Pathogen Reduction Technology (PRT) and a Treatment System for Whole Blood

Funded by US Department of Defense

Congressionally Directed Medical Research Program

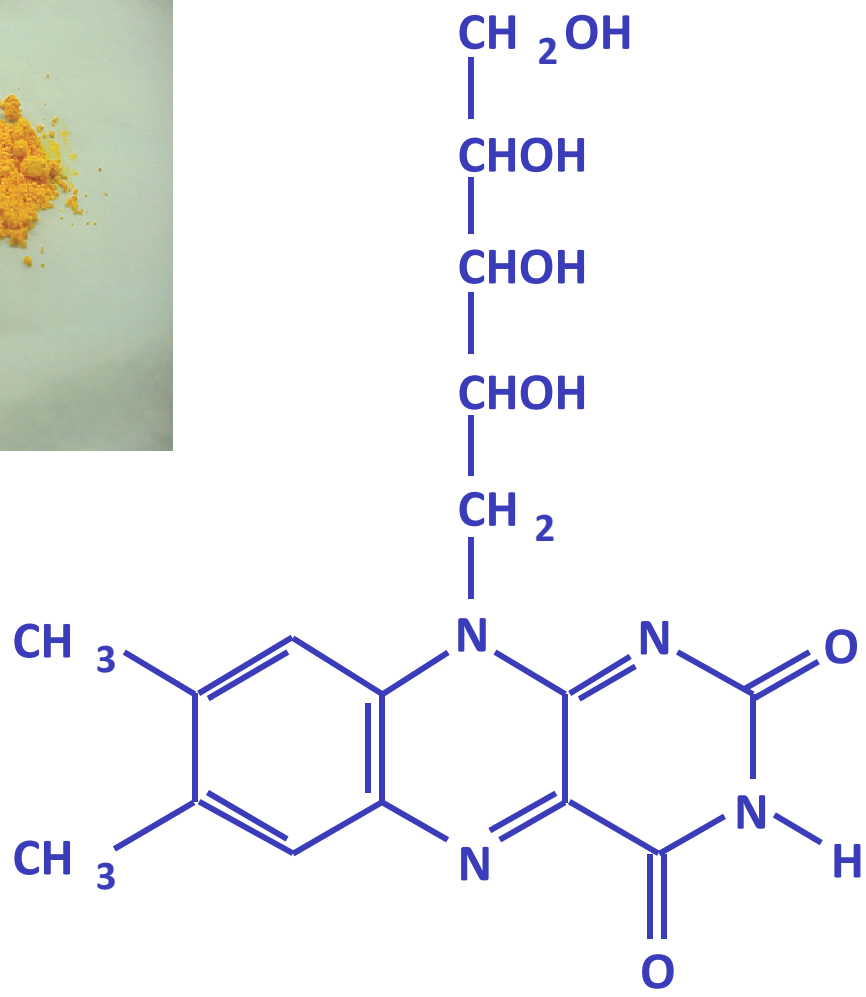
Goals of Pathogen Reduction

Pathogen Inactivation: The Definitive Safeguard for the Blood Supply

Bryant, Barbara J and Klein, H.

Conclusions-**The ultimate goal of pathogen inactivation is to maximally reduce the transmission of potential pathogens without significantly compromising the therapeutic efficacy of the cellular and protein constituents of blood. This must be accomplished without introducing toxicities into the blood supply and without causing neoantigen formation and subsequent antibody production**. Several promising pathogen inactivation technologies are being developed and clinically tested, and others are currently in use. Pathogen inactivation offers additional layers of protection from infectious agents that threaten the blood supply and has the potential to impact the safety of blood transfusions worldwide.

(Arch Pathol Lab Med. 2007;131:719-733)



Riboflavin (vitamin B_2)

PRT System Concept

Platelet or
Plasma product

+

Riboflavin
(Vitamin B2)
solution

+

UV Light

- ✓ Reduction of infectious levels of viruses, bacteria, parasites
- ✓ Inactivation of residual white cell

Pathogens Tested
White Blood Cell Inactivation Tested

- HIV
  - Extracellular
  - Proviral
  - Cell-associated
- Hepatitis A
- Hepatitis B
- Hepatitis C (ongoing)
- Influenza (H1N1)
- West Nile Virus
- Parvovirus
- Chikungunya

- S. epidermidis (4 Strains)
- S. aureus (5 Strains)
- E. coli
- B. cereus
- S. agalactiae
- S. mitis
- S. pyogenes
- E. cloacae
- P. acnes
- S. marcescens
- K. pneumoniae
- A. baumannii
- Y. enterocolitica

