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# Impact of Overnight Hold of Whole Blood on Plasma Protein Function

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2

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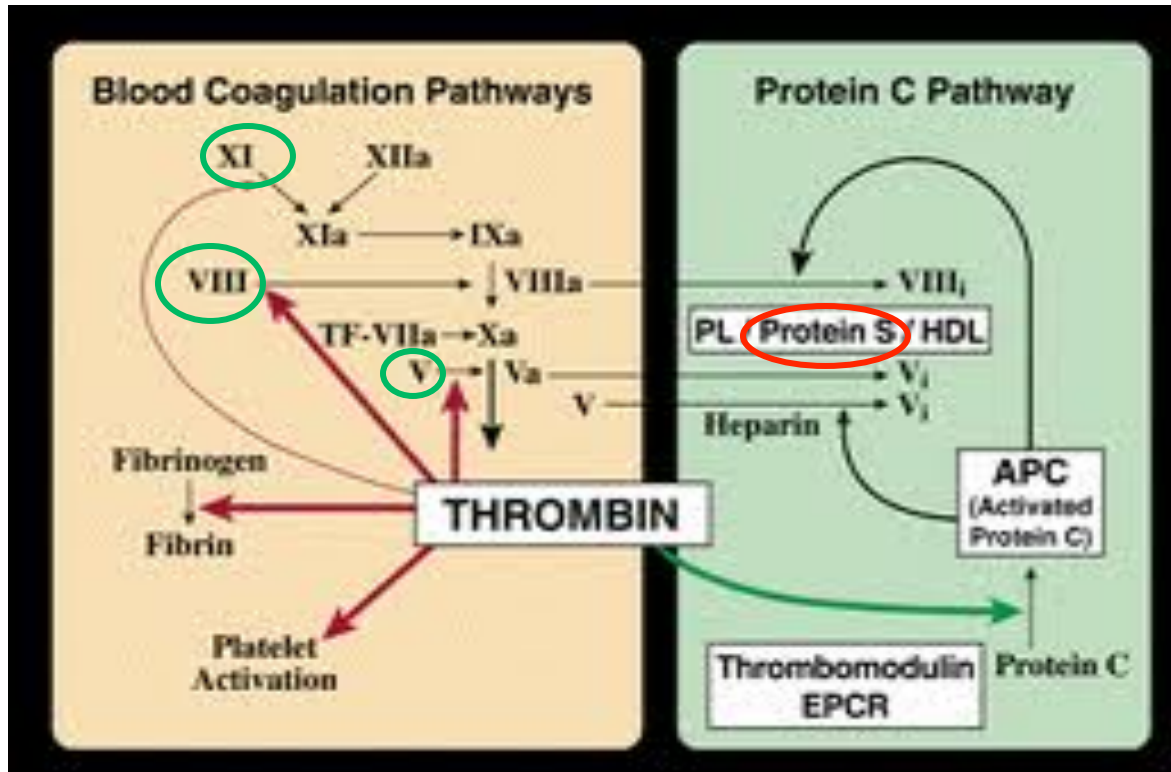
**Canadian Blood Services**  
*it's in you to give*

# Conflict of Interest Declaration

- Member, Medical Advisory Committee, Fresenius-Kabi
- Currently receive research support from Terumo BCT, Fresenius-Kabi and MacoPharma
- None of the data presented herein were collected in conjunction with any company sponsorship.

# Coagulation Simplified: It's All About Thrombin

Labile Factors:  
XI  
VIII  
V



Protein with a Past: Protein S

Generate Thrombin or Shut Down Thrombin Generation

# Plasma Production Methods at CBS

- Fresh frozen plasma prepared by apheresis
- FP24 - now called FP
  - Prepared from whole blood that was held overnight at RT on butanediol cooling plates (Buffy coat production method – B1)
  - Prepared from whole blood that was collected and placed into an insulated shipping container with cold packs within 1-3 hours of collection, cooled to 4°C until processed within 24 hours of collection. (B2)
- All plasma frozen in blast (-50°C) or contact (-60°C) freezers. Subsequent hospital storage at  $\leq -18^{\circ}\text{C}$ .

