22.59)******	0.1 (-8.16, 8.36)	3.62 (-4.64 , 11.87)	3.52 (-4.74 , 11.77)
Protein S	**-10.61 (-12.68, -8.54)****	**-8.98 (-11.73, -6.24) ****	**-13.45 (-16.66, -10.24)****	-0.07 (-6.16, 6.02)	**-7.13 (-13.22 , -1.04)***	**-7.07 (-13.16 , -0.97)***

[#] Data from Sponsors A, B, and C are paired, whereas data from Sponsor D are non-paired

+ An apparent outlier was excluded from the analysis for one plasma unit with the factor V value of 2. This was considered as a laboratory error.

Table 1 shows that Sponsor C's WB-PF24RT24 has the greatest number of potentially significant differences from FFP, as defined by more than 20% difference, for most factors compared to products stored after removal of the cellular components. Noteworthy are the potentially significant differences (WB-PF24RT24 < FFP by > 20%) in Factors V, VIII, XI, and Protein S. The other products have a fewer number of potentially significant differences from FFP, which are limited to Factor VIII and Protein S. The potential clinical significance of these findings is a central issue for discussion by the Advisory Committee. In cases where factor levels showed differences in pairs on both sides of the table (e.g. Factor VIIa and vWF), consistent differences between test and control were not demonstrated.

As is shown in Table 3, the mean Protein S level of the PF24RT24 products is significantly less than that in FFP for all four manufacturers' PF24RT24 (but not Sponsor D, Test-FFP) that were analyzed separately. Please note that for Sponsor D there is no difference between the Test-FFP with the Control FFP. However, there is a significant decrease in mean Protein S levels between WB-PF24RT24 and the Control-FFP or Test-FFP. When considering the overall data, paired (Table 1) and mean values (Table 3), the decreases in coagulation factors are greater with the WB-PF24RT24 than with the A-PF24RT24 especially regarding Factor VIII and Protein S. This observation suggests that the prolonged room temperature storage, combined with cell contact, contributes to loss of labile protein function.

Overall, the results demonstrate that plasma stored for 24 hours at RT, has reduced levels of both Protein S and Factor VIII compared to plasma stored for 8 hours or less at RT (FFP).

The reduced Protein S activities in the characterization study of the PF24RT24 raise potential safety questions for critically ill patients, in particular when PF24RT24 is used in certain clinical settings (e.g., liver transplantation, plasma exchange therapy in patients with thrombotic thrombocytopenic purpura (TTP), severe bleeding in trauma, neonates, and postpartum hemorrhage) and also when used for Protein S deficient patients (see detailed discussion below).

Discussion:

A. Comparison of the Sponsors' findings with those of the BEST collaborative study.[3,7]

Whole Blood PF24 (WB-PF24) is a licensed product but is presented here for reference purposes. Cardigan et al. compared non-paired coagulation factor data from WB-PF24 (plasma separated from Whole Blood which was held at room temperature for less than 8 hours, and then moved to the refrigerator for an additional 16 hours before freezing) and FFP (plasma, separated from Whole Blood, which was held at room temperature for 8 hours post collection, before freezing) as shown in Table 4 and Table 5.[3]

Table 4: Cardigan et al. 2005

TABLE 1. Coagulation variables in plasma frozen within 8 hours from donation or after storage of whole blood at 4°C overnight (18-24 hr from donation) in comparison to reference ranges*

Factor	Whole blood storage time		Reference range based on FFP separated <8 hr		Standard hematology reference ranges	
	<8 hr (n = 66)	18-24 hr (n = 60)	Range (n = 66)	Percent of units stored 18-24 hr in range	Range	Percent of units stored 18-24 hr in range
PT ratio (n = 110)	1.04 (0.94-1.18)	0.99 (0.89-1.17)	0.95-1.16	98	Varies	
APTT ratio (n = 110)	1.08 (0.83-1.97)	1.25 (1.03-1.46)[b]	0.86-1.36	83	Varies	
Fibrinogen (g/L)	2.69 (1.54-5.00)	2.37 (1.54-3.90)[a]	1.10-4.30	100	1.5-4.0	100
Prothrombin (IU/mL)	0.96 (0.72-1.26)	1.05 (0.82-1.40)[b]	0.70-1.20	100	0.50-2.00	100
FV (U/mL)	0.94 (0.35-1.48)	0.80 (0.53-1.12)[b]	0.50-1.40	100	0.50-2.00	100
FVII (IU/mL)	1.01 (0.58-1.56)	1.07 (0.42-2.65)	0.60-1.40	98	0.50-2.00	98
FVIII (IU/mL)	1.00 (0.48-1.85)	0.77 (0.38-1.40)[b]	0.40-1.60	98	0.50-2.00	92
F IX (IU/mL)	0.98 (0.60-1.45)	1.05 (0.60-1.58)	0.60-1.40	100	0.50-2.00	100
FX (IU/mL)	0.99 (0.71-1.29)	1.17 (0.80-1.77)[b]	0.70-1.30	100	0.50-2.00	100
FXI (U/mL)	0.95 (0.66-1.44)	0.88 (0.51-1.36)[a]	0.60-1.30	95	0.50-2.00	100
FXII (U/mL)	0.98 (0.20-1.47)	1.29 (0.51-2.39)[b]	0.40-1.50	100	0.50-2.00	100
C1-INH (U/mL)		0.87 (0.60-1.64)	NA†	NA	0.70-1.30	92
FXIIa antigen (ng/mL)	1.89 (0.40-4.12)	1.83 (0.67-3.51)	0.50-5.00	100	<2.9	92
Pro F1 + 2 (nmol/L)	0.65 (0.35-1.30)	0.88 (0.37-1.82)[b]	0.20-1.10	72	0.40-1.10	72
VWF:Ag (U/mL)	1.13 (0.66-1.75)	1.02 (0.39-2.21)	0.60-1.65	85	0.50-2.00	95
VWF activity (U/mL)	1.01 (0.57-1.63)	0.97 (0.30-1.85)	0.50-1.50	87	0.50-2.00	87

* Data are given as median (range). [a]$p < 0.05$ and [b]$p < 0.0001$ compared with FFP separated less than 8 hours. Data from FFP separated less than 8 and 18 to 24 hours are not paired (see Materials and methods). Reference ranges have been calculated with mean ± 2SD for normally distributed data and the geometric mean with 95 percent CI for skewed data. In range is defined as above the lower limit for coagulation factors and below the upper limit for PT and/or APTT and activation markers.