- Malaria
- Leishmaniasis
- Chagas
- Babesia
- Orientia

- xGVHD
- Alloimmunization
- Cytokine Production
- MLR
- Proliferation

References Available on Request
ray.goodrich@caridianbct.com

Pre-Clinical and Clinical Program with Mirasol PRT System

Mirasol PRT for Platelets

Design Exploratory
Recovery and Survival
Sample Radiolabelled Platelets
Healthy Volunteers (N = 18)
Cross-Over Design

Design Verification
6.2 J/ml
Recovery and Survival
Sample Radiolabelled Platelets
Healthy Volunteers (N = 24)
Cross-Over Design

Design Validation
6.2 J/ml
Safety and Performance
Full Unit Platelets
Subjects in Clinical Setting
Parallel Design

CE Mark

Core Toxicology Studies

- Ames (17.3 J/ml) ✓
- CHO (17.3 J/ml) ✓
- Acute Tox. (Rat) (17.3 J/ml) ✓
- Neoantigenicity (17.3 J/ml) ✓

- Ames (6.2 J/ml , ± γ) ✓
- Acute Toxicology (Rat) (6.2 J/ml , ± γ) ✓
- Acute Toxicology (Dog) (6.2 J/mL , ± γ) ✓
- Neoantigenicity (6.2 J/ml , ± γ) ✓

- Acute Toxicology (Saline Bolus Study, Riboflavin and Lumichrome)✓
- Antigen Profiling (Capture P Assay, 6.2J/ml) ✓
- Embryo – Fetal Development (6.2 J/ml) ✓
- MMN (6.2J/ml) ✓
- CHO (6.2J/ml) ✓
- ^{14}C-riboflavin PK (6.2 J/ml) ✓
- ^{14}C-riboflavin Protein Binding (6.2 J/ml) ✓
- Photochemistry (6.2J/ml, ± γ) ✓
- Subchronic Toxicology with Acute Toxicology Arm including Histopathology (Dog) (6.2 J/ml) ✓
- Hemocompatibility (6.2 J/ml) ✓
- Cytotoxicity (6.2 J/ml) ✓

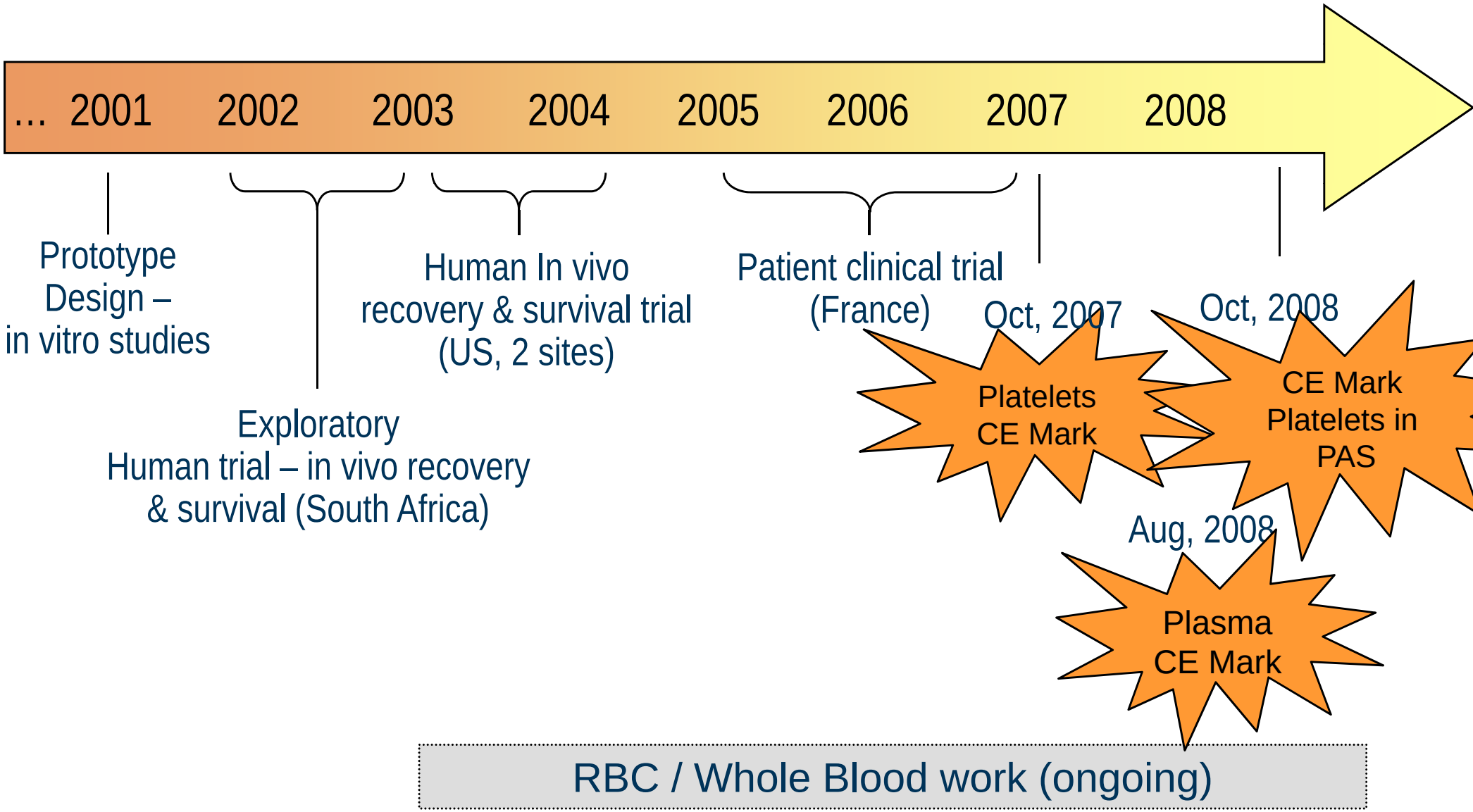
Studies of Platelet and Plasma Protein Function

