NHSBT Component Developments

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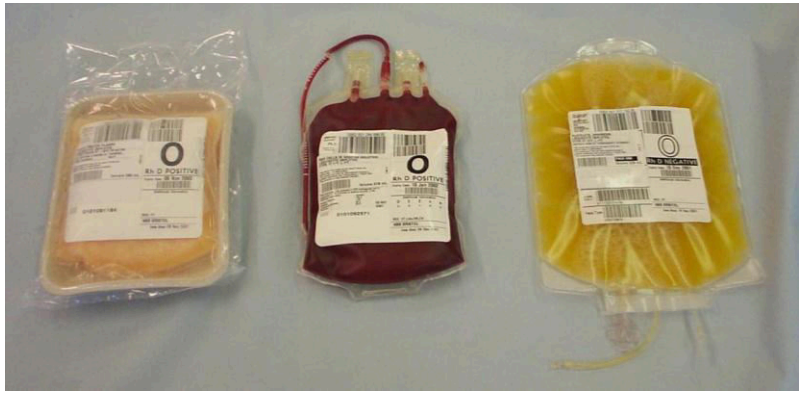
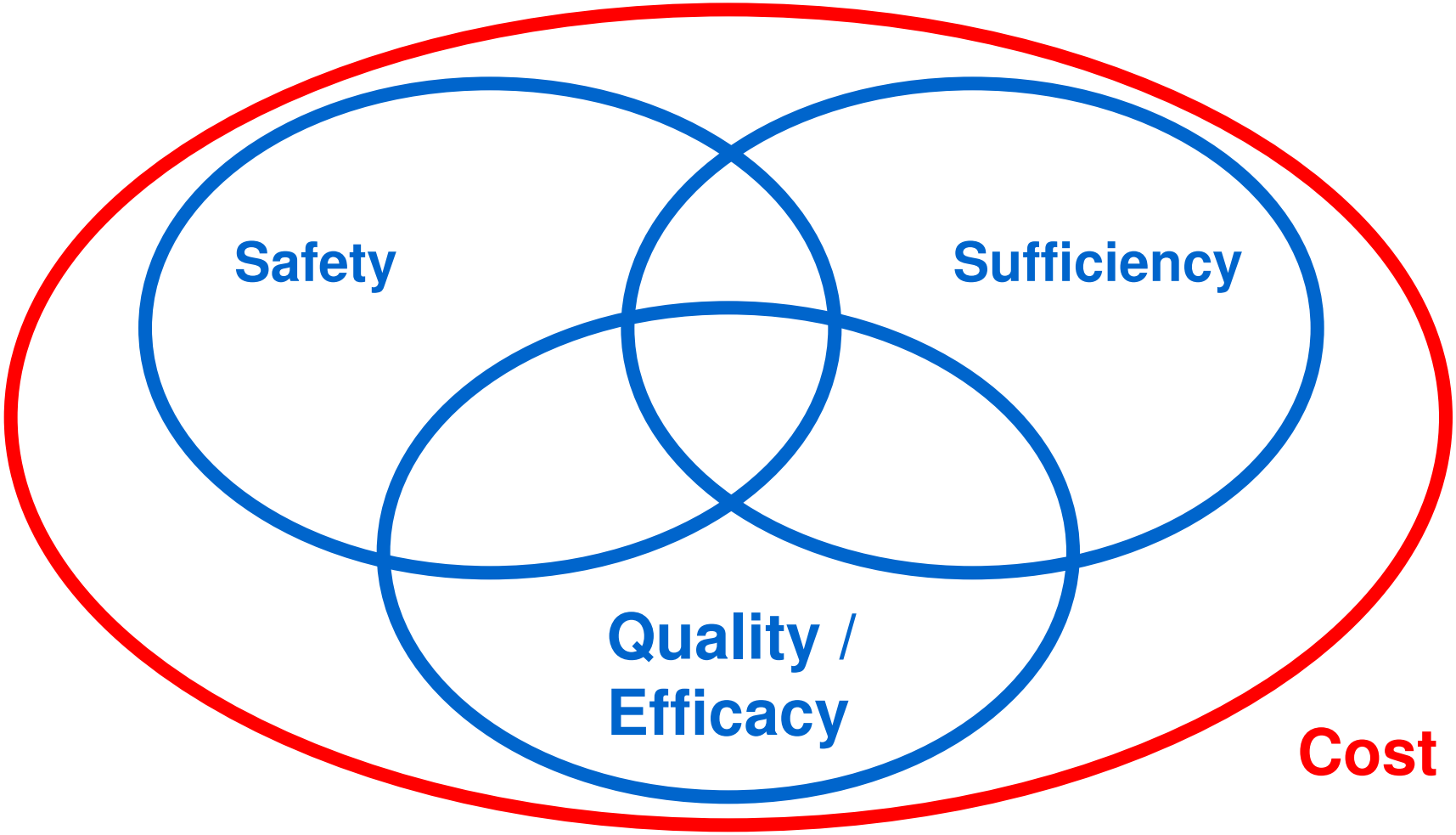
Interim CDL Manager
Components Development Laboratory



Outline

- Red cells:
  - Apheresis
  - New additive solutions
  - Pathogen inactivation
  - In vitro grown red cells
- Platelets:
  - Additive solutions
- Plasma:
  - Pathogen inactivation

What are we trying to achieve?



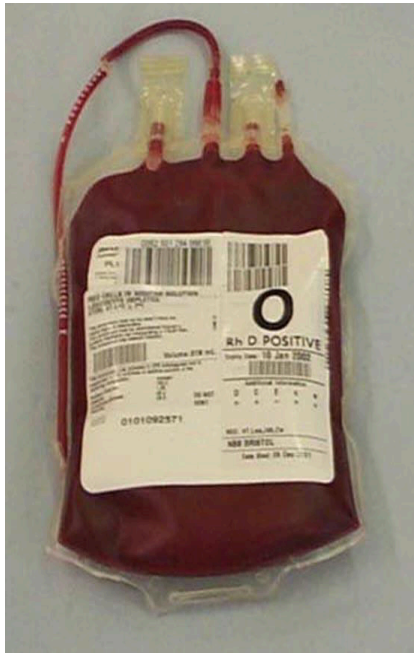
‘Conventional’ Blood Components

Each year NHSBT issues:

- Red cells (1.9 million)
- FFP/cryoprecipitate/cryosupernatant (400,000)
- Platelets (200,000)
- Granulocytes (1000)

- A major change could affect 100,000s of patients

Developments with Red Cell Components



Apheresis (Component Donation)

- Blood is collected into a sterile, closed system, centrifuged and separated into components
  - Required component(s) are retained and the rest is returned to the donor
- UK - Currently done in static clinic to collect platelets
- Allows collection of single/multiple components from one donor
  - Platelet collection 1, 2, 3 units
  - Granulocytes
  - Plasma
  - 1 red cell + 1 platelet
  - 1 red cell + 1 plasma
  - 2 units of red cells

Double red cells

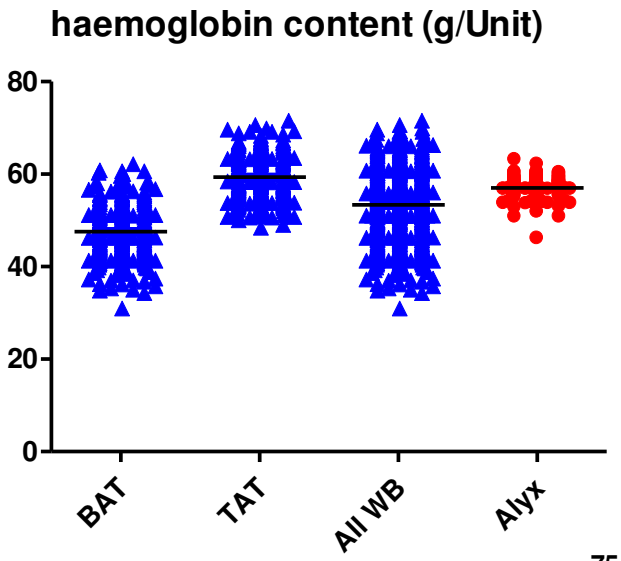
- Portable systems - 2 units per donor (30 mins)
- Not feasible for all red cells as only 40% of donors eligible

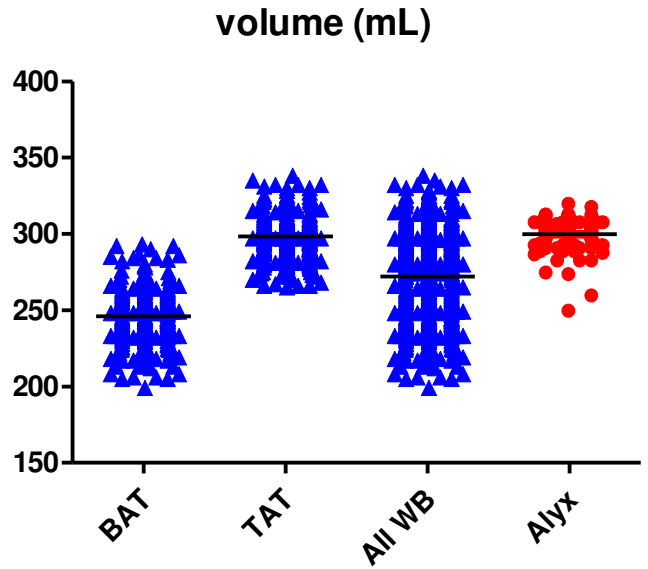
  >70kg weight

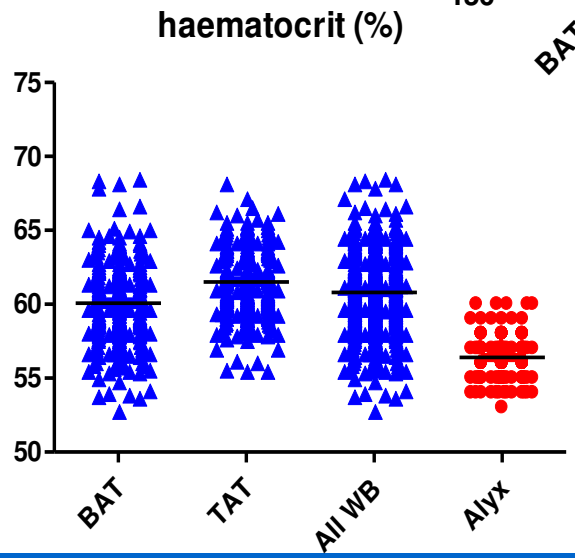
  >14g/dL Haemoglobin

- More consistent product

Apheresis v WB red cells







n = 159 Alyx
n = 502 WB

Does variation in RBC matter?

- Assumption is that 1 unit of red cells will raise Hb by 1g/dl in the patient
- Doesn't take account of varying size of patient or amount of Hb in the bag
- What happens if you match the Hb content of the unit to the individual patient?

TRANSFUSION PRACTICE

Hb content-based transfusion policy successfully reduces the number of RBC units transfused

Önder Arslan, Selami Toprak, Mutlu Arat, and Yasemin Kayalak

Transfusion 2004;44:485-488

- Can 3-4 unit orders be reduced to 3-2, and 2 unit orders be reduced to 1, if we match Hb to patient?
- Patient blood volume, current Hb and desired Hb are used to calculate Hb needed

Outcome

- 30 % fewer units transfused compared with original order

DRC for thalassaemia

- NHSBT study (Heffernan et al, 2004)
  - 44 thalassaemia patients needing 2 units every 3 weeks
  - Difficult to maintain Hb within narrow limits due to irregular patient attendance
  - Pre-transfusion Hb varied by 5-16g/l in regular attenders
- Consistent rise in Hb with standard product
- Hb kept within narrow limits if patients attend regularly
- 43/44 patients preferred DRC as time for Tx predictable

Benefits to NHSBT

- Donors can donate WB up to 3-4 times per year
  - Only 4 % donate 3 times a year
  - Mean = 1.5 donations/year
  - 60 % of donors give blood once a year or less
- If ‘once a year’ donors gave 2 units of red cells
  - Boost red cell stocks
- Could target blood groups in short supply
- Reduced donor exposure if both units go to same recipient

Disadvantages?

- Cost may be higher
- Increased collection time if multi-component
- Donor eligibility (only about 35-40 %)
- May contain more plasma (lower Hct)
  - Increased TRALI or vCJD risk?

