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Plasma produced from WB stored at ambient for 24 hours

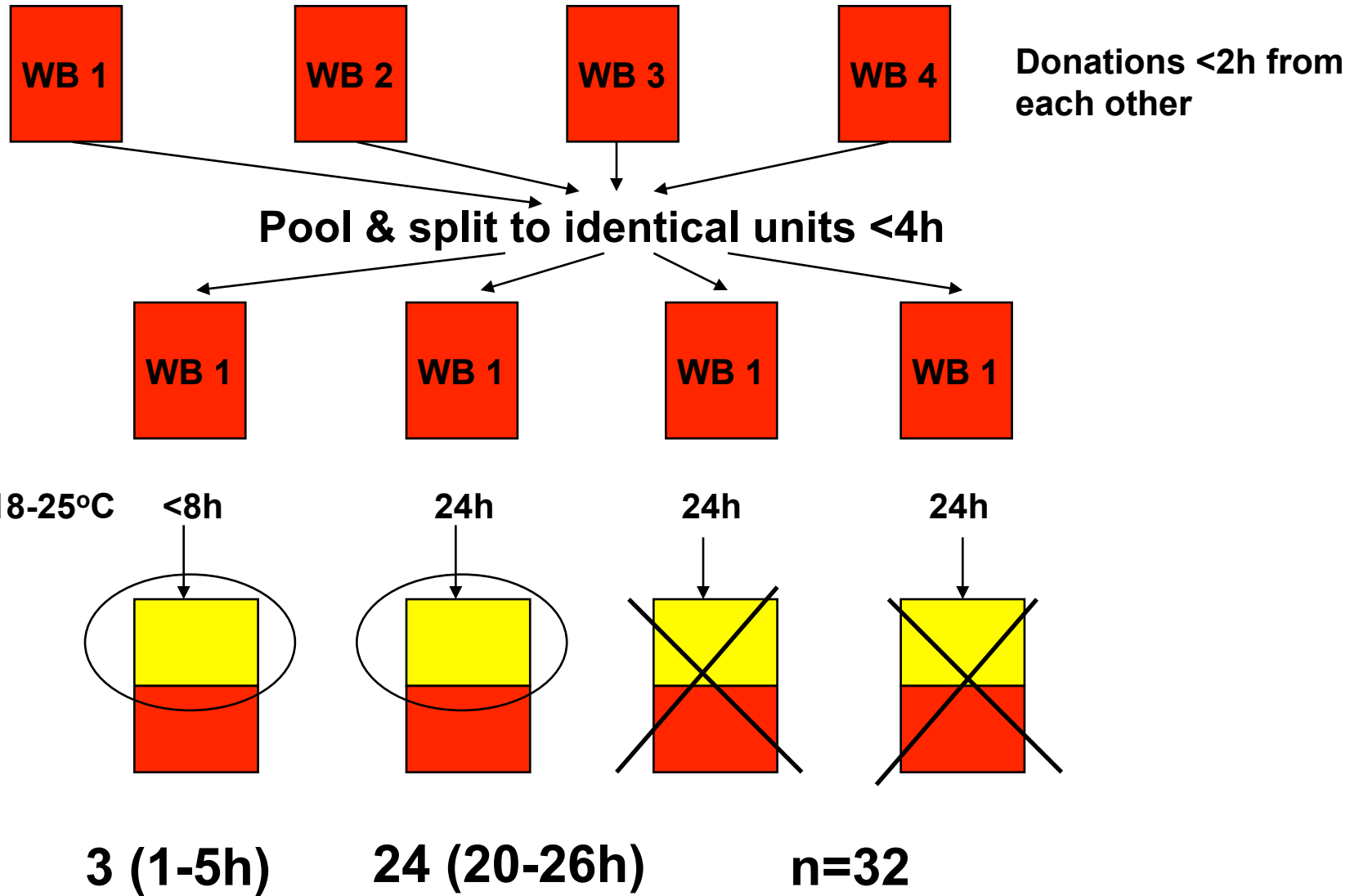
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Chair, UK Advisory Committee on Blood Components
Scientific Member, BEST Collaborative