- Platelet In Vitro Function Tests (17.3 J/ml) ✓
- Platelet Functionality (Escolar) (17.3 J/ml) ✓

- Platelet In Vitro Function Tests (6.2 J/ml , ± γ)✓
- Complement Protein Activation (6.2 J/ml , ± γ)✓
- Plasma Protein Profile (6.2 J/ml , ± γ) ✓

- Platelet Functionality (Escolar) (6.2 J/ml) ✓

Development Program – Overview



Problem to be Solved

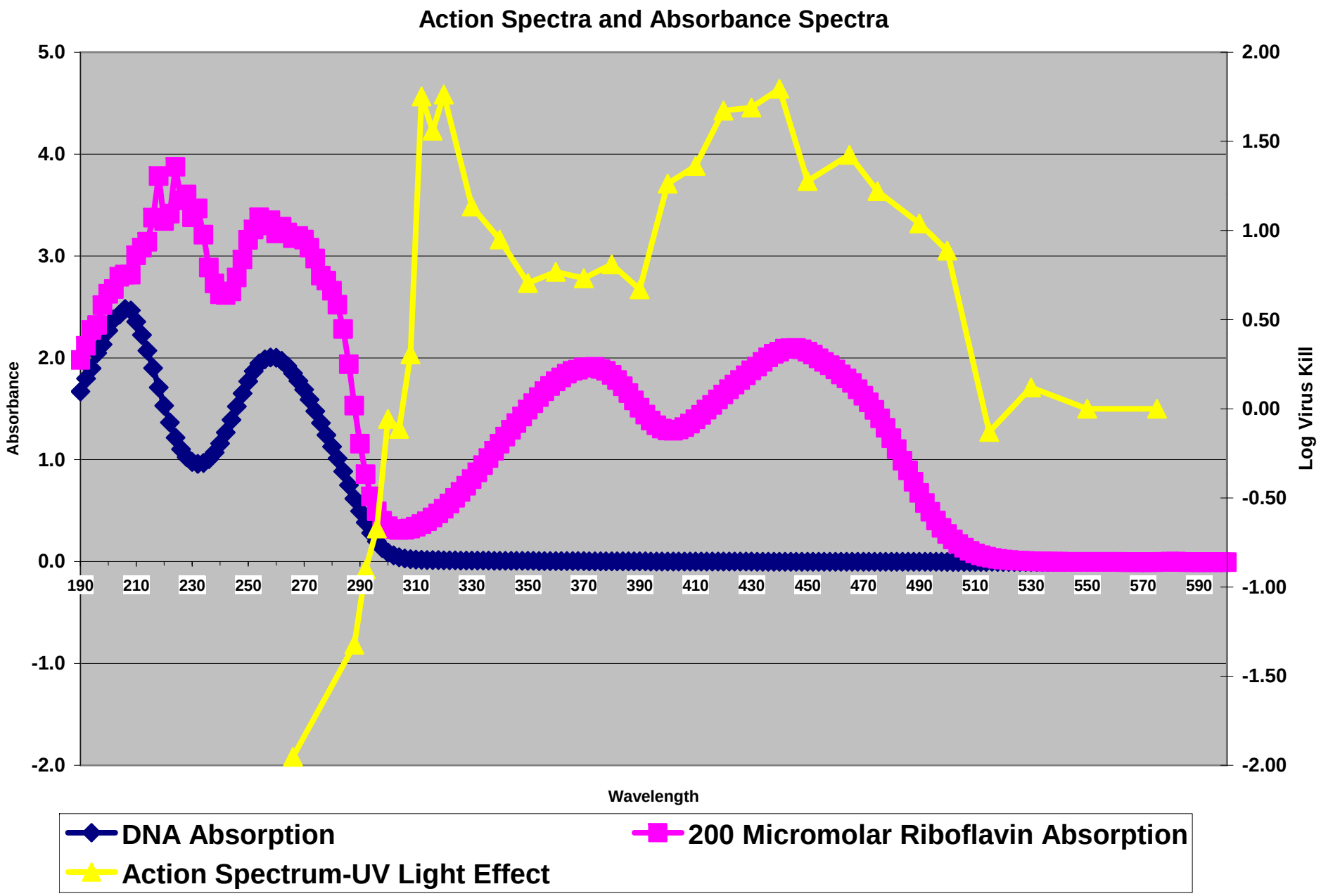
- **Defense Health Board Memorandum DHB 2007-04 dated June 2, 2008**
- **The use of Fresh Whole Blood in the Military**
  - Hemorrhage resulting from combat-related injuries is the most common cause of potentially preventable deaths in military operations [1].
  - Evidence indicates that the use of fresh whole blood (FWB) during massive transfusion (>10 units) is independently associated with improved survival [1].
  - The use of warm FWB currently persists only in emergency life-threatening scenarios when tested, stored blood components are not available, such as combat casualties.
  - Risks of non-leukoreduced warm FWB transfusions include the transmission of infectious agents, alloimmunization and the potential for microchimerism.
  - ***Pathogen reduction of FWB would increase the safety of FWB and provide an adequate and safe blood supply in a combat environment.***