† NA = not available.

Table 5: Cardigan et al. 2005

TABLE 2. Coagulation inhibitors in plasma frozen less than 8 hours from donation or after storage of whole blood at 4°C overnight (18-24 hr from donation) compared with reference ranges*

Factor	FFP storage time		Based on standard hematology reference ranges	
	<8 hr (n = 20)	18-24 hr (n = 20)	Range	Percentage of Day 1 units in range
ATIII (IU/mL)	0.96 ± 0.10	0.95 ± 0.10	0.80-1.20	95
PC (IU/mL)	0.98 ± 0.23	0.96 ± 0.22[b]	0.70-1.30	95
α_2-AP (U/mL)	1.02 ± 0.08	0.97 ± 0.11[b]	0.80-1.20	95
PS Bioclot (IU/mL)	0.99 ± 0.13	0.91 ± 0.09[b]	0.55-1.60	100
PS Staclot (IU/mL)	1.12 ± 0.16	1.09 ± 0.16[a]	0.77-1.43, men 0.55-1.23, women	100
PS free antigen (IU/mL)	0.80 ± 0.15	0.77 ± 0.15[a]	0.70-1.48, men 0.50-1.34, women	100

* Data are given as mean (SD). [a]$p < 0.05$ and [b]$p < 0.01$ compared with FFP separated less than 8 hours. Data from FFP separated less than 8 and 18 to 24 hours are paired. FFP was LD with a RZ2000 whole-blood filter.

WB-PF24 had reduced Factors V and VIII levels (14 and 23%, respectively), and lesser reductions in mean Protein S levels (8 and 3%, depending on the assay).

Subsequently, Cardigan et al. studied the coagulation factor content of plasma prepared from Whole Blood that was processed either on the day of collection (FFP) or after the Whole Blood was stored at ambient temperature for 24 hours (WB-PF24RT24) (Table 6).[7]

- "Plasma produced less than 8 hours from Whole Blood donation" is referred to by FDA as FFP.
- "Plasma produced less than 24 hours from Whole Blood donation" is referred to by FDA in this document as WB-PF24RT24.

Table 6: Cardigan et al. 2011:

TABLE 1. Coagulation screening tests and factors and/or control proteins in plasma produced less than 8 or 24 hours from whole blood donation*

Parameter	<8 hr	24 hr	p value (8 hr vs. 24 hr)
PT (sec)	10.7 (10.2-11.5)	10.7 (10.3-11.8)	0.547
PT ratio	0.97 (0.93-1.06)	0.97 (0.94-1.07)	0.623
APTT (sec)	28.3 (25.9-31.8)	30.0 (26.8-32.4)	<0.001†
APTT ratio	1.00 (0.91-1.12)	1.06 (0.94-1.16)	<0.001†
Fibrinogen (g/L)	2.41 (1.86-3.22)	2.42 (1.94-3.29)	0.017†
VWF : CBA	1.10 (0.68-1.31)	1.13 (0.69-1.31)	0.155
Factor XIII	1.17 (0.70-1.44)	1.30 (0.71-1.55)	<0.001†
Protein C	1.08 (0.87-1.20)	1.02 (0.79-1.16)	<0.001†
Protein S	0.74 (0.57-1.22)	0.64 (0.50-1.02)	<0.001†
Antithrombin	0.95 (0.86-1.06)	0.95 (0.85-1.06)	0.147
Prothrombin fragment 1+2	0.13 (0.07-0.39)	0.09 (0.06-0.24)	<0.001†

* Data are given as median (range), n = 32.
† Significant difference.
CBA = collagen-binding activity.

These data are most analogous to the data submitted to the FDA by Sponsors C and D, involving 24 hour cell contact, and less analogous to data from Sponsors A and B, not involving prolonged cell contact. Data in this Cardigan et al. study were generated using 32 groups of 4 pooled units of Whole Blood. The Cardigan study showed a 23% decrease in the activity of Factors (F) VIII, but no significant change in activity of coagulation factors FV, FVII, FXI, FXII, fibrinogen, antithrombin, or von Willebrand factor (these data are not listed in Table 6). The results also showed prolonged APTT (measured in sec. and as a ratio) compared to FFP, and free Protein S activity was reduced by 10% compared with FFP (0.74 vs. 0.64). In all three cases statistical significance was achieved at p values < 0.001 (Table 6). The low p values reflect the fact that the data were analyzed using mean differences of pairs and a paired t-test. Cardigan et al. stated "that the loss of coagulation factors observed is unlikely to be clinically significant and that plasma produced from whole blood stored at ambient temperature for up to 24 hours may be used for the same clinical indications as FFP or PF24."

The data submitted by Sponsors to the FDA were all based on a paired design (except Sponsor D) comparing units from the same donors in each pair (Tables 1, 2, 3). The Cardigan data were also paired, but the pairing was from pools of 4 units which may reduce the chance of observing potentially significant differences from FFP, by averaging of the effects that might be seen with single donor units. The data presented for Sponsor C in Table 1 demonstrate that in 30/66 (45.5%) of the pairs the Protein S level in the Test units were less than the Control unit by more than 20%. To determine the effect of pooling units in groups of 4 a simulation study was done by the FDA. Sponsor C data were pooled in groups of 4 units to simulate what was done in the Cardigan study, and analyzed for changes in protein S. This simulation showed that the number of "pairs" with potentially significant differences from FFP becomes 5/17 (29.4%), rather than 30/66 (45.5%), i.e. detection of about a third of the potentially significant differences from FFP was lost as a result of the pooling.