Content

- BEST study
- Experience from England

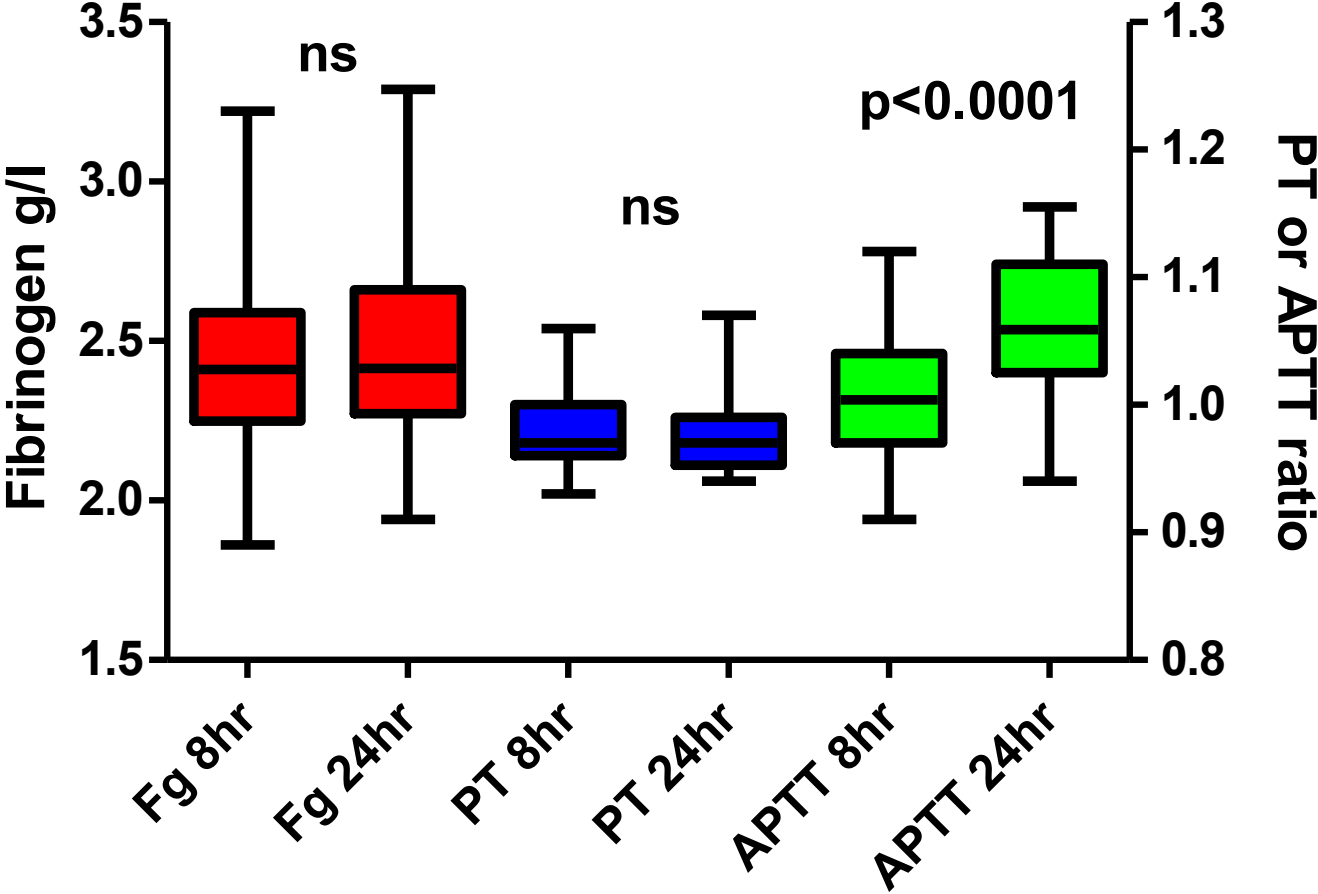
BEST study Design



BEST #41 plasma

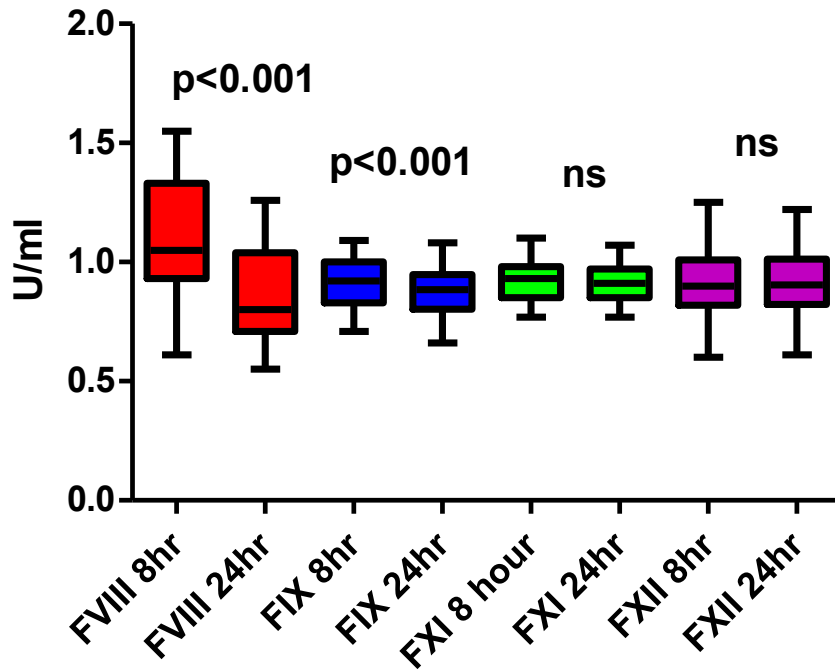
- All tested at single site (England)
- PT/APTT
- Fibrinogen (Clauss)
- Factors II, V, VII, IX, X, XI, XII - one stage -clotting assay
- Antithrombin, protein C, FXIII - chromogenic assay
- Protein S – free antigen
- Thrombin generation – calibrated automated thrombogram