[1]Spinella, P. C. , et al. Warm fresh whole blood transfusion for severe hemorrhage: U.S. military and potential civilian applications (2008) *Crit Care Med.* 36, S340-S345.

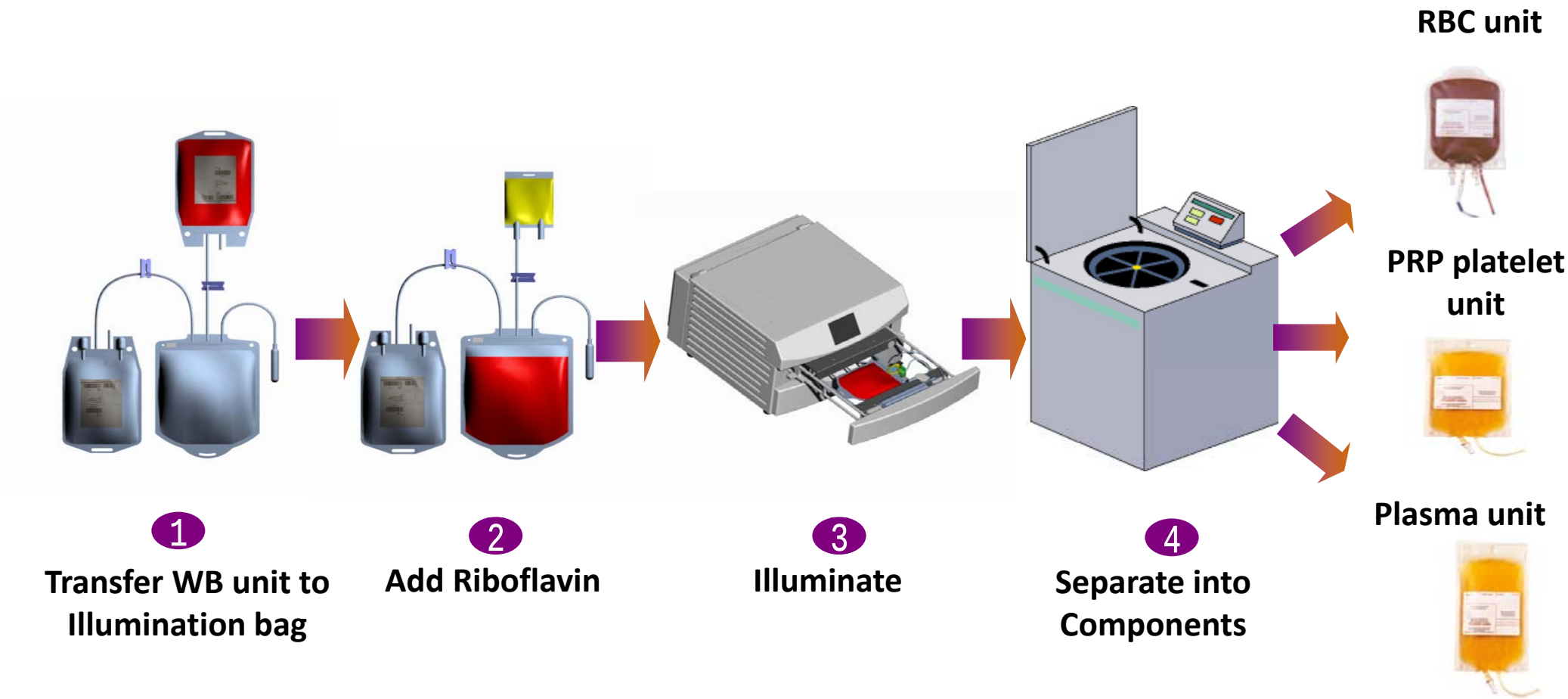
.