# Validation Studies: Comparison of Buffy Coat Plasma vs. PRP Plasma

- Twenty units of plasma were prepared from units collected in buffy coat systems held for 20-24 hours after collection on cooling trays. These units were compared to 20 ABO-matched plasmas produced from the PRP production method (8 hr).
- All measurements were made using functional assays.

	FFP from PRP	FP from BC
Factor V	1.15 ± 0.19 (0.69 – 1.50) U/mL	1.06 ± 0.19 (0.62 – 1.50) U/mL
Factor VIII	1.26 ± 0.32 (0.82 – 1.95) U/mL	0.91 ± 0.25 (0.55 – 1.51) U/mL
Factor XI	1.11 ± 0.16 (0.79 – 1.43) U/ml	0.94 ± 0.15 (0.65 – 1.36) U/mL

FP from overnight RT held whole blood had 72% of the FVIII activity of FFP from cooled whole blood, 85% of the FXI activity, and 92% of the FV activity.

FXI	1.11 ± 0.16 (0.79-1.43) U/mL	0.94 ± 0.15 (0.65-1.36) U/mL†	85
Fibrinogen	3.01 ± 0.48 (2.37-4.08) g/L	3.92 ± 1.95 (2.11-8.06) g/L‡	130
VWF	1.32 ± 0.45 (0.72-2.00) U/mL	1.26 ± 0.47 (0.63-2.00) U/mL	96
ADAMTS-13	0.91 ± 0.25 (0.55-1.22) U/mL	0.88 ± 0.28 (0.52-1.54) U/mL	97

	FFP from PRP	FP from BC
Protein S	1.17 ± 0.45 (0.64 – 2.93) U/mL	1.09 ± 0.21 (0.77 – 1.35) U/mL

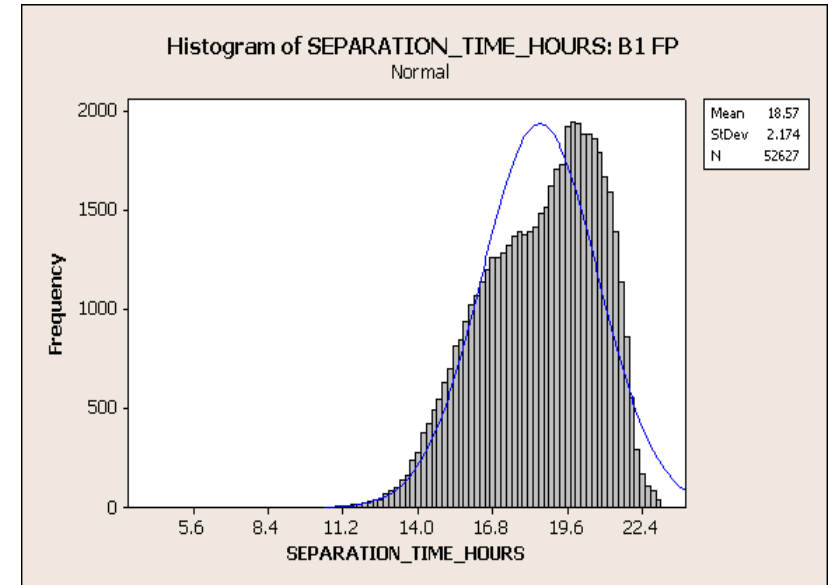
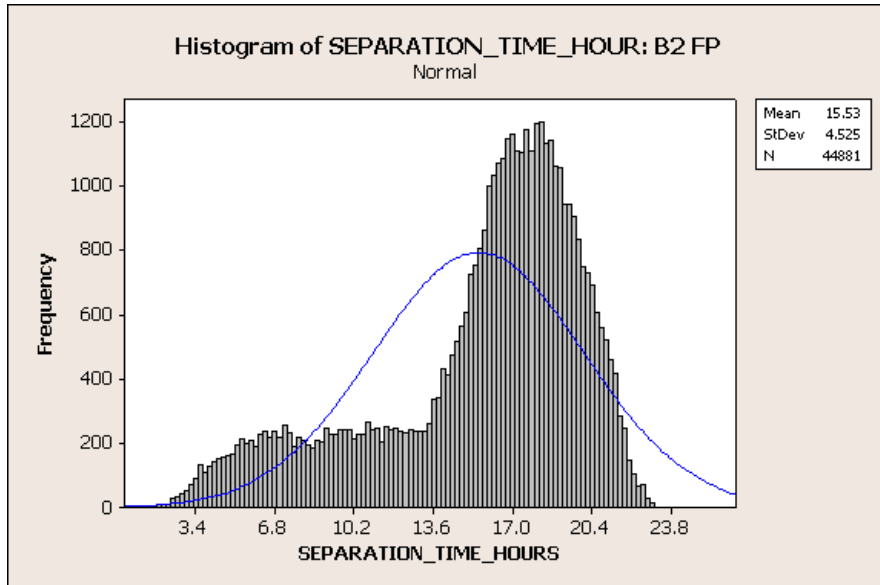
Plasma from overnight RT held whole blood had 94% of the protein S activity of immediately cooled whole blood. Difference was not significant.