screening tests

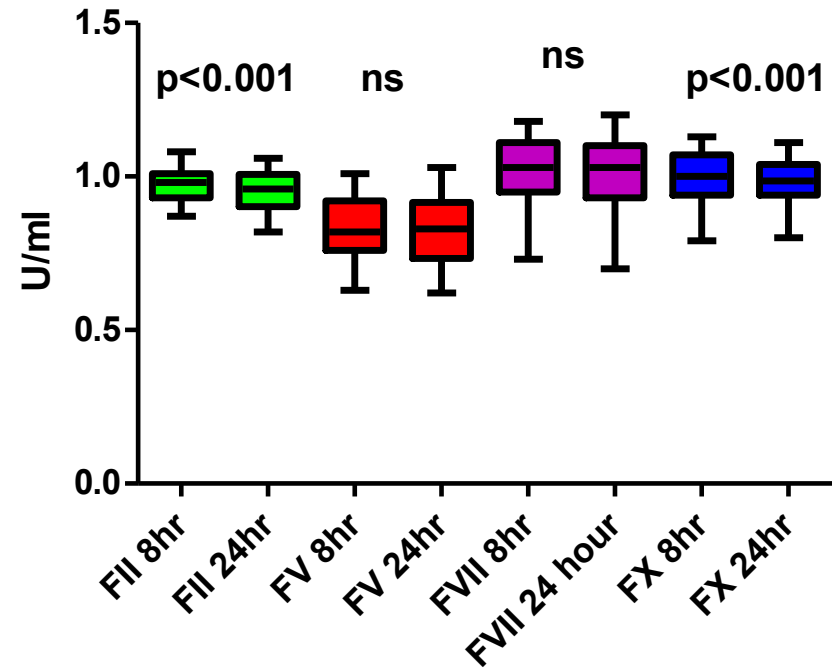


Factor assays

APTT based assays

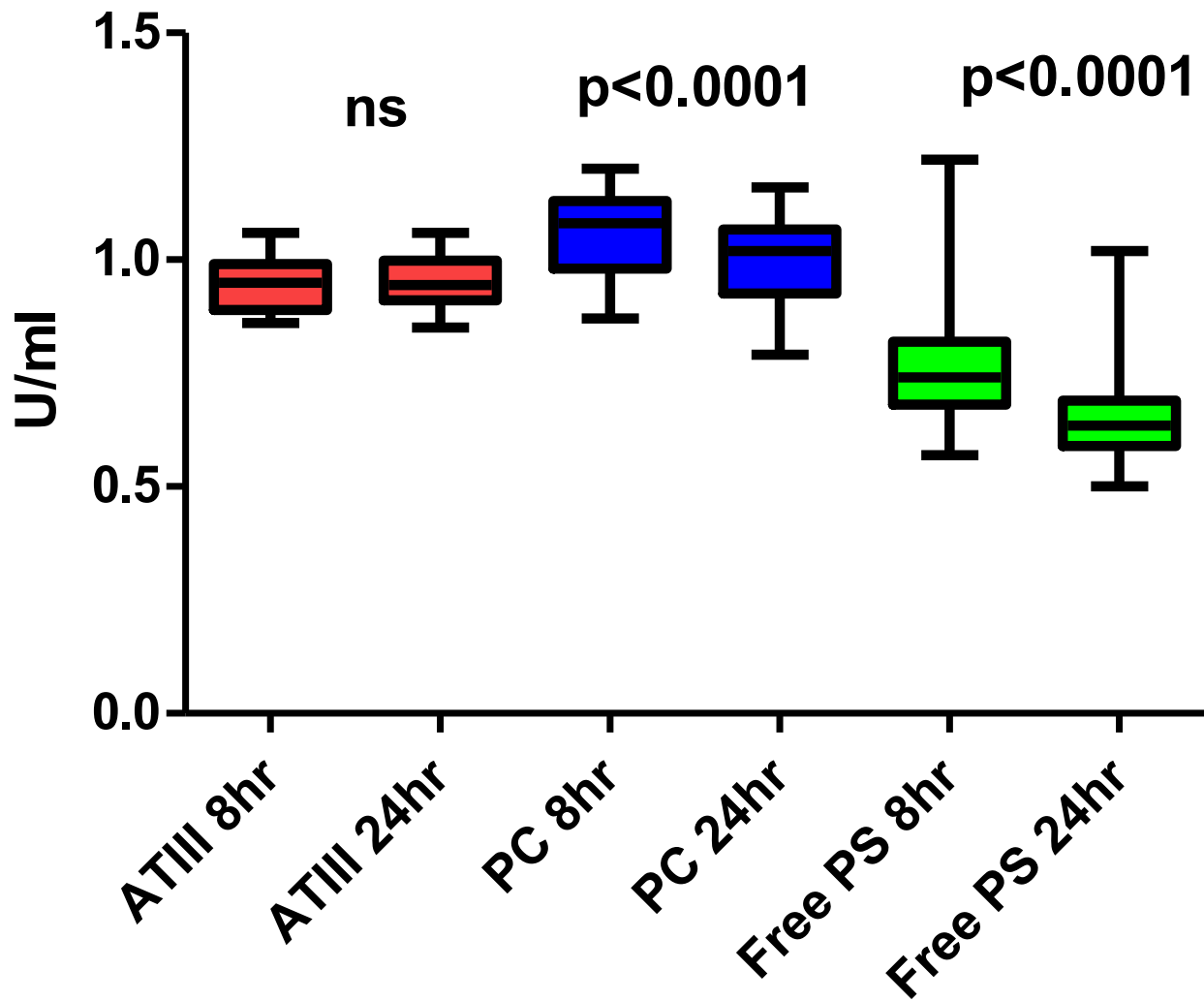


PT based assays



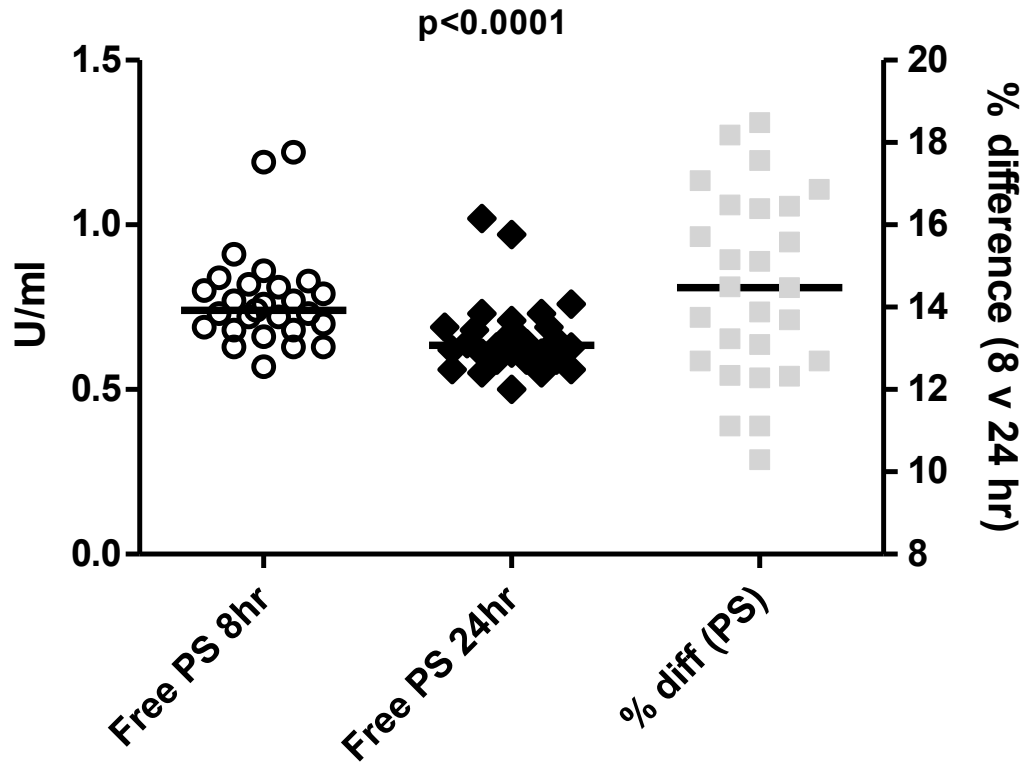
20% loss of FVIII activity, 27/32 > 0.70 IU/ml = 84%