Evolution of the System for Whole Blood

- System for Whole Blood
  - Illuminator – same illuminator as System for Platelets and Plasma, with minor software modifications
  - Illumination/Storage Set – equivalent to the Set for the Plasma system, which provides a DEHP-plasticized storage bag
  - Riboflavin Solution content and volume identical to that in the System for Platelets and Plasma



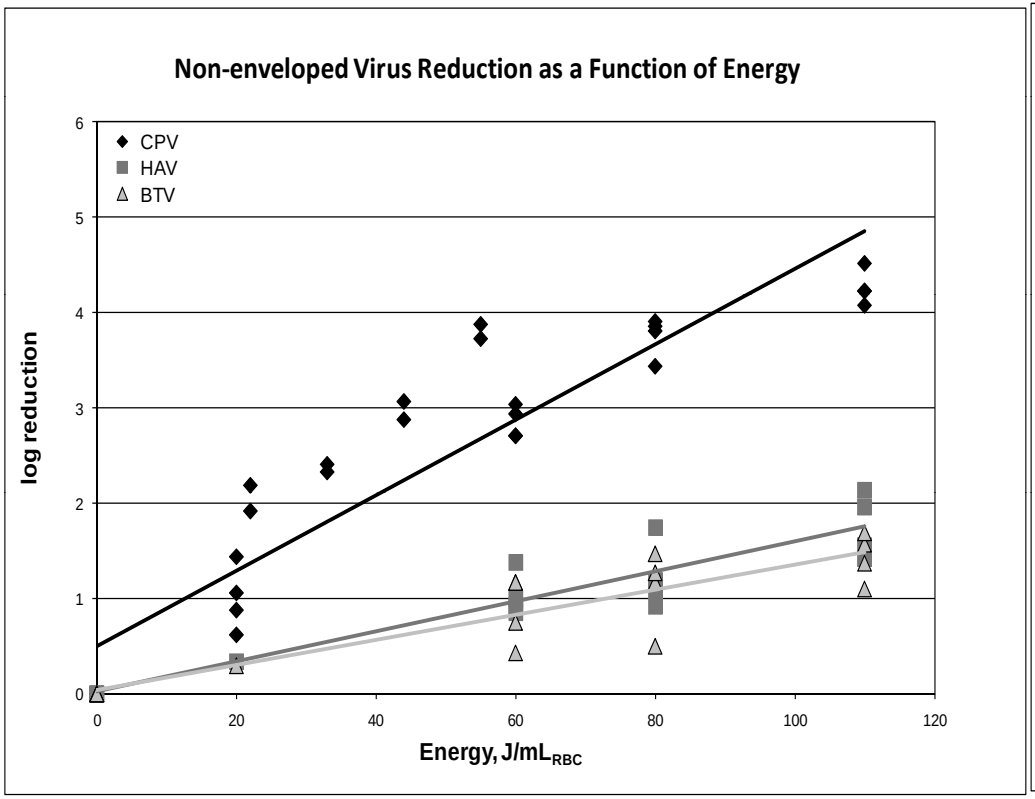
Martin et al. (2004) ***Photochem. Photobiol.;*** 81: 474-480