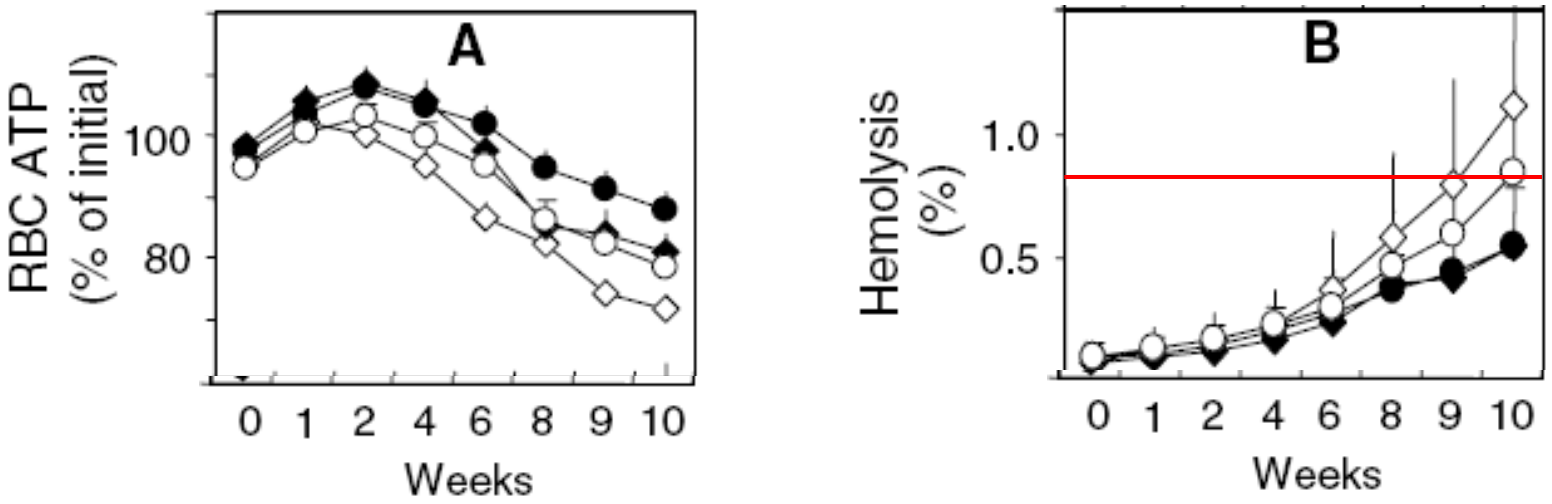
New red cell additive solutions

- In the UK and most of Europe, red cells are stored in SAGM (1970 – 1980ies)
- In the US red cells are generally stored in AS1 or AS3
- Despite lots of development work, little has changed in routine practice
- NHSBT in colaboratoin with Sanguin are planning to assess a range of ‘new’ additive solutions in 2013:
  - SAGM, AS1, PAGSSM
  - PAG3-M, Eryrothrosol 4 & SOLX (EAS81)

Buffering and dilution in red blood cell storage

50 TRANSFUSION Volume 46, January 2006

J.R. Hess, N. Rugg, A.D. Joines, J.F. Gormas, P.G. Pratt, E.B. Silberstein, and T.J. Greenwalt



AS-3, (◇); EAS-81, (○).

SOLX

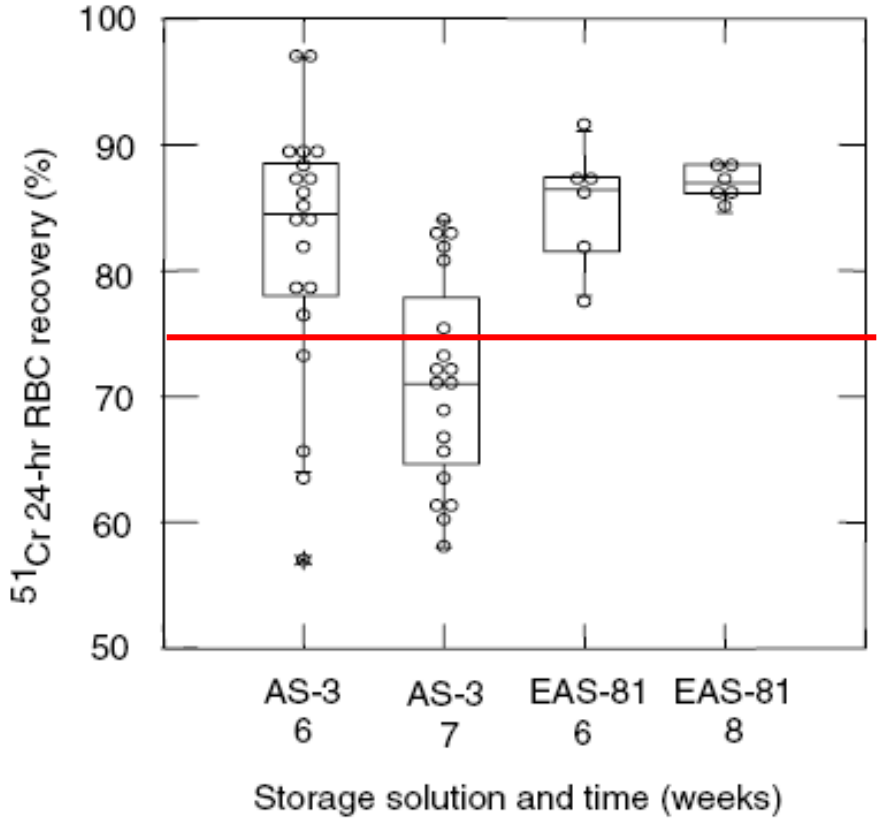


Fig. 2. RBC 24-hour in vivo recovery measured after storage in EAS-81 for 6 weeks (n = 6) or 8 weeks (n = 6) and compared to a historic control, the licensure study for AS-3 published by Simon and colleagues in 1987. Both studies used the ^{51}Cr single-label method.