Inhibitors/anticoagulants

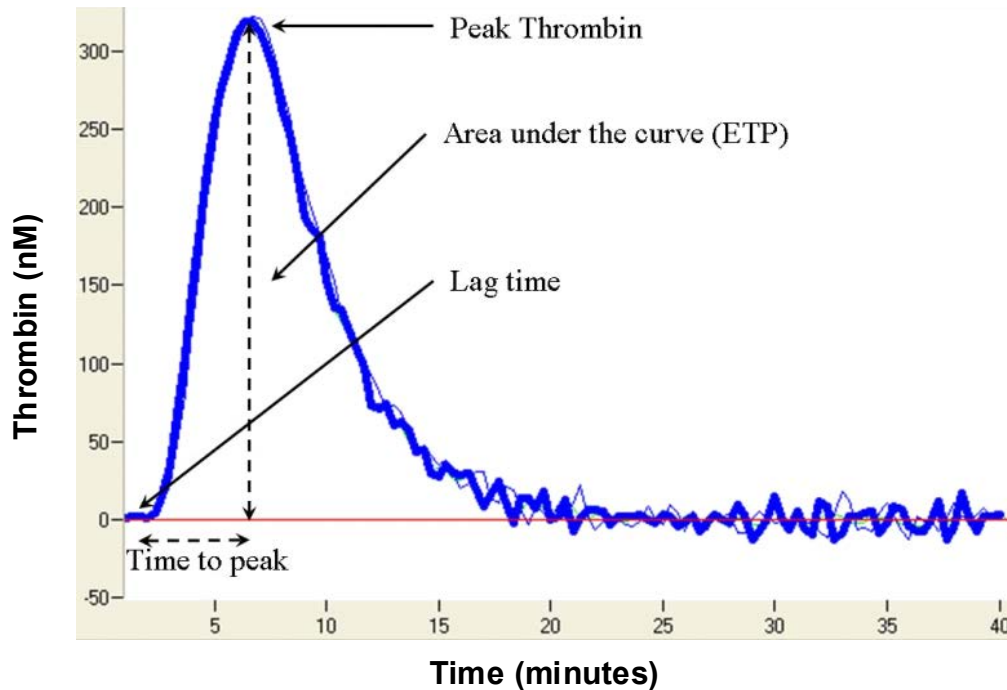


Protein S

Free Protein S all centres



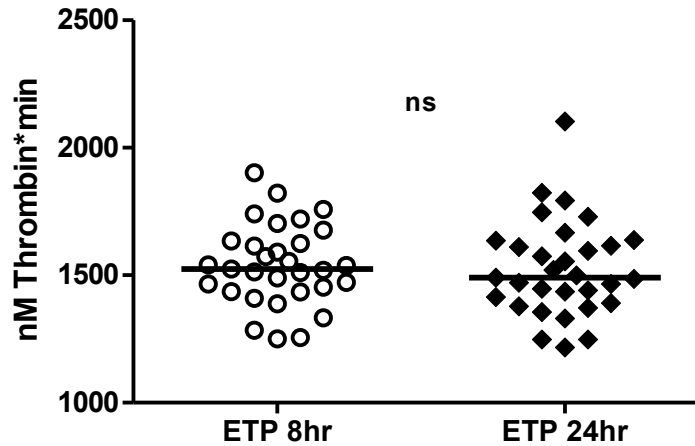
Real time thrombin generation



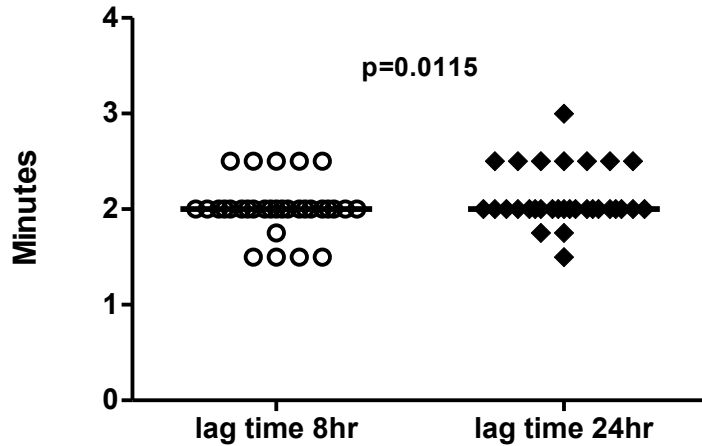
- Low dose TF (pM)
- Exogenous PL
- Ca²⁺
- Fluorogenic substrate cleaved by thrombin

Thrombin generation

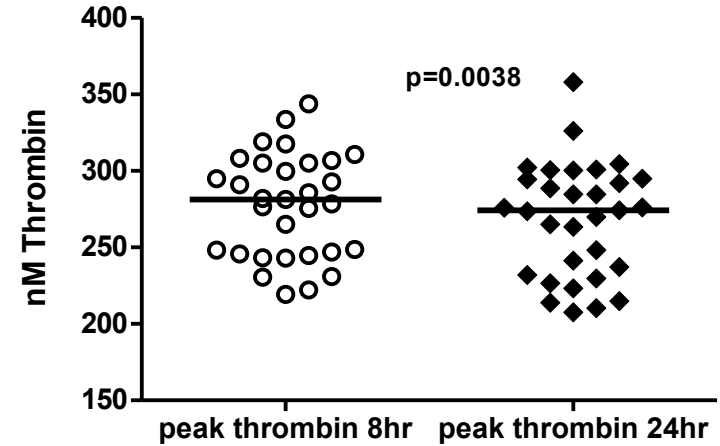
a) Endogenous thrombin potential



b) Lagtime



c) Peak thrombin



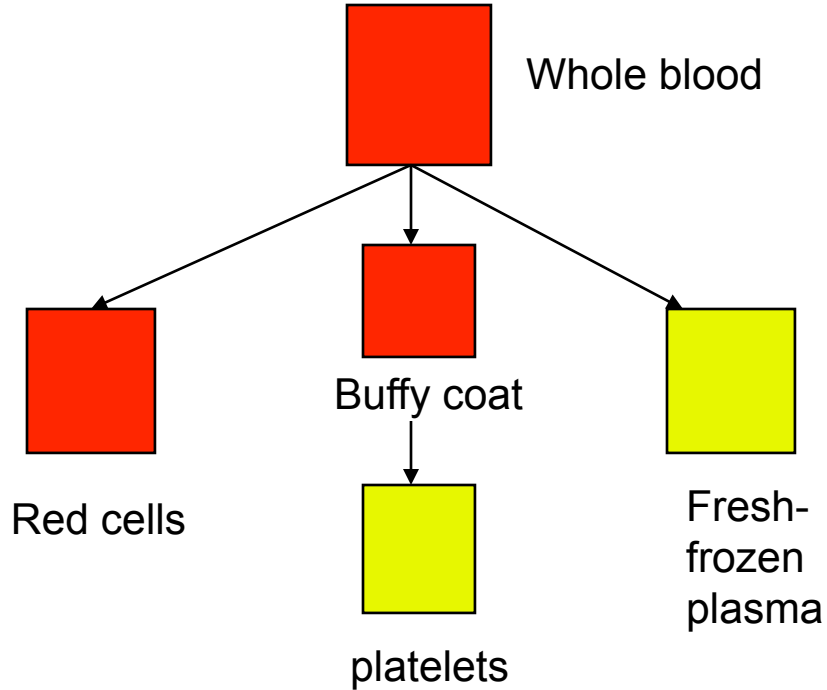
Data on Plasma from NHS Blood & Transplant