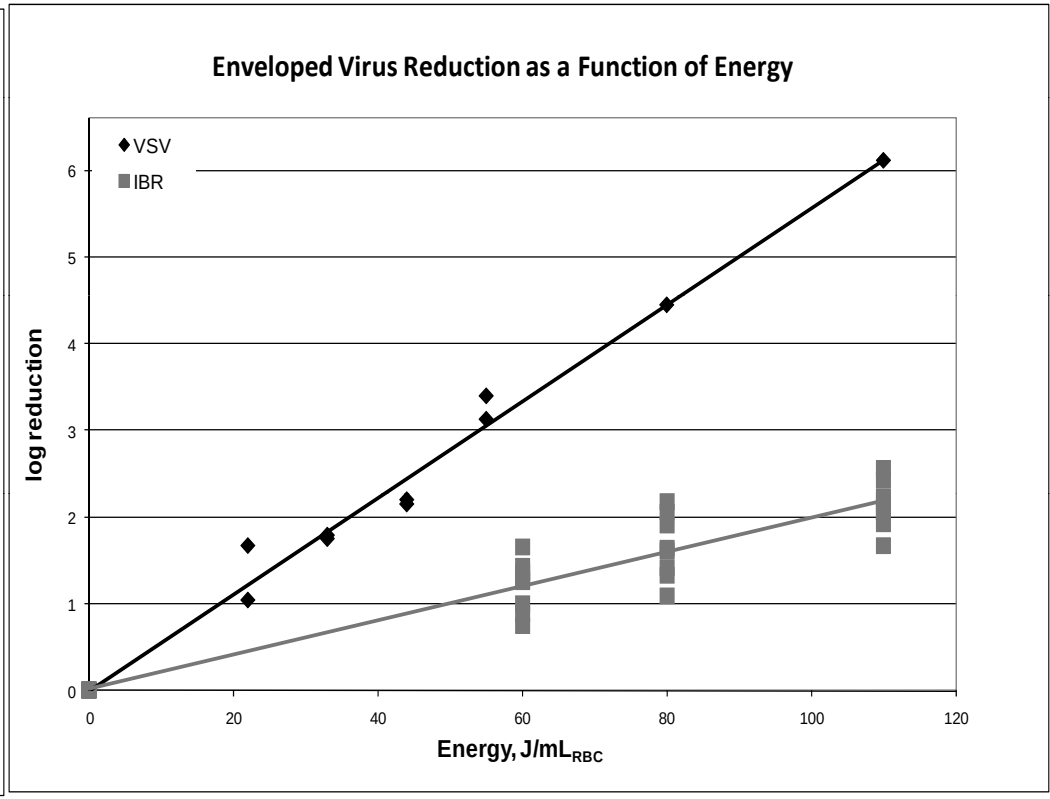

RBC unit
PRP platelet unit
Plasma unit
1
Transfer WB unit to Illumination bag
2
Add Riboflavin
3
Illuminate
4
Separate into Components

Status of System Development

- Pathogen Reduction
  - Virus reduction evaluations over a range of energies
    - Enveloped and Non-Enveloped viruses
  - Bacteria reduction over a range of energies
    - Gram positive and negative bacteria
  - Parasite reduction studies over a range of energies
- WBC inactivation
  - *in vitro* data obtained over range of energies
- Blood component quality
  - RBC quality – room temperature storage of whole blood as well as storage of refrigerated RBCs for 42 days
  - Platelet quality (PRP) – evaluated post-treatment and on Day 5
  - Plasma quality (FFP) – evaluated post-treatment and on Day 28
- Toxicology
  - Baboon immunization and R&S model
  - Neutrophil activation studies

Virus Reduction in Whole Blood





Bacteria Reduction in Whole Blood

	Replicates Positive for Bacteria Growth / Total # of Replicates			
	80 J/mL$_{RBC}$		**110 J/mL$_{RBC}$**	
	RBC	**PRP**	**RBC**	**PRP**
Y. enterocolitica	0/6	0/6	0/3	0/3
S. liquefaciens	0/7	0/7	0/3	0/3
A. baumannii	1/5	1/5	0/3	0/3