# What Happens in the Real World?

- QC of routine production – factor VIII
- Extended quality monitoring – assess production differences across 12 production sites using an extended functional coag panel.
  - Data are collected for both overnight hold products (B1) and immediately cooled (B2).



# How Long from Collection to Separation?



B2 Frozen Plasma	
	Hour of Separation
Mean	15.5
Median	16.8
Min	0.9
Mac	23.0
SD	4.5
n	44881

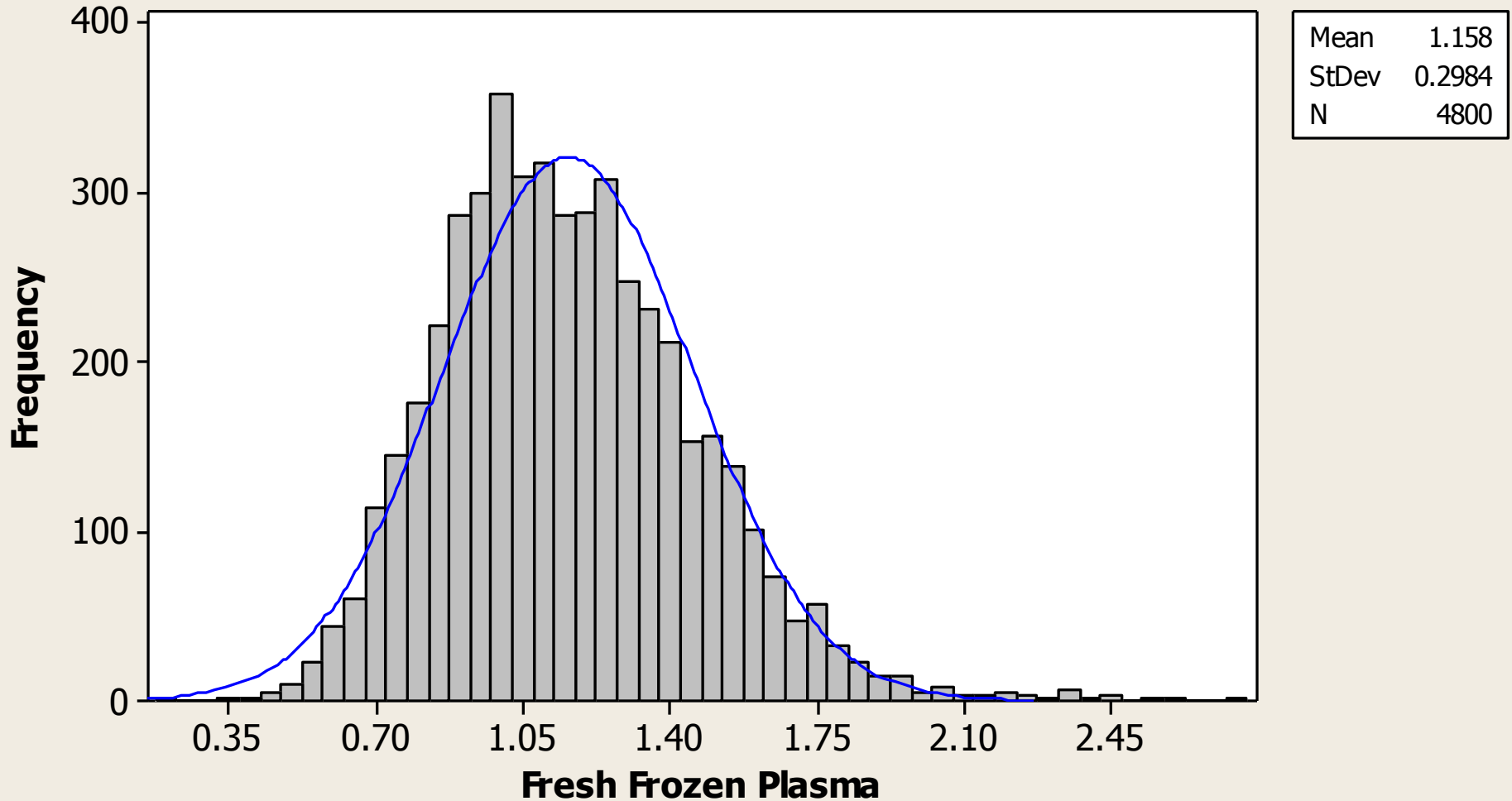
B1 Frozen Plasma	
	Hour of Separation
Mean	18.6
Median	18.9
Min	3.6
Max	23.0
SD	2.2
n	52627