B. Clinical Experience with Plasma Products Containing Reduced Functional Protein S

The products referred to above are all single units from a single donor. Pooled solvent detergent treated plasma products were made in the US (and later voluntarily withdrawn, see below) and are currently made in Europe. Data from these products made from plasma stored at RT for different times are relevant to this discussion, although the manufacturing process is different and may result in changes in other plasma proteins not

observed in the new products presented in this report. Hellstern noted that when "recovered plasma" was stored at room temperature for 15 hours after donation and then frozen, the Protein S activities decreased significantly compared to plasma frozen 4 hours after collection (Table 7).[8] He stated, "On the basis of the results summarized in Table 1 {in this summary Table 7, below}, PS activity levels below 60 U/100 mL were found in 21 of 60 recovered plasma (RP) units frozen 15 hours after collection, but only in 2 of 60 AP (apheresis plasma) units and in none of 100 RP units frozen within 4 hours after donation ($P < 0.0001$, chi-squared test). This may explain why markedly lower PS activity potencies were recently found in Octaplas distributed in Ireland and produced from US RP frozen 15 hours after donation, compared with Octaplas placed on the German market." The German product was only made from RP stored for 4 hours. These findings suggest that prolonged room temperature hold of plasma may be associated with lower Protein S levels. Factors V, VIII, and XI also decreased with longer storage at RT.

Table 7: Hellstern, 2004

Table 1. Levels of clotting factors, inhibitors, and citrate in blood group A plasma units frozen within 4 h (recovered plasma, RP 4 h) and 15 h (recovered plasma, RP 15 h) after blood collection, respectively, and in blood group A apheresis plasma units frozen within 4 h after collection (AP)

Measure	RP 4 h (n = 100)	RP 15 h (n = 60)	AP (n = 60)	Reference range (n = 100)
FV, U/100 mL	78 (38–166)	72 (18–113)	107[a] (63–159)	54–145
FVIII, U/100 mL	87[d] (32–247)	70 (28–148)	93[b] (52–160)	52–140
FIX, U/100 mL	96 (43–156)	89 (39–152)	121[a] (69–170)	45–148
FXI, U/100 mL	90[e] (43–151)	80 (26–154)	111[a] (66–168)	61–122
Protein S, U/100 mL	87[f] (51–145)	67 (28–122)	80[c] (51–106)	56–168
Plasmin inhibitor, U/100 mL	107 (80–215)	102 (87–114)	114[c] (93–155)	72–132
Citrate, mM	21.8 (19.3–23.3)	20.8 (15.8–23.7)	13.1[a] (11.9–15)	

Data are expressed as mean value (minimum–maximum).
[a] $P < 0.0001$ *vs* RP 4 h and RP 15 h.
[b] $P < 0.001$ *vs* RP 4 h and RP 15 h.
[c] $P < 0.0001$ *vs* RP 15 h.
[d] $P < 0.001$ *vs* RP 15 h.
[e] $P < 0.01$ *vs* RP 15 h.
[f] $P < 0.0001$ *vs* RP 15 h.

Lower levels of Protein S may lead to thromboembolism. Previous US clinical experience with a solvent/detergent-treated plasma (PLAS+SD, V.I. Technologies Inc., Melville, NY) associated with thromboembolic adverse events has raised concerns about low Protein S levels, especially in liver transplantation and liver disease.

In 2002, the Centers for Disease Control and Prevention (CDC) reported an association between receipt of PLAS+SD (Protein S activity from ---b(4)-- and death from pulmonary embolism (PE) in liver transplant (LT) patients at Hospital A[9]. The report indicated that intra-operative thromboembolic complications, especially PE, were very rare events prior to the introduction of this product. Hospital A is a non-profit, 1,100-bed hospital, which includes a Liver Disease and Transplantation Center. In 1999, 42 LT were performed at this center. From April 2 to December 15, 1999, 6 of 31 (20%) patients undergoing LT procedures at Hospital A developed PE; all six died in the operating room (OR). Because of the association between the adverse events and PLAS+SD use, Hospital A personnel reported the adverse events to the FDA and stopped using this product on December 15, 1999. FDA conducted an on-site investigation and notified the other US LT centers of the adverse events. In a multivariate analysis, the strongest independent predictors of PE incidence were: (1) the quantity of PLAS+SD received, and (2) a pre-operative hypercoagulable state. Lots of PLAS+SD used in Hospital A had low Protein S levels ranging from –b(4)-- activity. A number of

publications reported that solvent detergent-treated plasma (SDP) has decreased activity levels for Protein S, Protein C, von Willebrand Factor, antiplasmin, and antitrypsin. According to these reports, the decrease in anti-coagulation factor activity, Protein S in particular, may have contributed to thrombosis and SDP should not be considered a biochemical equivalent of FFP or PF24, despite generally similar attributes.

Flamholz et al. reported a study of three patients with thrombotic thrombocytopenic purpura (TTP) exchanged with PLAS+SD who developed venous thromboembolism.[10] The authors studied two pools (not used to treat the patients) of PLAS+SD and found functional Protein S levels of 24.8% and 15%. They suggested that when used as replacement fluid for repetitive therapeutic plasma exchange, e.g., in patients with TTP, PLAS+SD could lead to lowered Protein S levels and, possibly, a risk of hypercoagulable complications. They described three patients with TTP who had low functional Protein S (FPS) levels during plasma exchange (PEX) for TTP. Each developed one or more deep vein thrombosis (DVT) while receiving 100% PLAS+SD or alternating 50% PLAS+SD and 50% cryosupernatant plasma (CSP) as replacement fluid. FPS levels rose when 100% CSP was substituted for PLAS+SD. They concluded, "Our observations suggest that use of SDP alone or in 50% combination with CSP as replacement fluid in PEX (plasma exchange) for TTP may lead to difficulty in maintaining safe Protein S levels. Determination of risk of resulting clinically significant thrombotic events requires further study."