Results of Low-Titer Bacteria Reduction in Mirasol-treated Whole Blood

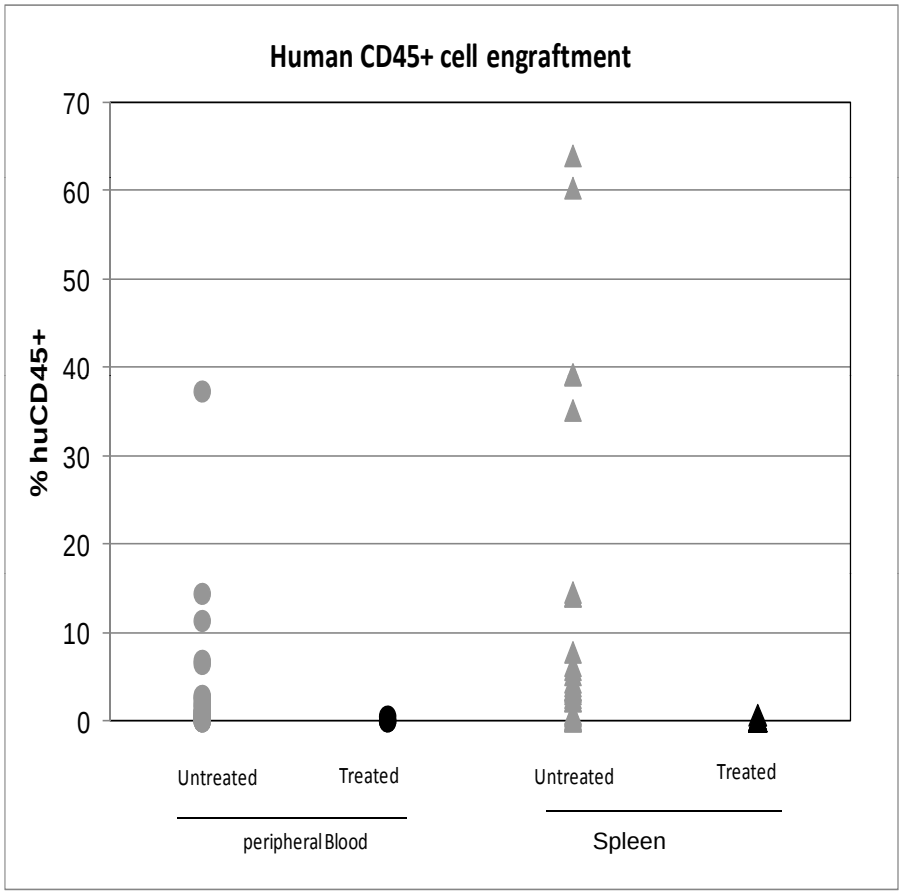
Spike levels at 20-100 CFU per product

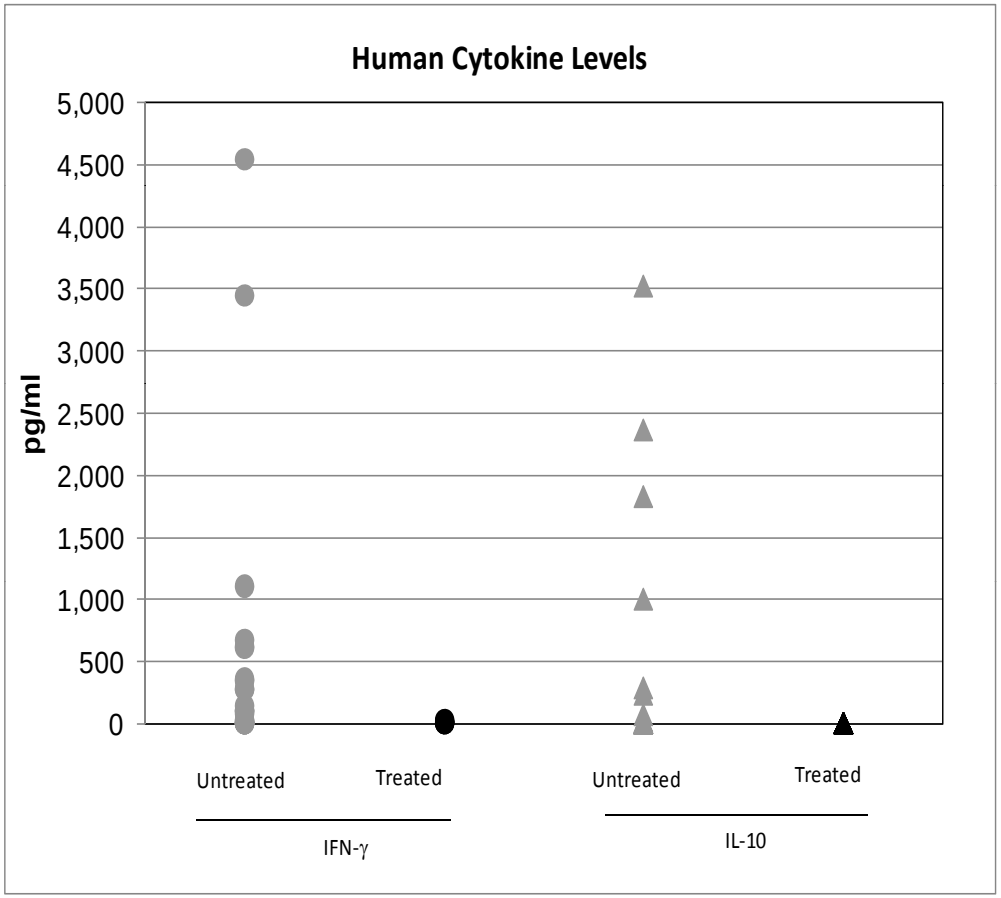
White Cell Inactivation in Whole Blood

	Untreated	**22 J/ml$_{RBC}$**	**33 J/ml$_{RBC}$**	**44 J/ml$_{RBC}$**
CD69 expression [%]	56 p=0.00004	1 **NS**	0 **NS**	0 **NS**
Proliferation and antigen presentation [cpm]				
PHA	85,667 p=0.0004	1,234 p=0.0001	592 **NS**	419 **NS**
CD3/28	98,428 p=0.000002	7,264 p=0.0007	732 p=0.02	631 **NS**
Allorecognition	17,459 p=0.003	703 **NS**	714 **NS**	495 **NS**
Allostimulation	16,015 p=0.006	1034 **NS**	506 **NS**	606 **NS**