NHS Blood & Transplant

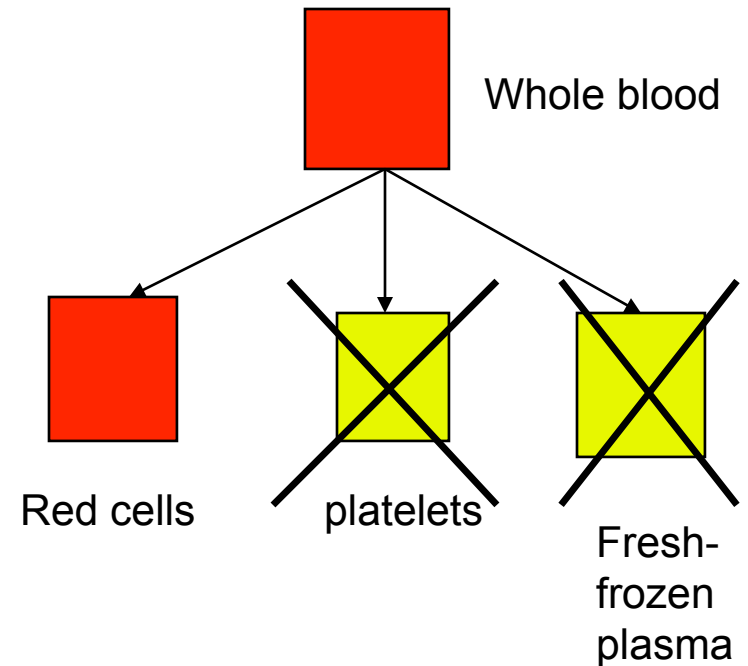
- Supply 100% platelets & red cells in England
 - 250,000 platelets (80% apheresis)
 - 1,800,000 red cells
- Supply most plasma for transfusion
 - 260,000 FFP
 - 100,000 cryoprecipitate
 - 20,000 MB FFP
- SD FFP from Octapharma (19% of FFP in England)
- Introduced ambient storage of WB in 2008
 - Driver was male only FFP (TRALI) & efficiency
 - 80% of FFP now produced on day 1
 - 24 hour post-thaw shelf-life

Current processing methods, England

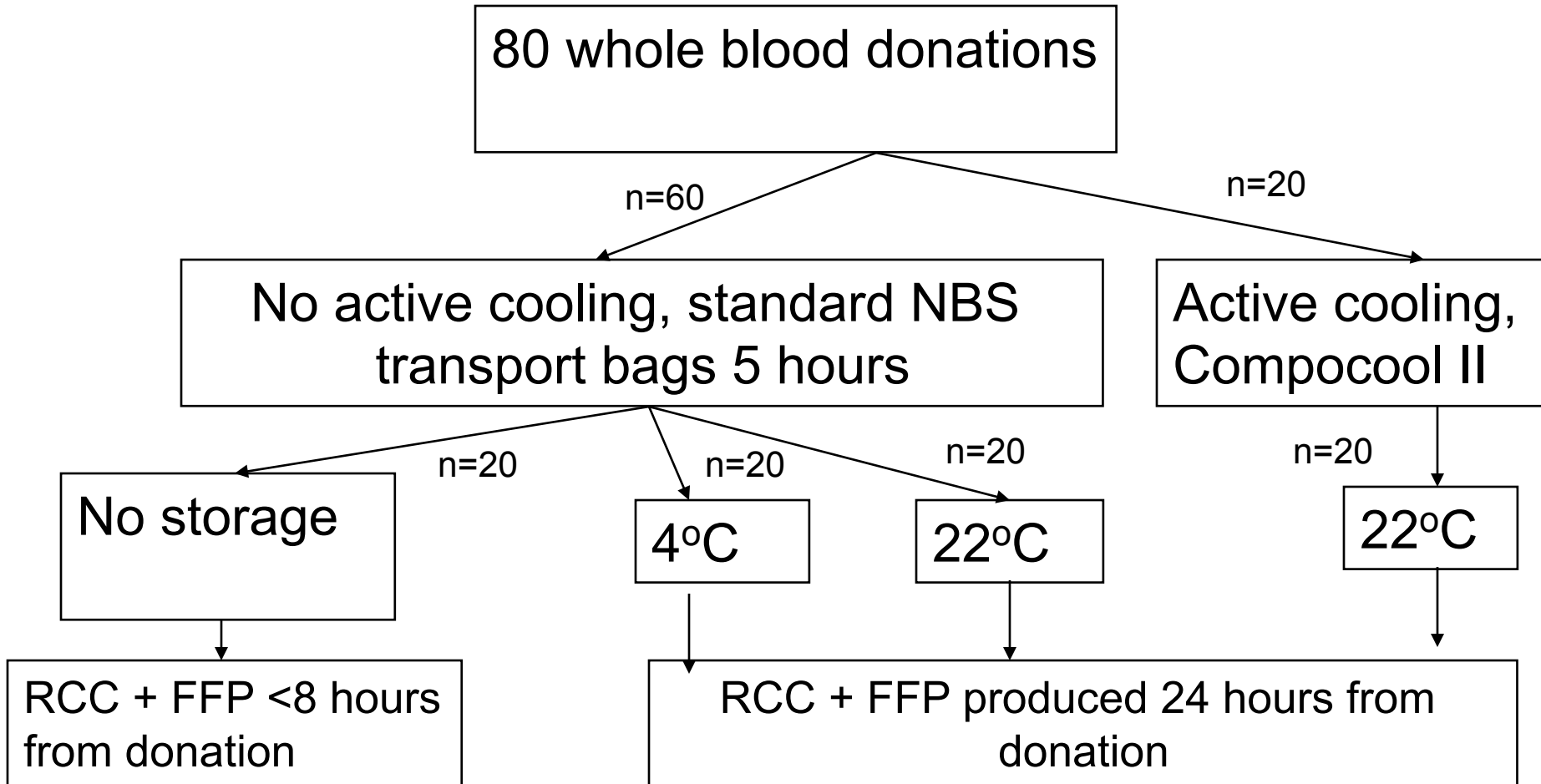
Day of collection (day 0) or after ambient storage (day 1)



After 4°C storage (day 1)