FDA requirement
>75 % recovery

New red cell additive solutions

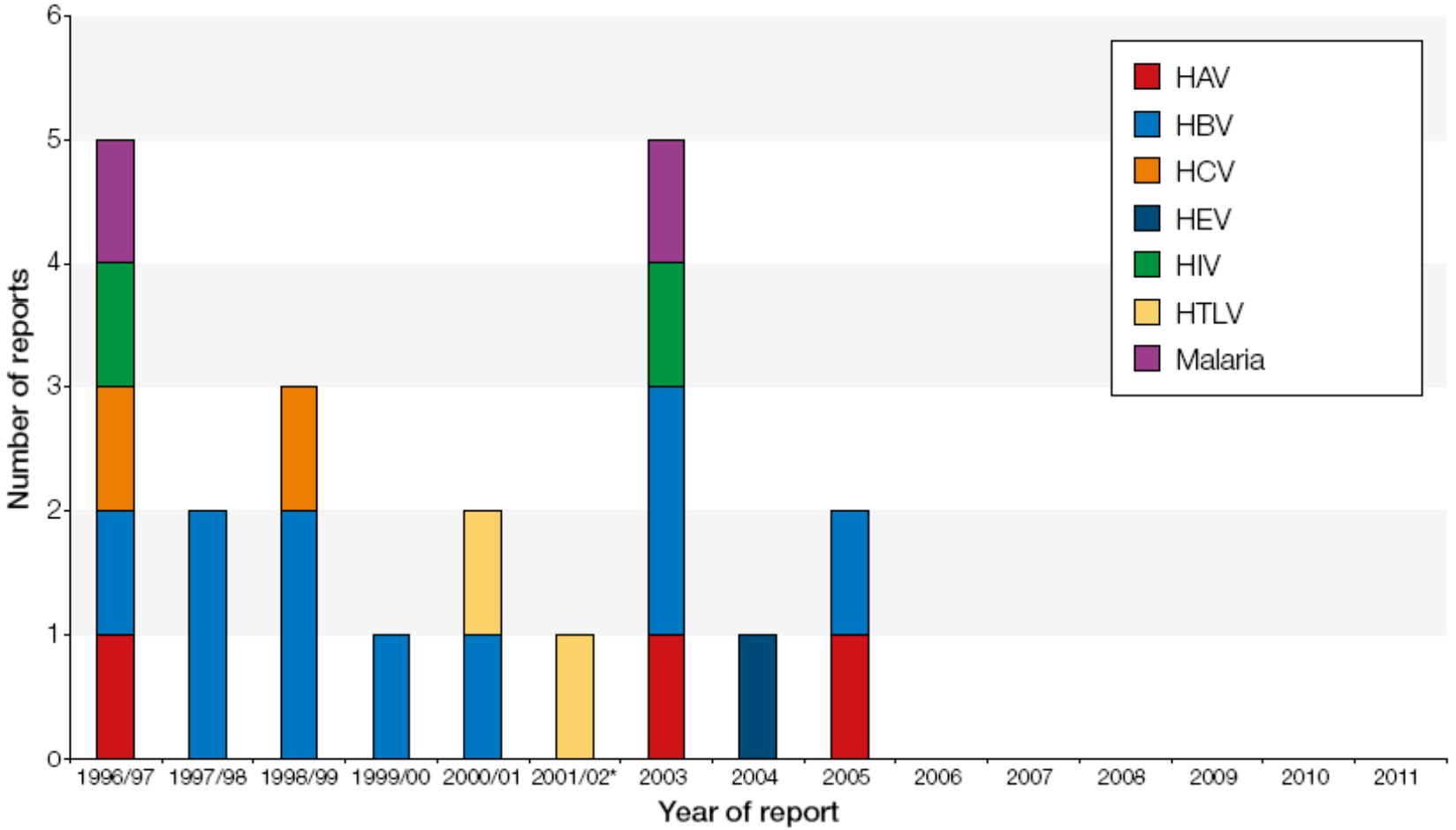
- Potential shelf-life of 56 days (8 weeks)
  - Not the driving force behind any potential change
  - Concerns over the safety of ‘old’ blood versus ‘fresh’ blood
- Improved quality over 35-42 days (5-6 weeks)
  - Should offer a benefit to patients

Pathogen inactivation

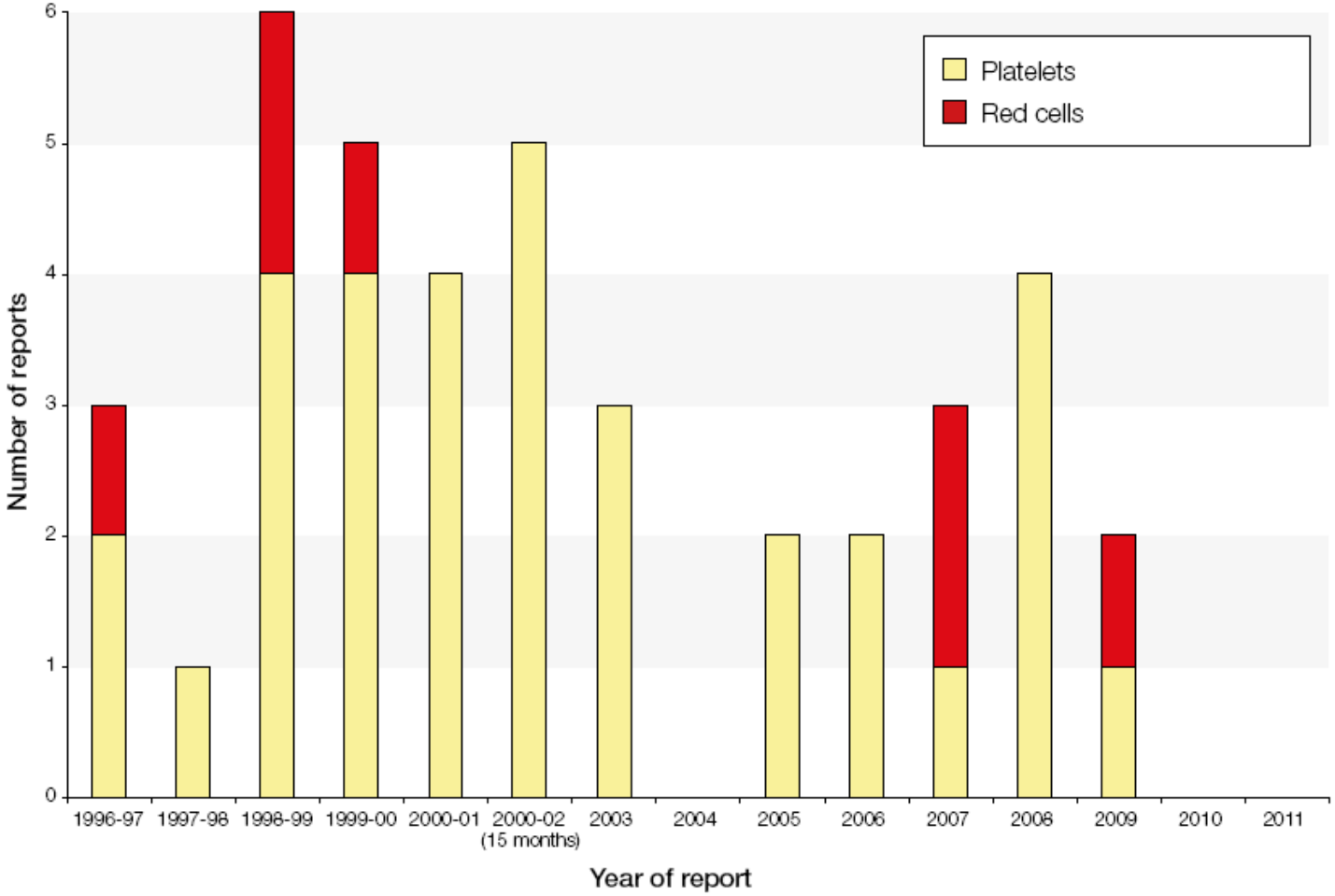
TTI represent a significant risk to the UK blood supply