Yarranton et al. reviewed the occurrence of venous thromboembolism (VTE) in 68 consecutive patients with TTP (25 men, 43 women). Eight documented VTE events (six deep venous thrombosis, three pulmonary emboli) were identified in seven patients (all female) during plasma exchange (PEX) therapy.[11] The patients were all treated initially with daily 1.0 plasma volume exchange (except patient 2 who received 0.5 plasma volume exchange). Octaplas (pooled SD treated plasma frozen within 4 hours of collection) was the last plasma to be used in plasma exchange (PEX) prior to VTE in 7/8 events. The authors indicated that VTE is a multifactorial disease and, although several known precipitating factors were present in all patients in this study, the use of large volumes of SD plasma in PEX may be an additional risk factor. The functional Protein S levels in 10 batches of Octaplas tested in this study showed a median 0.58 IU/ml (range 0.55–0.63). Protein S activity levels were not consistently measured in their patients during PEX using Octaplas. Archived samples were available in one patient in whom there was a trend to lower functional Protein S levels during PEX using Octaplas (mean 0.56 IU/ml) compared with CSP (mean 0.68 IU/ml) as the replacement fluid.

Doyle et al. studied the coagulation factor content of Octaplas compared with fresh frozen plasma and they concluded that "All routine coagulation screening tests, factor VII and Protein C levels were within the normal reference range for both Octaplas and FFP. However, we found significant reductions in factor V (31%), factor VIII (28%) and Protein S (50%) in S/D plasma".[12]

Norway has reportedly used Octaplas universally for many years. There are publications from this country documenting safe use of this SDP in patients undergoing cardiac surgery or liver resection who underwent routine clinical monitoring.[13,14] Solheim also stated that "in the US, the SD-treated plasma named PLAS + SD (V.I. Technologies, Watertown, MA), was licensed in 1998, but has subsequently been voluntarily withdrawn from the market. Even though both products are SD-treated by similar methods,

significant product differences, ascribed to production methods and type of plasma used, have been reported. Such differences could explain the complications observed in the US".[13] Hellstern also indicated that "The quality of the starting material influences clotting factor and inhibitor activities in the final plasma bags. PLAS+SD, formerly marketed by V.I. Technologies (Watertown, MA, U.S.), and Octaplas from Octapharma (Vienna, Austria) distributed to Ireland and Great Britain is manufactured from US recovered plasma (RP), frozen 15 hours after donation. By contrast, Octaplas manufactured for the Norwegian and German markets is produced from national RP or a mixture of RP and apheresis plasma (AP), all frozen within 4 to 8 hours after collection. "[8]

The current product label for Octaplas has the following statement under "Precautions".

"OCTAPLAS contains reduced concentrations of protein S compared to normal plasma. In clinical situations where large volumes of OCTAPLAS are infused (e.g. plasma exchange for TTP or during liver transplantation), patient plasma Protein S concentrations may be reduced, predisposing the patient to the development of deep venous thrombosis (DVT). Consideration should be given to the use of prophylactic measures against DVT in such patients, especially for those with other risk factors. OCTAPLAS should not be used to treat patients with Protein S deficiency."

Although there have been limited reports of adverse events with Octaplas in Europe such as thrombosis (cited above), the overall experience appears to be acceptable, with the caveat that these products have not been studied in controlled clinical trials with carefully monitored subjects. Most of the lots tested in the reference cited above had Protein S levels >50%.

It should be noted that the Canadian Standards Association allows production of frozen plasma for transfusion that has been held at RT for up to 24 hours before being frozen ("PF24RT24" in this Issue Summary). In practice, a considerable amount of plasma derived from WB stored at RT for more than 8 hours prior to freezing is used for transfusion purposes in Canada. This plasma comes from blood that has been held overnight on controlled cooling trays (butane diol plates) that lowers the blood to room temperature with a consistent cooling rate in the bag. This WB is collected in bags that permit the manufacture of platelets derived from buffy coats (BC), whereas bags in the United States are configured for the manufacture of WB-derived platelets from platelet-rich plasma (PRP). However, both are subjected to a single centrifugation (when platelets are not harvested in the US and albeit at different speeds), and the plasma is subsequently expressed into a storage container. The amount of time spent in the collection container with cellular contact at RT is a variable of particular importance and is not inherently different in the two procedures.

Serrano et al. studied Protein S levels in plasma prepared by both the PRP and the BC methods. In this unpaired study, plasma derived from the PRP method was frozen between 7 and 10 hours after collection and plasma derived from the BC method was frozen between 22 and 24 hours after collection. The mean Protein S level in the PRP-prepared plasma was 1.17 U/mL ($\pm$ 0.45) (n=20) and the Protein S level in the BC-prepared plasma was 1.09 U/mL ($\pm$ 0.21) (n=20).[15]

C. Potential Safety Implications for use of Thawed Plasma

In addition to concern about reduced Protein S levels in PF24RT24 products, the common clinical practice of keeping thawed plasma at 1-6^{o}C for up to 5 days could compound the effect by additional loss. Many hospitals are holding Thawed Plasma (TP) in their inventories. Although TP is not approved or licensed by FDA, according to the AABB Standards, TP is permitted for transfusion for up to 5 days after thawing, when stored at 1 ^{o}- 6 ^{o}C. Yazer et al. found a 30.5% decrease of Protein S in thawed PF-24 after 5 days storage at 1 ^{o}C to 6 ^{o}C, and Matijevic et al. found a 23.4% decrease of Protein S in thawed FFP after 5 days storage at 1 ^{o}C to 6 ^{o}C.[4,16] Since Protein S levels are already decreased for PF24RT24, compared to FFP, additional storage after thawing at 1 ^{o}C to 6 ^{o}C for 5 days would be expected to further diminish functional Protein S availability. In spite of these concerns, we are not aware of thrombotic complications associated with the use of thawed plasma derived from FFP and PF24PF24 and refrigerated for up to 5 days after thawing [4,15] in clinical practice. However, to our knowledge, controlled studies comparing plasma thawed and used within 6 hours to plasma stored for 5 days have not been performed.

D. Potential Paths Forward

1. Labeling

FDA initially thought that plasma products under discussion, namely PF24RT24 could be characterized and labeled based upon coagulation factor assay results. However, results from the studies discussed above revealed variable decreases in coagulation factors, especially in Protein S levels. The latter raises potential safety concerns. This, coupled with the knowledge that currently licensed PF24 plasma is used interchangeably with FFP, despite the AABB Circular of Information stating that PF24 should not be used to replace labile coagulation factors, led to concerns that PF24RT24 with reduced coagulation factor levels would likely be used in coagulopathic patients. The concern would be for use of PF24RT24 in all coagulopathic patients, and particularly in cases where large volumes are required, such as liver transplantation, TTP, and for severe bleeding following trauma. Thus, FDA's current consideration is that labeling may not be sufficient to allay potential safety concerns.