# Routine Quality Control of Apheresis Plasma

**FVIII IU/mL**

Normal

Fresh Frozen Plasma, Apheresis

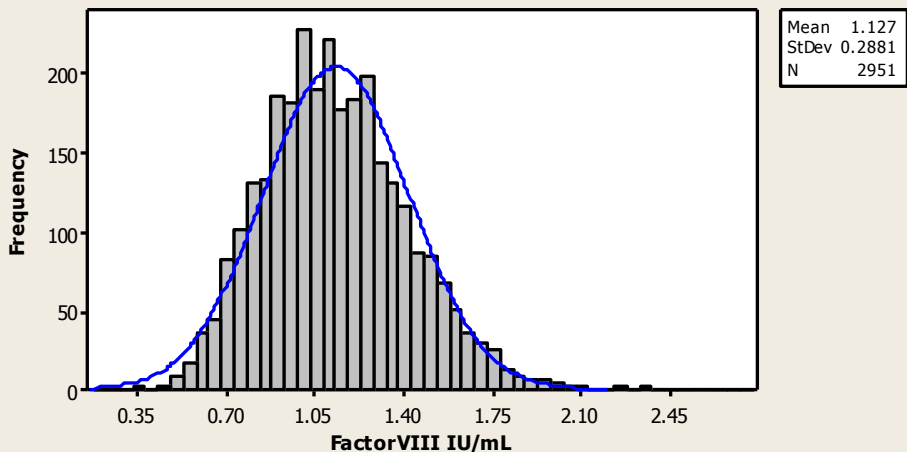


# FVIII QC (October 2009 to February 2012)

**Histogram of FactorVIII IU/mL**

Normal

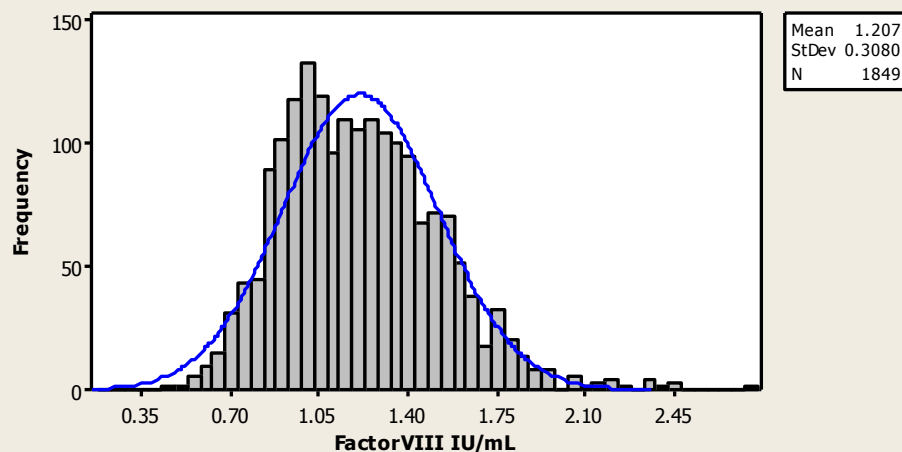
Component = ACD Fresh Frozen Plasma, Apheresis