Validation studies



FFP data

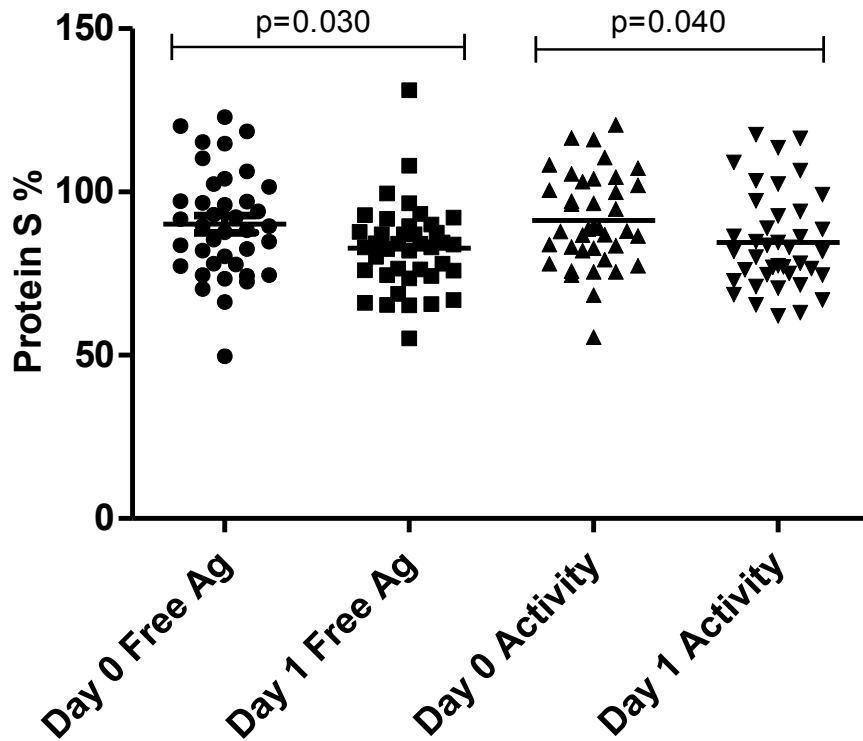
	Fibrinogen (g/L)	Factor V (IU/mL)	FVIII (IU/mL)	Percentage with >0.70 IU/mL FVIII levels
Day 0	2.76	0.96	1.17	100%
	(2.50-3.02)	(0.91-1.01)	(1.10-1.25)	
			B, C, D	
Day 1, Ambient, no cooling	2.65	1.00	0.98	85%
	(2.47-2.84)	(0.95-1.06)	(0.87-1.09)	
		C	A, D	
Day 1, Ambient, cooling plates	2.61	0.87	0.92	88%
	(2.39-2.83)	(0.79-0.92)	(0.83-1.00)	
			A, D	
Day 1, 4°C	2.76	0.89	0.74	55%
	(2.52-3.00)	(0.84-0.95)	(0.65-0.83)	
			A, B, C	

N=20 for each group, 10 group O, 10 A

Data from a further 126 units 22°C hold, no cool FVIII = 0.97 (SD 0.38)

From Wilshire et al, Transfusion 2008; 48: 2338-2347

FFP from routine production- 22°C storage WB



- Unpaired samples
- All male donors
- n=40 day 0 or 1
 - Day 0 8hr (4-11)
 - Day 1 21hr (16-26)
- Day 1 values 7% lower by either method

After 4°C storage of WB

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 Staciot (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). ^ap < 0.05 and ^bp < 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.

3-9% reduction in PS clotting activity dependent on assay
4% reduction in free antigen

From: Cardigan et al, Transfusion 2005; 45:1342-1348

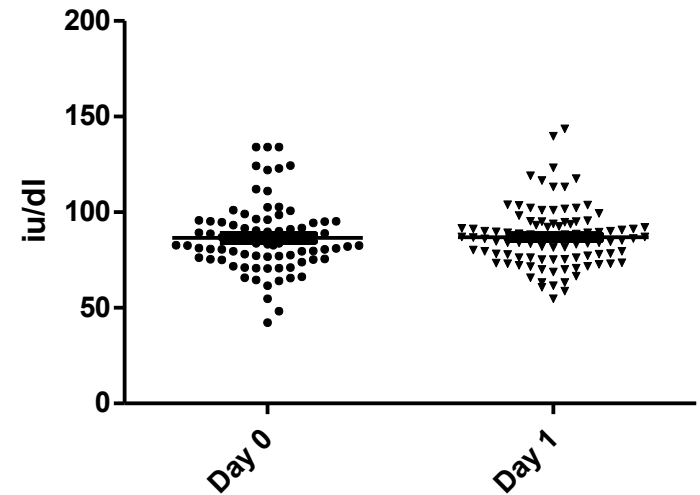
Storage of WB at 4°C - all male donors, unpaired

Table 1 Levels of coagulation factors and physiological inhibitors in fresh-frozen plasma (FFP) (for all ABO blood groups)

Analyte	Day 0 (n = 82)	Day 1 (n = 99)	Day 0 vs. Day 1
Prothrombin time (second)	12.0 (11.7–12.5)	12.1 (11.8–12.5)	NS
Activated partial thromboplastin time (second)	27.8 (26.9–29.0)	29.1 (27.4–31.1)	P = 0.0007
Fibrinogen (g/l)	2.54 (2.28–2.79)	2.51 (2.21–2.78)	NS
Factor II (iu/dl)	92.7 (85.4–99.8)	91.0 (85.7–100.5)	NS
Factor V (iu/dl)	102.0 (92.4–115.6)	101.9 (86.5–112.9)	NS
Factor VII (iu/dl)	102.3 (91.0–114.6)	95.1 (82.3–107.5)	P = 0.013
Factor VIII (iu/dl) clotting	83.5 (70.3–106.4)	68.4 (53.5–86.7)	P < 0.0001
Factor VIII (iu/dl) chromogenic	92.5 (77.6–121.9)	75.5 (56.6–109.2)	P = 0.0008
von Willebrand factor:Ag (iu/dl)	115.8 (87.2–143.5)	116.7 (88.5–145.6)	NS
Factor IX (iu/dl)	93.2 (83.8–105.0)	88.2 (81.8–100.0)	P = 0.04
Factor X (iu/dl)	102.6 (94.8–113.2)	103.7 (93.1–112.3)	NS
Factor XI (u/dl)	96.7 (88.6–105.7)	93.6 (83.0–105.5)	NS
Factor XII (u/dl)	114.5 (90.1–137.2)	109.0 (89.3–131.9)	NS
Factor XIII (u/dl)	110.5 (97.1–122.1)	113.2 (101.0–126.4)	NS
Microparticles (nM, phosphatidylserine)	4.34 (2.93–5.51)	5.7 (4.55–7.19)	P < 0.0001
C1 esterase inhibitor (μ/dl)	104.5 (97.0–119.7)	104.6 (96.9–116.8)	NS
α ₂ -macroglobulin:Ag (g/l)	1.30 (1.04–1.78)	1.33 (1.12–1.87)	NS
α ₂ -macroglobulin activity (g/l)	1.15 (1.02–1.47)	1.17 (1.03–1.31)	NS
Antithrombin-III (iu/dl)	98.4 (94.0–104.6)	99.0 (93.4–103.8)	NS
Protein C (iu/dl)	110.4 (100.7–119.5)	109.3 (97.2–119.6)	NS
Free protein S:Ag (iu/dl)	83.8 (76.1–95.2)	87.1 (76.2–93.5)	NS
Heparin cofactor II	97.7 (86.3–111.7)	93.3 (86.3–103.4)	NS
α ₂ -antiplasmin (u/dl)	101.6 (95.9–107.3)	102.2 (96.1–108.9)	NS
Free tissue factor pathway inhibitor (ng/ml)	10.6 (7.9–13.3)	11.4 (9.9–13.9)	P = 0.04