2. Clinical studies

Based on the changes in Protein S during storage, the question that arises is whether PF24RT24 is safe and effective. The in vitro data imply that thromboembolic adverse events might occur in patients more frequently, but this cannot be determined with any certainty without data from adequately controlled, randomized, clinical trials.

In order to resolve the potential safety concerns regarding PF24RT24, clinical trials may be required. The design of such trials poses ethical and logistic challenges. The FDA has considered development of products for treatment of severe bleeding and helped organize a workshop that discussed possible paths forward. (Severe Bleeding Workshop held in December, 2010, and sponsored by FDA/DoD/NHLBI.)

However, an ethical question arises whether clinical studies of PF24RT24 could place patients at unreasonable risk. To address this concern, the clinical development program could begin with low risk conditions and progress incrementally to higher risk conditions depending on the safety and efficacy seen in the early studies.

A staged approach could be pursued as follows:

- Stage 1: Healthy volunteers would undergo apheresis and the plasma would be divided into FFP and PF24RT24 products. The donors would then receive autologous plasma with and without the administration of anticoagulation with warfarin. In addition to INR, a panel of pro- and anti-coagulation factors will be measured with sampling at different times to determine PK parameters.
- Patients with the following clinical situations requiring plasma transfusions could be randomized into an FFP or PF24RT24 group.
    - Stage 2: Patients on warfarin anticoagulation requiring emergent interventions (e.g. surgery and invasive procedures)
    - Stage 3: Conditions requiring massive transfusions (excluding trauma)
        - TTP
        - Liver transplantation
    - Stage 4: Severe bleeding in trauma

Progression from one stage to the next would depend on careful monitoring at each stage and only allowing the next stage to go forward if the safety profile of the earlier stage did not reveal any signals of concern.

E. FDA Considerations

In approaching the issue whether PF24RT24 products should be approved, FDA considers the activity of coagulation proteins in plasma to be a reasonable predictor of the therapeutic effectiveness of plasma products. In the data presented herein, reductions in Protein S activity in the PF24RT24 products were demonstrated, which may pose thromboembolic risks to recipients.

There are several reasons for focusing mainly on Protein S in this Issue Summary rather than the other heat-labile coagulation factors. Decreased Protein S levels in solvent-detergent treated plasma products have been associated with thromboembolic SAEs and fatalities (e.g. in liver transplant and TTP patients) (see above), whereas the decreases in other factors (i.e. V, VIII, XI) detected in these products have not been associated with bleeding or other SAEs. The magnitude of Protein S decreases in products stored under different conditions exceeds that seen with other clotting factors, suggesting that Protein S is more susceptible to storage conditions (e.g. time and temperature). The range of Protein S levels in the PF24RT24 products tested overlaps the range of levels associated with SAEs and fatalities, but also overlaps those seen with FFP.

Currently, many blood banks and transfusion services store WB-PF24 commingled with FFP without maintaining a separate inventory. In these institutions, WB-PF24 is used for all, or almost all, patients interchangeably with FFP by physicians. If PF24RT24 is approved by FDA, it will likely be used interchangeably with FFP as well, so that restrictive labeling may not impact use. In addition, there is the potential concern that, if approved, PF24RT24 plasma will then also be treated as thawed plasma, and transfused for up to 5 days after thawing.

In addition, the PF24RT24 plasma products will be transfused to the patients as individual units so it would be important to ensure that individual units contain sufficient amounts of coagulation factors and proteins to meet patient needs without introducing additional risk. However, large volume transfusions to adults will necessarily include units of plasma from different donors, so a single unit with a very low level of Protein S will contribute only a fraction of the total plasma transfused. It appears that in some populations, adequate Protein S activity is important, especially in light of the reports that liver transplant patients treated with SDP, and TTP patients undergoing plasma exchange with SDP, may be more likely to experience DVT, with low Protein S activity as a possible contributing factor. The low levels of Protein S identified in the PF24RT24 products studied raise questions about their potential safety and efficacy because the threshold level of a low Protein S causing thrombosis has not been established.

An assessment of the risks and benefits of the availability of PF24RT24 plasma products also requires consideration of the impacts on safety of the blood supply with respect to transfusion-related acute lung injury (TRALI). FDA data has documented that TRALI is the major cause of transfusion mortality in the US[17]. The mortality from TRALI varies from 5-25% in different studies[18]. A recent prospective study in the US showed that introduction of TRALI mitigating strategies (mainly switching to male donors), in two academic institutions, resulted in a decrease of the annual TRALI incidence from 2.57 (95% CI, 1.72-3.86) per 10,000 units transfused (23 cases/89 321 units) in 2006 to 0.81 (95% CI, 0.44-1.49) per 10,000 units transfused (10 cases/123 731 units) in 2009 ($p = 0.002$). There was an estimated 35% reduction in TRALI per year by trend analysis (95% CI, 21%-47%, $p = 0.0001$).[19] In a UK study, many cases (52%) of TRALI, were associated with the finding of anti-HLA, anti-neutrophil or both antibodies, in the donor product[18]. These antibodies originate predominantly from female donors, presumably due to sensitization during pregnancy. Thus, FDA is aware that plasma from male donors has been conclusively demonstrated to reduce the risk of transfusion-related acute lung injury (TRALI) owing to the significantly reduced prevalence of HLA antibodies in males.[18] A safety benefit also has been shown for preferential use of single donor apheresis platelets collected from male donors, which contain a large plasma volume.