**Histogram of FactorVIII IU/mL**

Normal

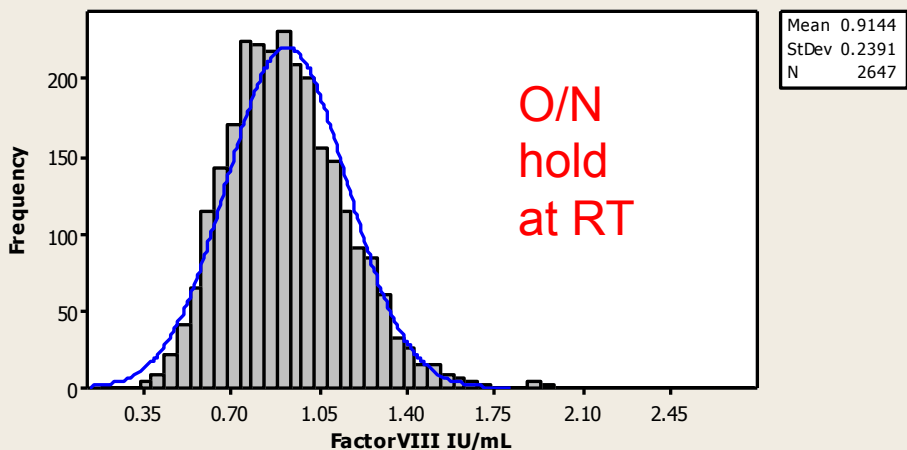
Component = Trisodium Citrate Fresh Frozen Plasma, Apheresis



**Histogram of FactorVIII IU/mL**

Normal

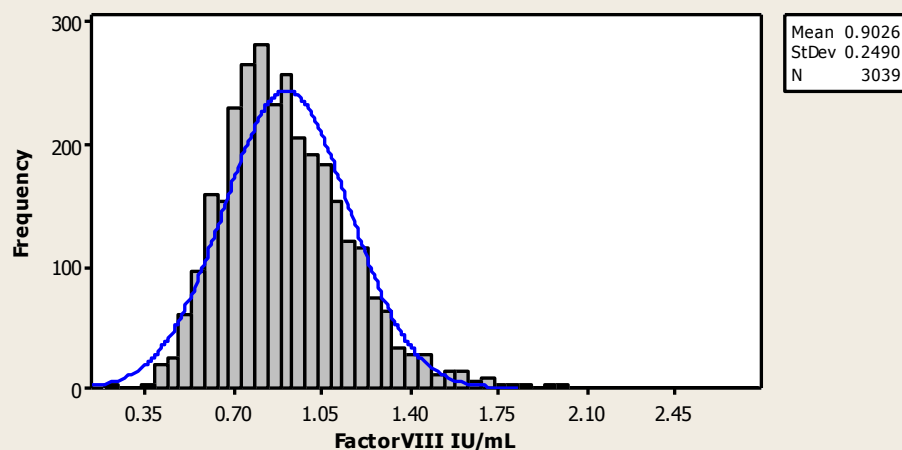
Component = CPD Frozen Plasma (B1)



**Histogram of FactorVIII IU/mL**

Normal

Component = CPD Frozen Plasma (B2)