- Viruses — Test all donations
  - HIV, HBV, HCV, HTLV
- Parasites — Test selected donations
  - Malaria
- Bacteria — Screen platelets components only
  - Staphylococcus, Yersinia, Pseudomonas

UK viral and parasitic TTI incidents (SHOT 2011)



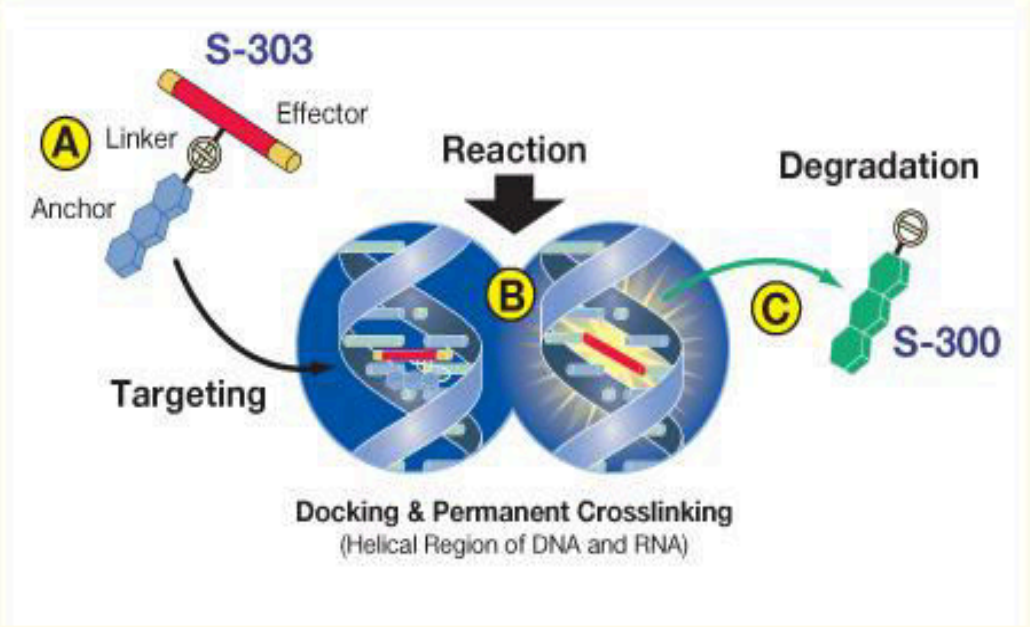
UK bacterial TTI incidents (SHOT 2011)

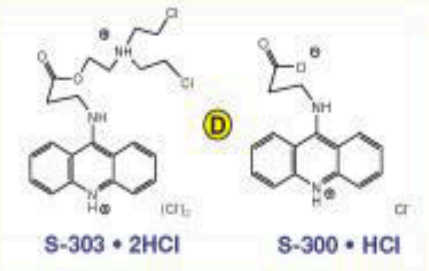


INTERCEPT for red blood cells

- Reduces the risk for a large spectrum of pathogens, including agents for which testing does not exist

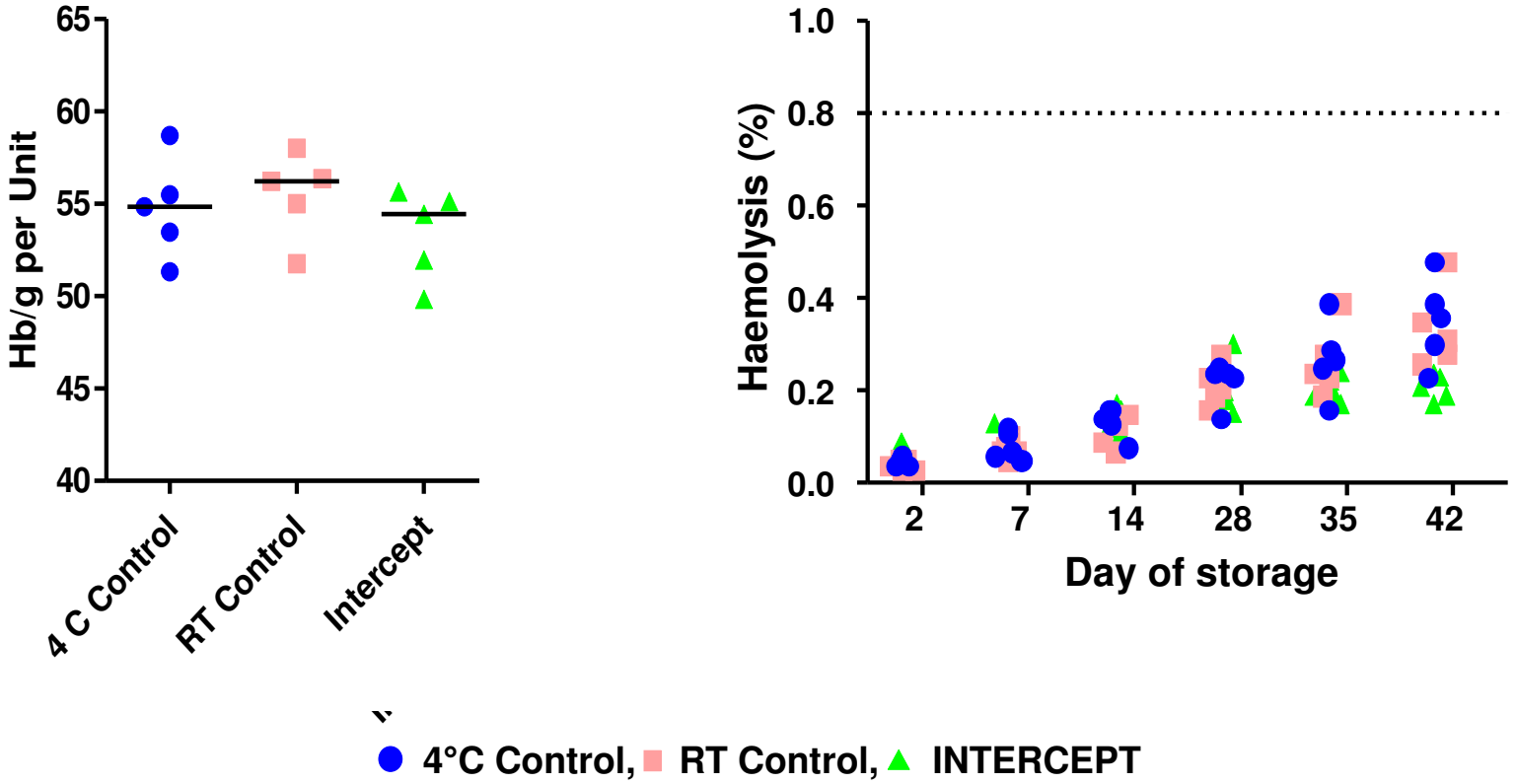
Figure 1: S-303 Treatment Process Mechanism of Action