FDA has also become aware that some blood establishments have been unable to provide all distributed AB plasma from male donors commensurate with the current demand. The American Red Cross (about 40% of the US blood supply) has recently stated publicly that 42% of their distributed AB plasma is from female donors and that 10.4% of distributed plasma is group AB (up from 7.4% in 2006). Current distribution is about twice the prevalence of group AB in the general population. This is presumably owing to urgent plasma transfusion to patients whose blood group is unknown and who are placed on a massive transfusion protocol for trauma or who are on warfarin and have intracranial hemorrhage, since group AB is a "universal donor" component. In addition, group AB patients can only receive group AB plasma. The extent to which the availability of PF24RT24 plasma components would lead to a greater proportion of male AB plasma is unknown, but is worthy of consideration. Other approaches, such as screening donors for anti-HLA and anti-neutrophil antibodies would likely also reduce TRALI incidence.

In summary, FDA recognizes that approval of PF24RT24 plasma products would offer a logistic benefit to blood collectors, especially by expanding options for mobile collections. Such approvals could improve the supply of blood products, for example by

facilitating increased collections and availability of AB plasma from male donors. However, in considering the available data on the level of Protein S in the PF24RT24 plasma products that have been studied, and the current practice of interchanging use of FFP and PF24RT24 plasma, FDA is concerned that potential safety risks might outweigh the potential benefits. Consequently, FDA seeks the advice of the BPAC on the interpretation of the available scientific data and how best to manage the potential risk that we perceive.

Questions to Committee:

1. Do the lower Protein S levels detected in the PF24RT24 products in these studies raise safety concerns?

    i. If so, what decrease in the mean protein S level is of concern?
    ii. Would a marked decrease in the protein S level (i.e., greater than 2 standard deviations below the mean) in a single unit be of concern?
    iii. Even if the level is acceptable at the time a unit is first thawed, is there concern regarding further degradation of the protein S levels in products held refrigerated for up to 5 days prior to transfusion?

2. If lower Protein S levels detected in PF24RT24 plasma products raise safety concerns, and taking into consideration the potential benefit of having more male plasma available (especially AB), which of the following approaches should be considered to resolve these concerns?

    i. Product labeling to include the results of the paired studies and warnings of the potential for thromboembolic events (i.e., without requiring additional clinical studies).
    ii. A clinical trial program using a staged approach starting with low risk patients and progressing incrementally to higher risk patients as appropriate?