# FP from Overnight Hold versus Immediate Cooling

**Table 2: Effect of collection set type on test parameters**

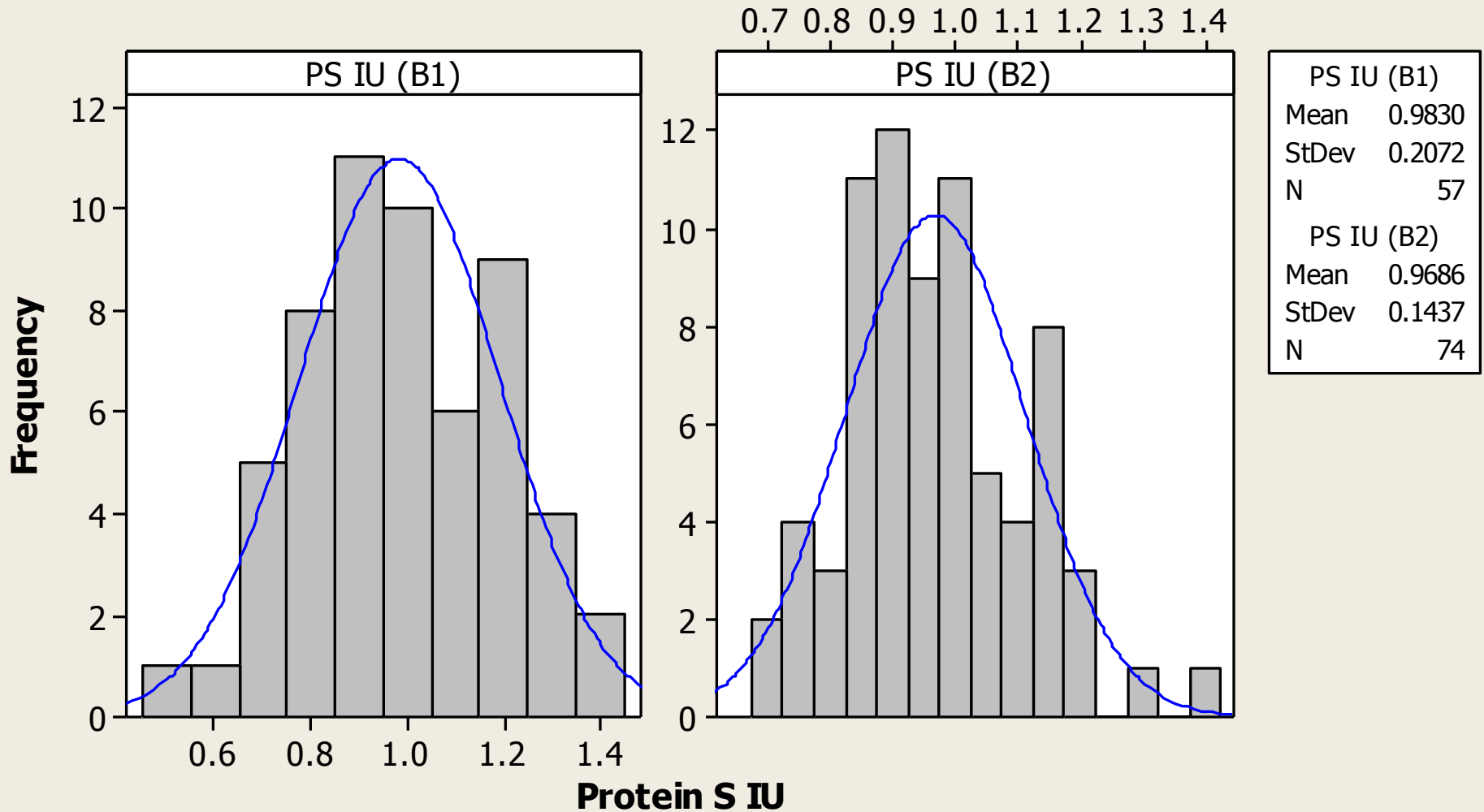
TEST	B1 FP	B2 FP
n	57	74
Factor V (IU/mL)	0.895 ± 0.17	0.861 ± 0.18
Factor VII (IU/mL)	0.981 ± 0.24	0.980 ± 0.20
Factor VIII (IU/mL)	0.913 ± 0.34	0.808 ± 0.33
Fibrinogen (grams/liter)	3.00 ± 0.73	2.90 ± 0.54
APTT (seconds)	36.8 ± 3.7	38.6 ± 4.9**
PT (seconds)	13.4 ± 0.72	13.3 ± 0.69
Alpha-2-antiplasmin	0.91 ± 0.10	0.91 ± 0.08
Protein S	0.983 ± 0.21	0.969 ± 0.15
Factor X	0.96 ± 0.05	0.94 ± 0.07
Factor XI	1.20 ± 0.27	1.18 ± 0.26