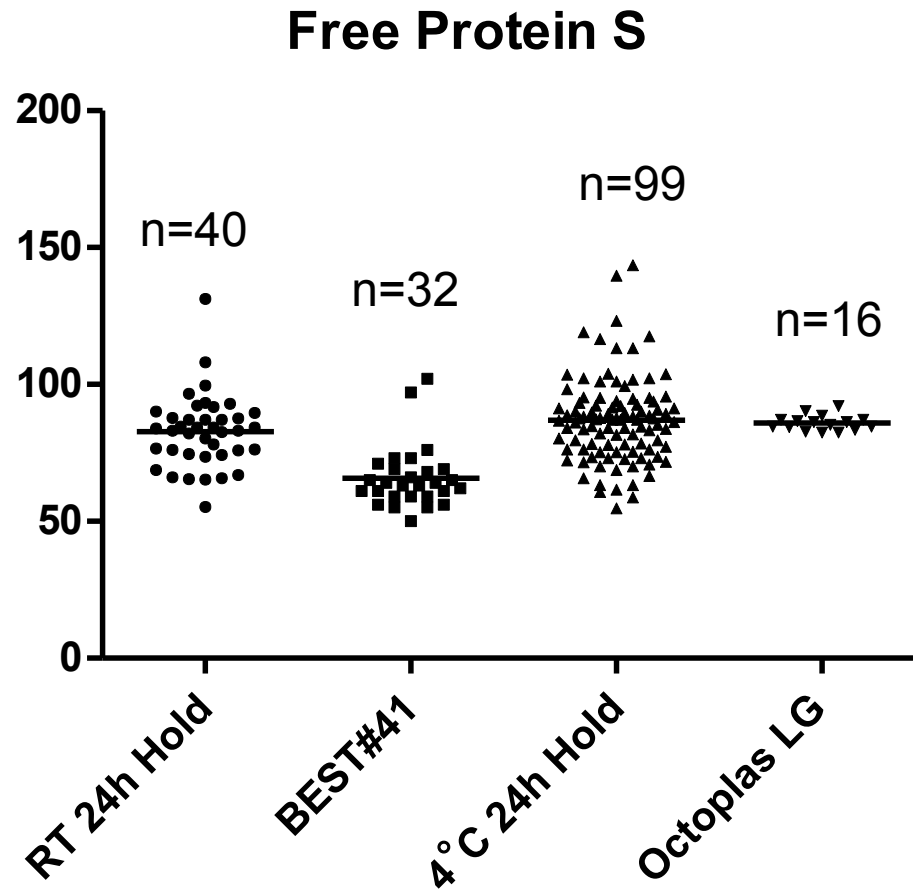
Data are presented as median values with the 25th–75th percentile range in parentheses.
Mann–Whitney *U*-test was employed to compare data derived from Day 0 and Day 1 samples.
NS, not significant at the 5% level.

Free PS antigen



From: Lawrie et al, Vox 2008; 45:306-314

PS in final product

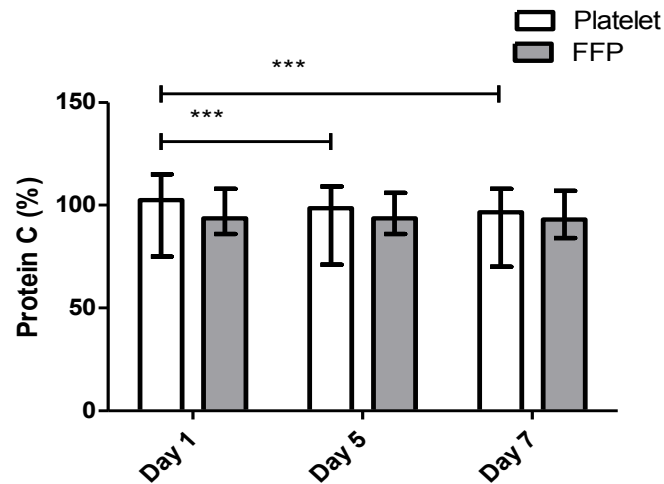
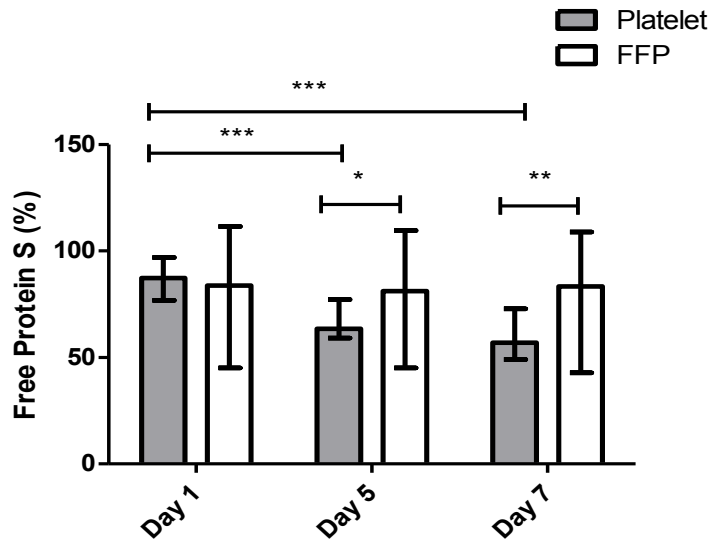


From: Cardigan et al 2005, Cardigan et al 2011, Lawrie et al 2010

Octaplas and PLAS+SD are not the same

	PS (IU/ml)	Reference
PLAS+SD	0.25 & 0.15	Flamholz
PLAS+SD	0.24 (0.14-0.37)	Solheim
PLAS+SD	0.20 & 0.15	Salge-Bartel
Octaplas	0.39 \pm 0.02	Doyle
Octaplas	0.64 (0.55-0.71)	Solheim
Octaplas	0.64 (0.61-0.66)	Svae
Octaplas	0.58 (0.55-0.63)	Yarranton

POST-THAW DATA



- N=10 units
- WB stored 22°C for 24 hrs
- FFP made
- Plasma stored 4°C following thawing
- Large variation in published studies on effect of post-thaw storage on PS