3. Please discuss whether any other of the study findings raises concerns.

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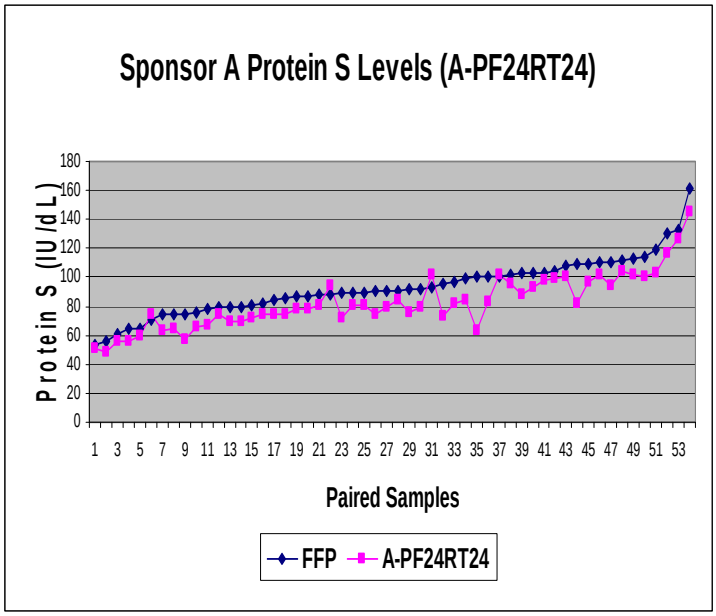
Appendix: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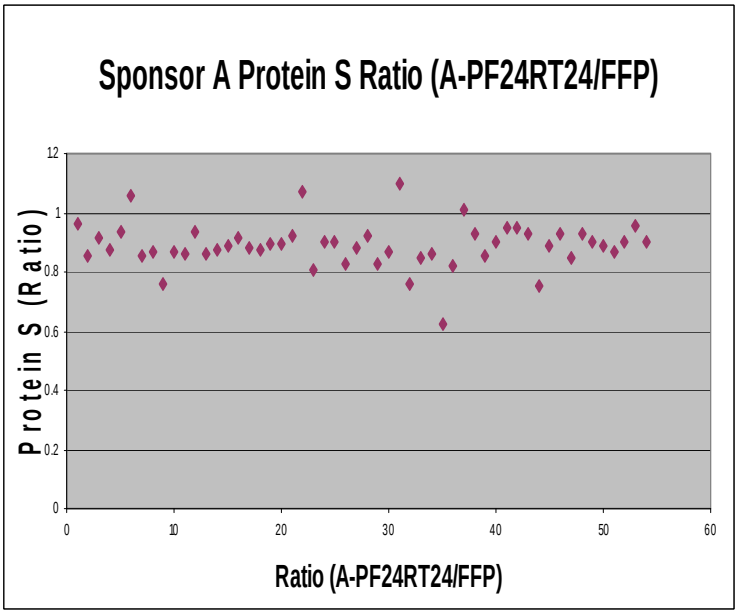
Protein S (IU/dL)
Paired Samples
FFP
A-PF24RT24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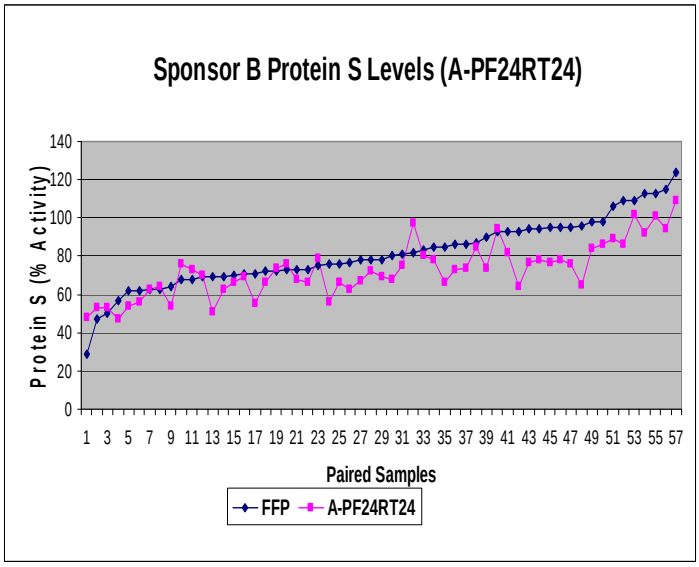
Protein S (Ratio)
Ratio (A-PF24RT24/FFP)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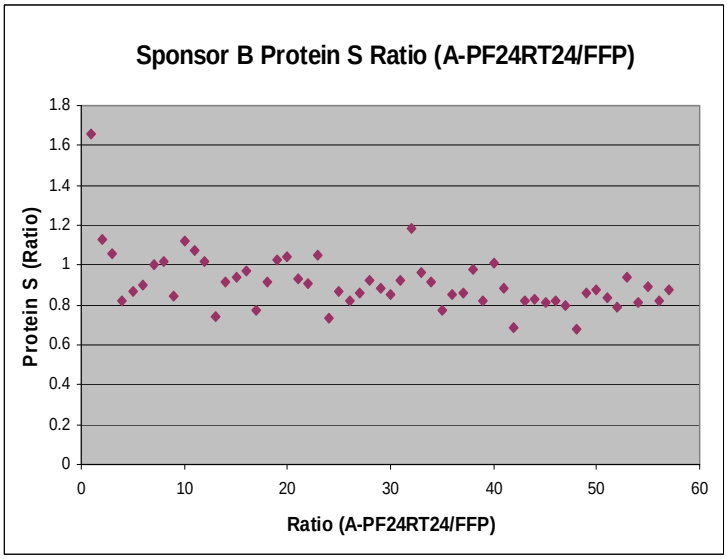
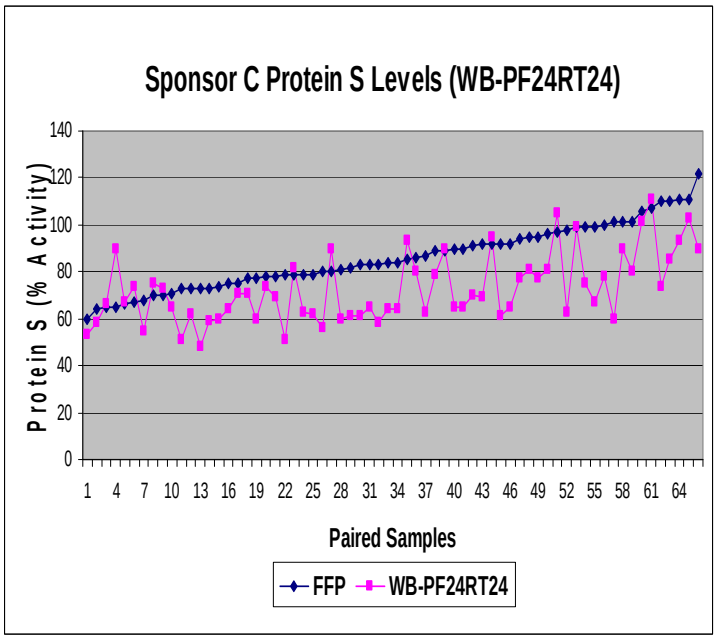
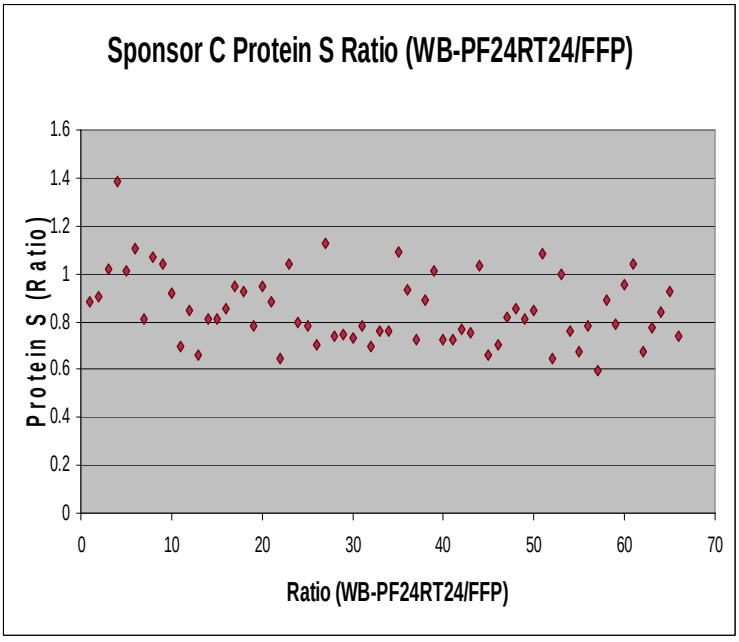
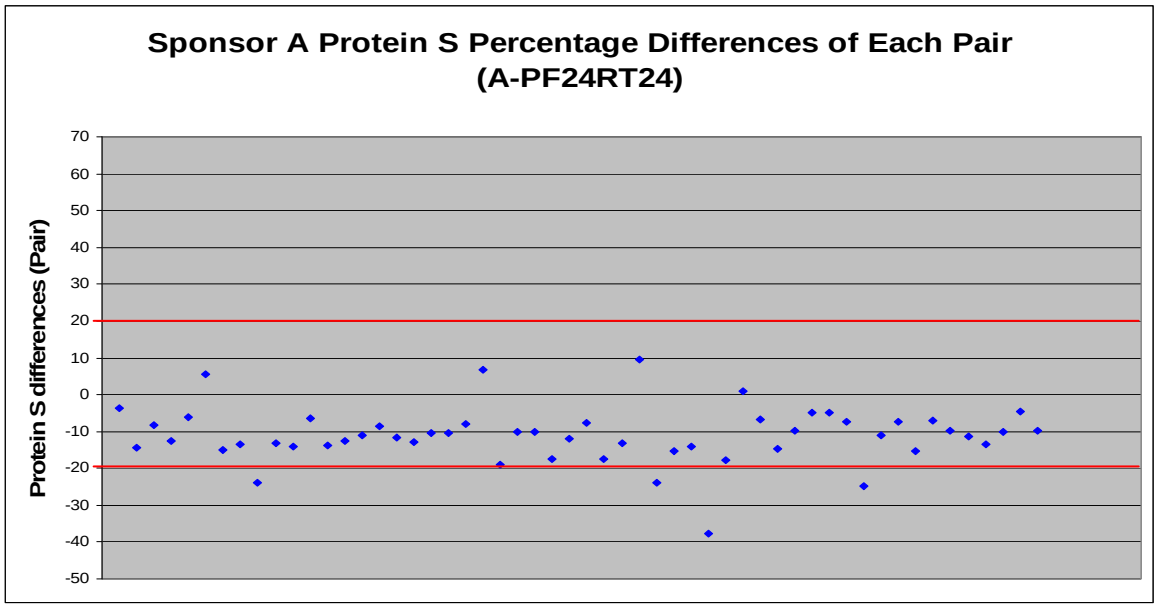
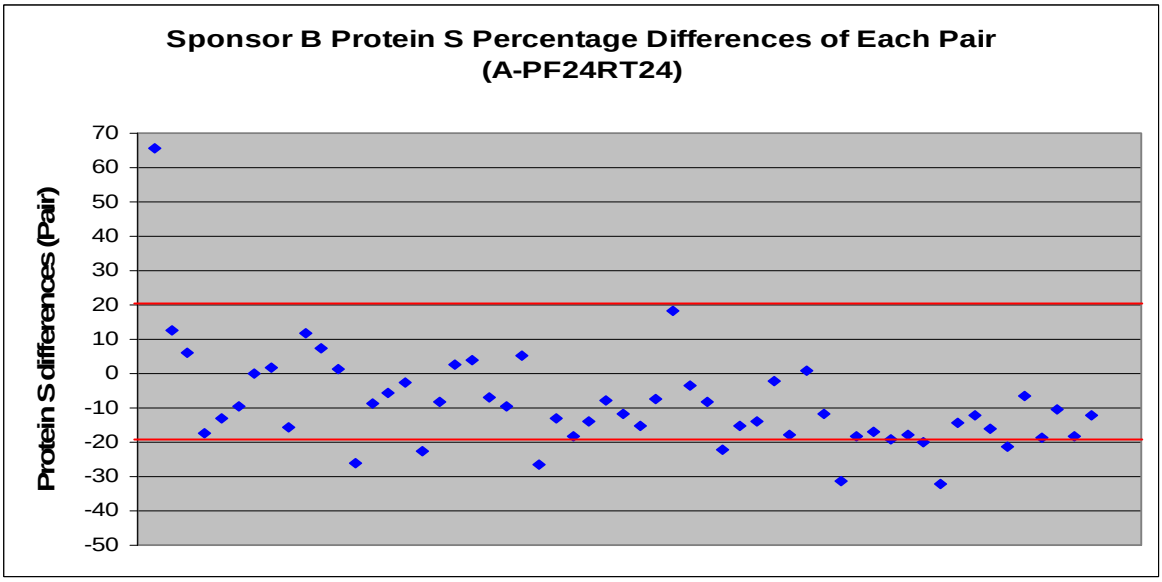
Paired Samples
FFP
A-PF24RT24