- Anchor selectively targets nucleic acids
- Effector crosslinks nucleic acids
- Linker temporarily joins anchor and effector
- Cross-linking reaction is faster than linker degradation
- Degradation yields unreactive by-products

Haemoglobin and haemolysis



Summary

- INTERCEPT treated red cells **met specification** for:
  - Volume
  - Haemoglobin content
  - Haemolysis
- Compared to control units, INTERCEPT treated red cells had **improved**:
  - Haemolysis
  - Supernatant potassium
  - Red cell microvesicles
  - ATP
- Compared to control units, INTERCEPT treated red cells had **reduced**:
  - Deformability

In vitro ‘grown’ red cells

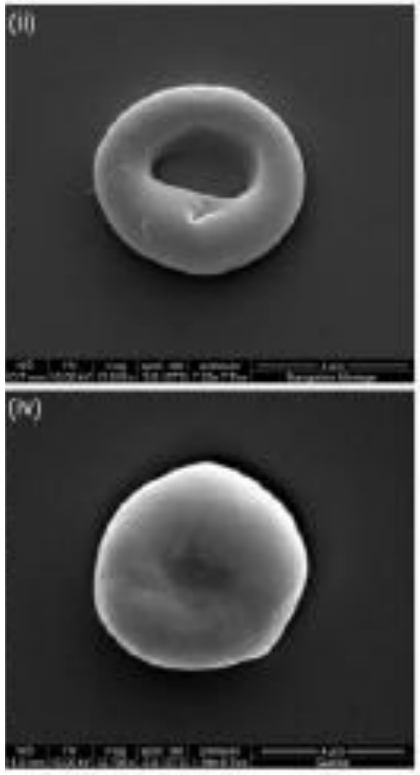
- Several groups, including in the UK are attempting to culture red cells in vitro
  - CD34+ peripheral blood
  - Cord blood
  - Embryonic stem cells
- Work is still in its infancy
- Significant problems still exist
  - Maturation and enucleation
  - Proliferation
  - Cost

blood

2012 119: 6296-6306
Prepublished online April 6, 2012;
doi:10.1182/blood-2011-09-376475

Maturing reticulocytes internalize plasma membrane in glycophorin A–containing vesicles that fuse with autophagosomes before exocytosis

Rebecca E. Griffiths, Sabine Kupzig, Nicola Cogan, Tosti J. Mankelow, Virginie M. S. Betin, Kongtana Trakarnsanga, Edwin J. Massey, Jon D. Lane, Stephen F. Parsons and David J. Anstee



- R2 reticulocyte
- An almost mature RBC

In vitro ‘grown’ red cells

Plenary paper

Proof of principle for transfusion of in vitro–generated red blood cells

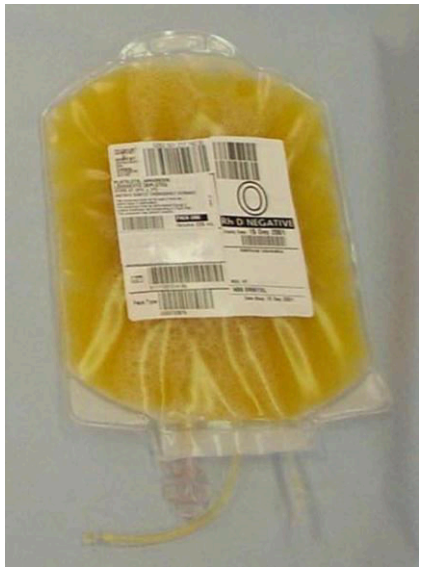
*Marie-Catherine Giarratana,[1,2] *Hélène Rouard,[3,4] Agnès Dumont,[5] Laurent Kiger,[6] Innocent Safeukui,[7] Pierre-Yves Le Pennec,[8] Sabine François,[1,2,9] Germain Trugnan,[10] Thierry Peyrard,[8] Tiffany Marie,[1-3] Séverine Jolly,[1-3] Nicolas Hebert,[1-3] Christelle Mazurier,[1-3] Nathalie Mario,[11] Laurence Harmand,[1-3] Hélène Lapillonne,[1,2,12] Jean-Yves Devaux,[5] and Luc Douay[1-3,13]

- Cultured CD34+ peripheral blood
  - Radiolabelled and reinfused into a human volunteer
    - Levels in circulation at 26 days = 41-63 %
    - Compares favourably with the reported half life for native RBCs of 28 days