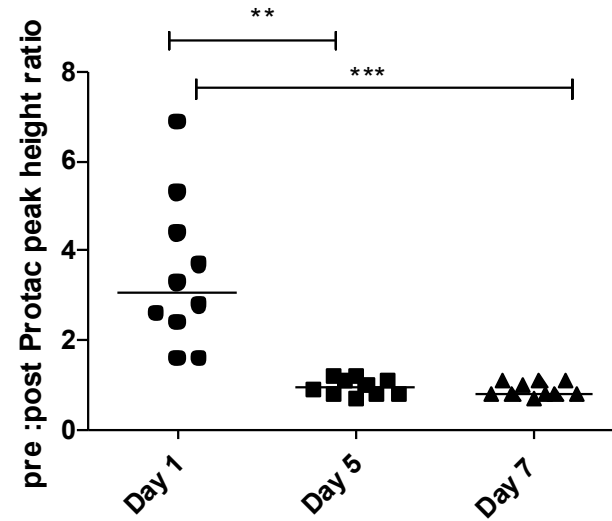
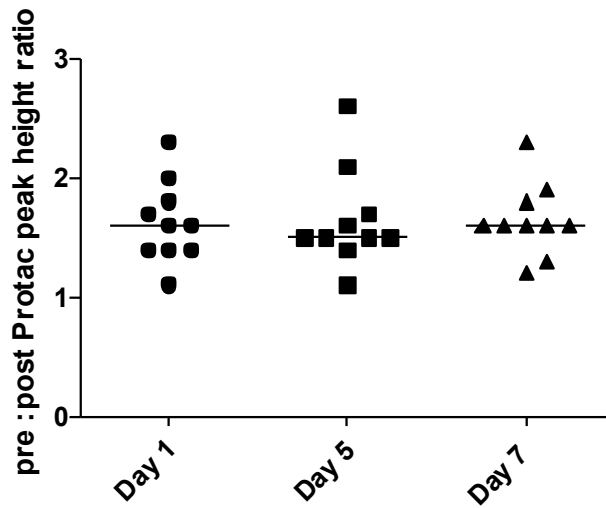
Post-thaw studies measuring PS

n	Storage of WB	Summary of findings	Reduction over 5-6 days	Assay	Reference
20 (5 of each ABO)	<1 hr (apheresis)	Post-thaw (0.95) Day 6 (0.95)	None	Free ag	Von Heymann et al, 2009
9 not stated	<8h?	Post-thaw (0.85 ± 14) Day 5 (0.63 ± 14)	26%	Activity-free ag?	Pati et al 2010
50	<6h apheresis	61% reduction in PS over 7 days	61%	Clotting	Thiele et al 2011
5 one of each	<24hr 4oC	Post-thaw (0.86) Day 5 (0.31)	64%	clotting	Nifong et al 2002
18 (5 of each A,B, O, + 3AB)	<24hr 4oC	Post-thaw (0.74) Day 5 (0.52)	31%	'activity'	Yaza et al, 2008
15 (5A, 5B, 5O)	<8 hr <24hr 4oC	FFP (0.97 ± 18) PF24 (0.92± 18) Day 5 FFP (0.90± 22) Day 5 PF24 (0.78± 18)	7% for FFP 15% for PF24	Clotting	Scott et al, 2009
30 (13 O: 17 non-O)	< 8 hour n=14 <24hr 4oC n=16	Post-thaw (0.88 ± 14) Day 5 (0.64 ± 14)	28%	Free ag	Matijevic et al 2011
15 (8A, 4O, 2B, 1AB)	<24 hr 'room temp'	FFP (1.02 ± 14) RT PF24 (1.04± 11) Post-thaw (0.65) Day 5RT PF24 (0.72)	None	Free ag	Alhumaidan et al 2010

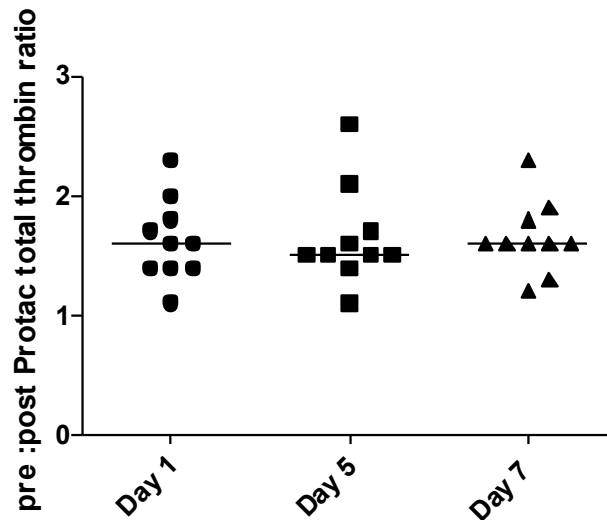
Thrombin generation (APC)

- Real time thrombin generation
- In absence/presence of activator of protein C (Protac)
- Increases sensitivity of method to reductions in activity of protein C/S
- Used to assess APC resistance

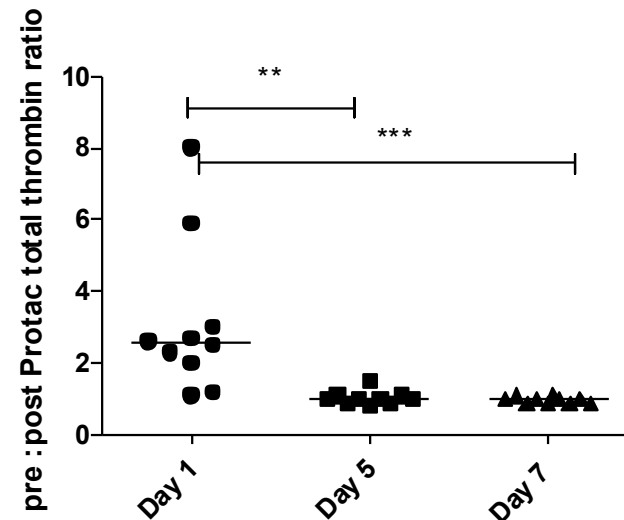
Thrombin generation in presence of APC



Thawed plasma



Platelets



Our experience

- Storage of whole blood at 4°C for 24 hours
 - 3-9% loss of PS paired study - depends on assay
 - No difference in unpaired samples
- Storage of WB at 22°C 24 hours
 - 14% loss PS paired BEST study
 - 7% loss unpaired study routine use
 - Lower limit of range above lower limit in Octaplas
- No loss of PS activity following subsequent 4°C storage of thawed plasma for 7 days
- Minimal effect of RT 24 hold on thrombin generation
- Product used widely throughout Europe, labelled as FFP
- PS activity lower in females
 - Loss of PS due to WB storage might be offset by selection of male only plasma
 - Male plasma also less prone to contact activation