Values are reported as the mean ± the standard deviation; \*\*, p = 0.015 versus the corresponding B1 value by Mann-Whitney test.

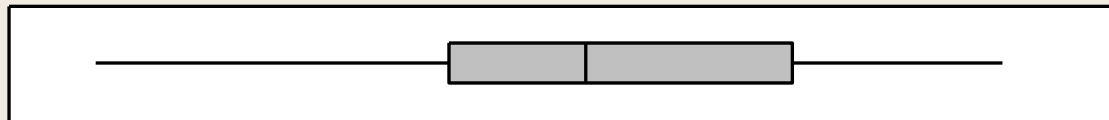
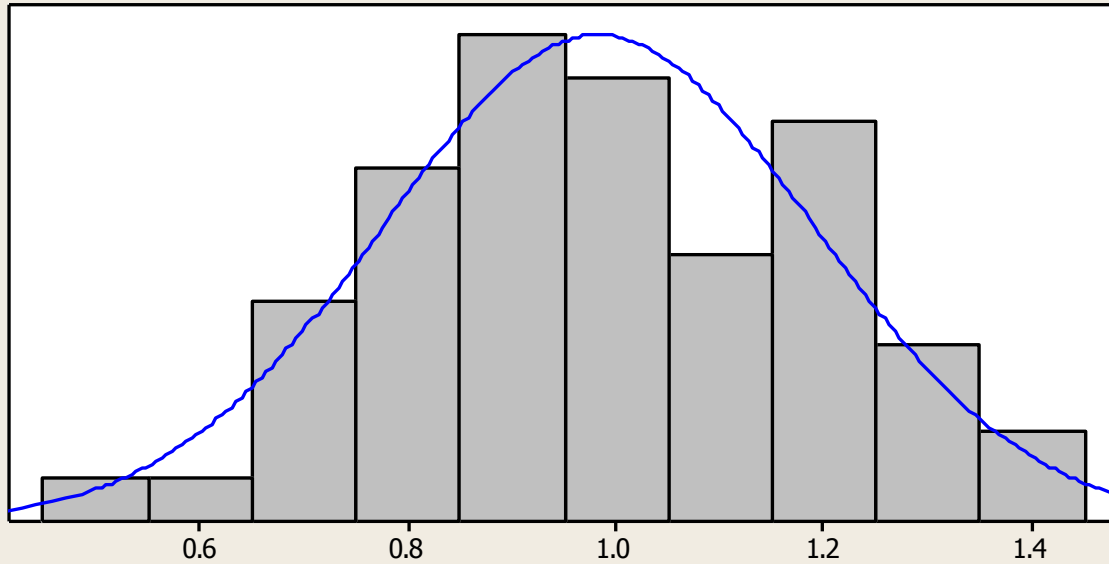
No statistically significant differences in factor levels were detected between FP prepared by immediate cooling to 4°C prior to freezing, and FP prepared from blood held overnight at room temperature.