In vitro ‘grown’ red cells

- Years away from the first ‘transfusion’
- Future potential?
  - Use as reagents for group/screening
  - May never replace the requirement for blood donors
  - It is possible that cultured red cells could provide a solution for patients who are carriers of rare blood groups or who are polyimmunized (1-3% of the transfused population)

Developments with platelet components



Platelet additive solutions

- NHSBT platelets are stored in plasma
  - Bacterial monitoring allows for a 7 day shelf-life
- Platelet additive solutions (70/30 %) have been shown to have an advantage over platelets stored in plasma

Vox Sanguinis (2008) **94**, 103–112

ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2007.01008.x

In vitro function of buffy coat-derived platelet concentrates stored for 9 days in CompoSol, PASII or 100% plasma in three different storage bags

R. Cardigan,[1] J. Sutherland,[1] M. Garwood,[1] S. Bashir,[1] C. Turner,[1] K. Smith,[1] V. Hancock,[1] M. Wiltshire,[1] C. Pergande[1] & L. M. Williamson[2,3]

- No desire to move to routine use of platelet additive solution

Washed platelets

- Indicated for patients susceptible anaphylactic and febrile non-haemolystic transufusion reactions (e.g. IgA or cytokines released during storage)
- Washed platelets are stored in 95 % additive solution
  - 24 hour shelf-life
- Newer platelet additive solutions have the potential to increase this shelflife

In vitro variables of apheresis platelets are stably maintained for 7 days with 5% residual plasma in a glucose and bicarbonate salt solution, PAS-5

Katherine Radwanski, Stephen J. Wagner, Andrey Skripchenko, and Kyungyoon Min

Washed platelets

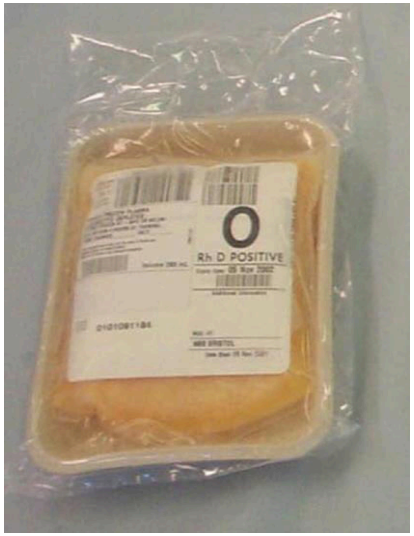
TABLE 2. PLT storage variables in suspensions containing 100% plasma (control) and 5% plasma/95% PAS-5 (test)*

Variable	Day 1	Day 5	Day 7
PLT count ($\times 10^9$/L)			
Control	1466 ± 113	1450 ± 134	1434 ± 130
Test	1411 ± 121†	1443 ± 137	1410 ± 128
pH (22°C)			
Control	7.24 ± 0.09	7.25 ± 0.14	7.13 ± 0.12
Test	7.23 ± 0.10	7.24 ± 0.19	7.21 ± 0.18
HSR (%)			
Control	60.7 ± 16.9	61.2 ± 13.6	57.4 ± 14.1
Test	65.5 ± 20.1	64.1 ± 12.9	55.1 ± 12.7
CD62P (% positive PLTs)			
Control	51.9 ± 15.6	45.7 ± 9.8	52.3 ± 11.7
Test	52.1 ± 14.8	39.5 ± 10.8†	41.8 ± 13.1†

Washed platelets

- NHSBT recently completed a study comparing the quality of washed platelets stored in various additive solutions including:
  - PAS-5 and PAS-G
- Results pending publication

Developments with plasma components



Manufacture of plasma components

This hasn't really changed over time:

- Biggest recent change – Ambient hold of whole blood
  - Most FFP is now prepared:
    - From male plasma
    - On day 1
- Increased efficacy and reduced cost
- Quality?
  - Dependent on time lines

Plasma components

- NHSBT import FFP for children:
  - Currently from Austrian (previously USA)
- Imported FFP treated with Methylene Blue (PI)
  - MB FFP also used for the production of cryoprecipitate
- All pathogen inactivation systems have an impact on component quality
- 2013 NHSBT assessing alternative methods of pathogen inactivation
  - MB, Mirasol, Intercept
    - Quality of FFP and cryoprecipitate

Methylene Blue FFP

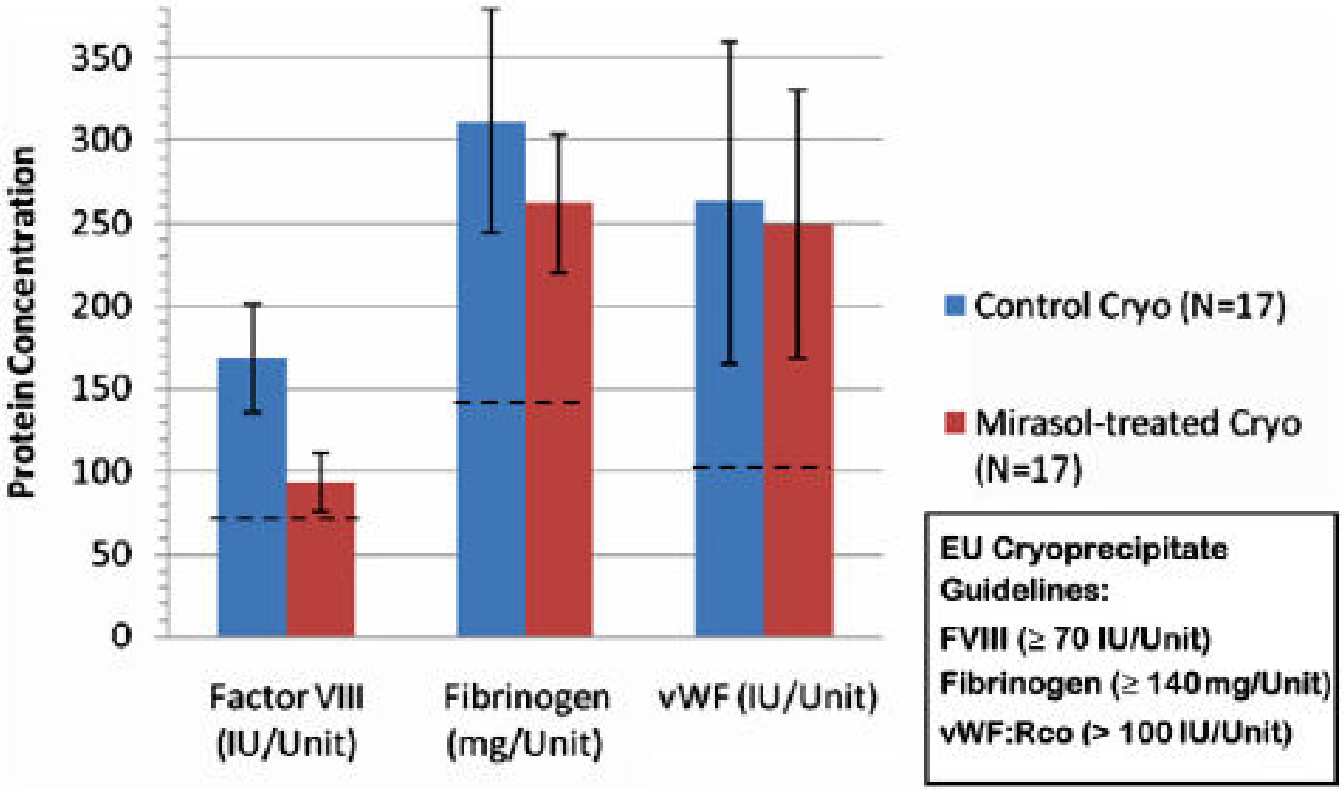
Factor	MB FFP After thaw, 0 hr	Standard FFP After thaw, 0 hr
PT (sec)	13.5 (0.65)	12.6 (0.55)
APTT (sec)	36.0 (2.81)	30.1 (2.21)
Fibrinogen (g/L)	2.14 (0.35)	2.89 (0.53)
FVII (IU/dL)	111.1 (19.65)	119.0 (20.85)
FVIII (IU/dL)	78.3 (15.69)	118.3 (24.07)
Protein C (IU/dL)	94.8 (8.49)	100.9 (9.59)
Protein S (IU/dL)	95.6 (17.54)	99.4 (16.99)