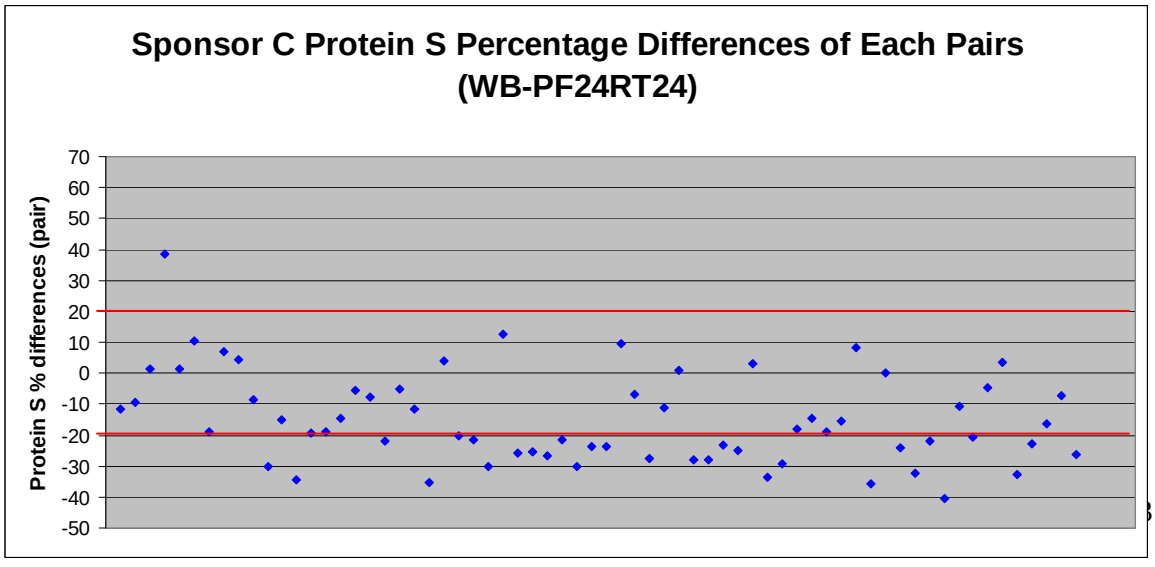

Sponsor B Protein S Ratio (A-PF24RT24/FFP)
Protein S (Ratio)
Ratio (A-PF24RT24/FFP)


Sponsor C Protein S Levels (WB-PF24RT24)
Protein S (% Activity)
Paired Samples
FFP
WB-PF24RT24


Sponsor C Protein S Ratio (WB-PF24RT24/FFP)
Protein S (Ratio)
Ratio (WB-PF24RT24/FFP)


Sponsor A Protein S Percentage Differences of Each Pair
(A-PF24RT24)
Protein S differences (Pair)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50


Sponsor B Protein S Percentage Differences of Each Pair
(A-PF24RT24)
Protein S differences (Pair)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50


Sponsor C Protein S Percentage Differences of Each Pairs
(WB-PF24RT24)
Protein S % differences (pair)
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50