# Histogram of PS IU (B1), PS IU (B2)

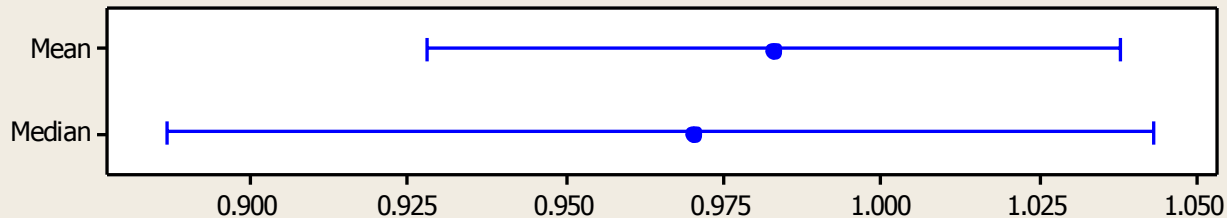
Normal



# Summary for PS IU (B1)



## 95% Confidence Intervals



### Anderson-Darling Normality Test

A-Squared	0.37
P-Value	0.423

Mean	0.98298
StDev	0.20723
Variance	0.04295
Skewness	-0.025362
Kurtosis	-0.634203
N	57

Minimum	0.50000
1st Quartile	0.84000
Median	0.97000
3rd Quartile	1.17000
Maximum	1.37000

95% Confidence Interval for Mean	
0.92800	1.03797

95% Confidence Interval for Median	
0.88666	1.04334

95% Confidence Interval for StDev	
0.17496	0.25422

# FP Compared to SD Plasma

**Table 4: Comparison of FP to SD Plasma**

TEST	FP (CBS)	Doyle et al (Octaplas)	Svae et al (Octaplas)
n	131	16	12
Fibrinogen (grams/liter)	2.94 ± 0.63	2.73 ± 0.20**	2.49 ± 0.65*
Factor V (IU/mL)	0.872 ± 0.17	0.69 ± 0.06***	0.861 ± 0.18***
Factor VII (IU/mL)	0.981 ± 0.22	1.13 ± 0.12**	1.08 ± 0.12*
Factor VIII (IU/mL)	0.850 ± 0.34	0.69 ± 0.18**	0.68 ± 0.18**
Factor X	0.944 ± 0.06	ND	0.78 ± 0.04***
Factor XI	1.19 ± 0.26	ND	0.99 ± 0.07***
Alpha-2-antiplasmin	0.909 ± 0.088	ND	0.23 ± 0.053***
Protein S	0.975 ± 0.17	0.39 ± 0.03***	0.64 ± 0.088***
APTT (seconds)	37.8 ± 4.5	35.6 ± 2.1**	35.2 ± 1.8**
PT (seconds)	13.3 ± 0.69	13.1 ± 0.30*	13.2 ± 0.53

Values are reported as the mean ± the standard deviation: ND, not determined; \*, p < 0.05; \*\*, p < 0.01; and \*\*\*, p < 0.001 versus the corresponding FP value by one-way ANOVA with Tukey's post-test.



# Conclusion

- FP from the routine inventory of a blood system that utilizes overnight RT hold of whole blood prior to producing rapid-frozen transfusion plasma does not show abnormally low levels of protein S. Units at the low end of the distribution are still well above the low end of protein S levels found in SD plasma.



# Should SD Plasma History Cause Concern?

- All units of plasma given to patients were S/D, therefore all units had low Protein S (and several other factors).
- With “FP24”, even if a unit was collected from a donor with unidentified heterozygous protein S deficiency, it would be diluted by other units with normal levels.
- Heterozygous protein S deficiency in the population has an incidence of approximately 1:500 (levels between 30 and 70 IU/mL).
- Clinical penetrance of heterozygous protein S deficiency is seen primarily in conjunction with a second cause (eg, factor V Leiden, oral contraceptives, etc).

# Thoughts about Protein S

- Does contact with cells overnight remove protein S from plasma? Binding should be through anionic PL binding, yet there is no evidence of increased annexin V binding to RBCs or platelets after O/N hold.
- Is this decrease reported to FDA by sponsors an assay artifact or a sample preparation artifact?
  - Functional assays measure free protein S only. Does O/N hold alter the proportion bound to C4bp?
  - Were plasma units prepared with rapid (blast) freezing in the sponsor studies?

# Thank you