Thrombin generation and clot formation in methylene blue–treated plasma and cryoprecipitate

696 TRANSFUSION Volume 49, April 2009

Rebecca Cardigan, Katherine Philpot, Philip Cookson, and Roger Luddington

Mirasol Cryo

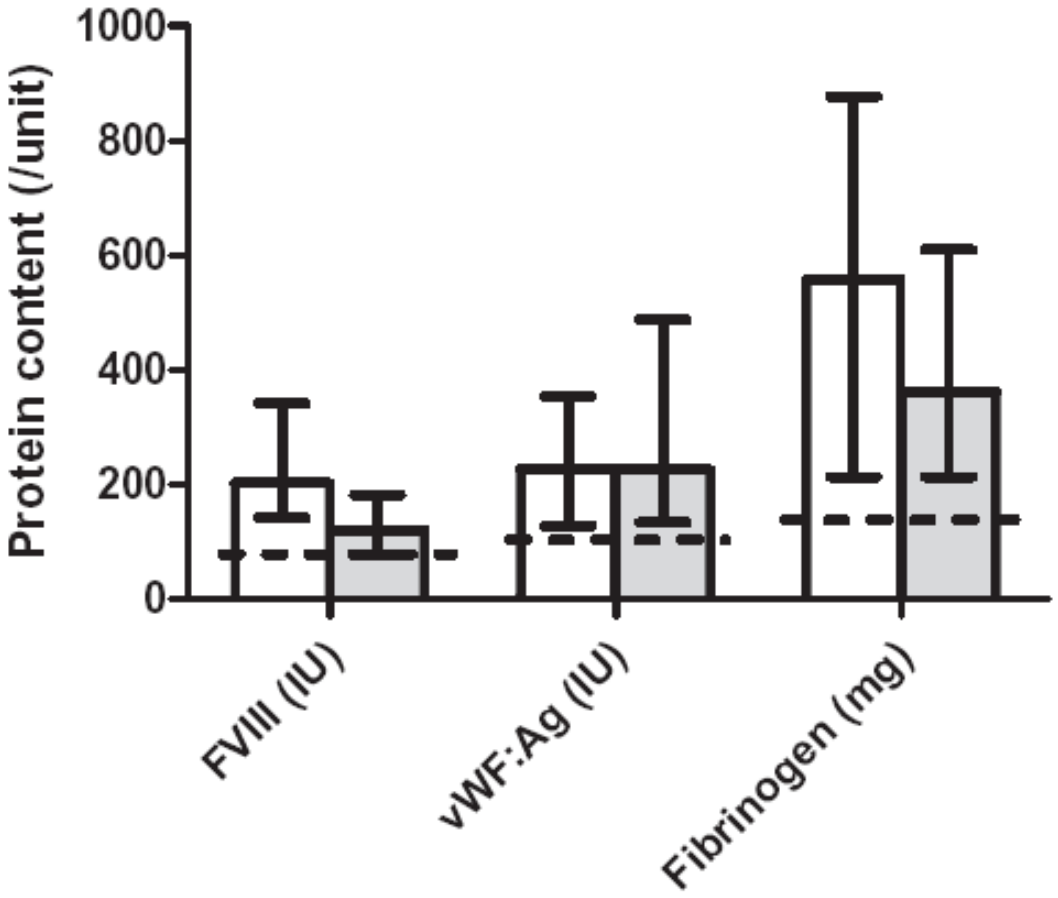


Preparation of cryoprecipitate from riboflavin and UV light-treated plasma

Transfusion and Apheresis Science 46 (2012) 153–158

Anna Ettinger*, Meghan M. Miklauz, David J. Bihm, Gabriela Maldonado-Codina, Raymond P. Goodrich

Intercept Cryo



Quantitative and qualitative analysis of coagulation factors in cryoprecipitate prepared from fresh-frozen plasma inactivated with amotosalen and ultraviolet A light

Joan Cid, Carolina Caballo, Marc Pino, Ana M. Galan, Nuria Martínez, Ginés Escolar, and Maribel Diaz-Ricart

Pathogen inactivation of plasma

- NHSBT study is ongoing
- Results will influence the next PI tender

Other future changes

- Smaller granulocye component (5 donations)
- Washed red cells – Washing / storage solution
- Automated blood processing
  - Whole blood
  - Pooled platelets