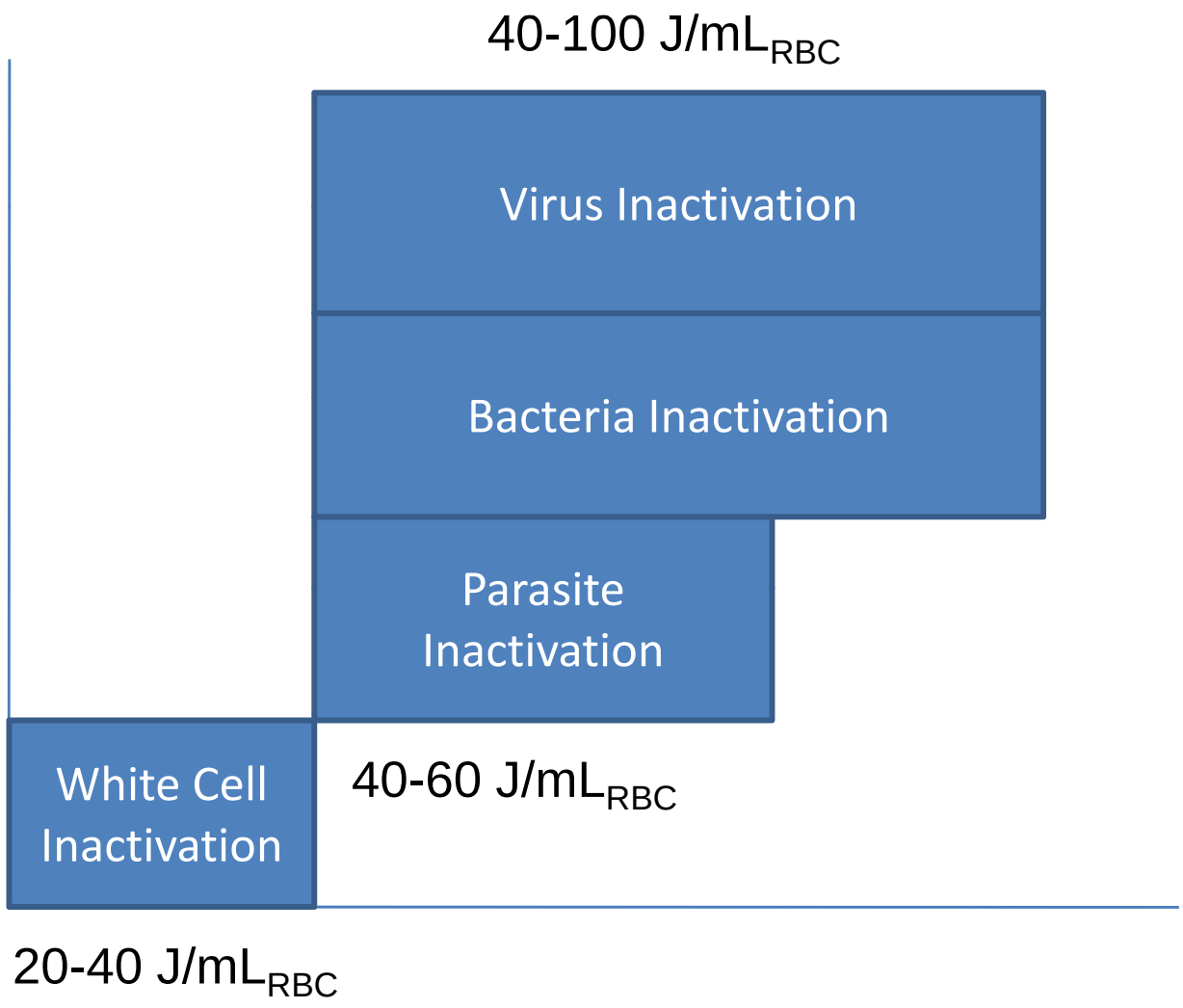
NS: not significantly different than zero

White Cell Inactivation in Whole Blood Results From a GVHD-Animal Model

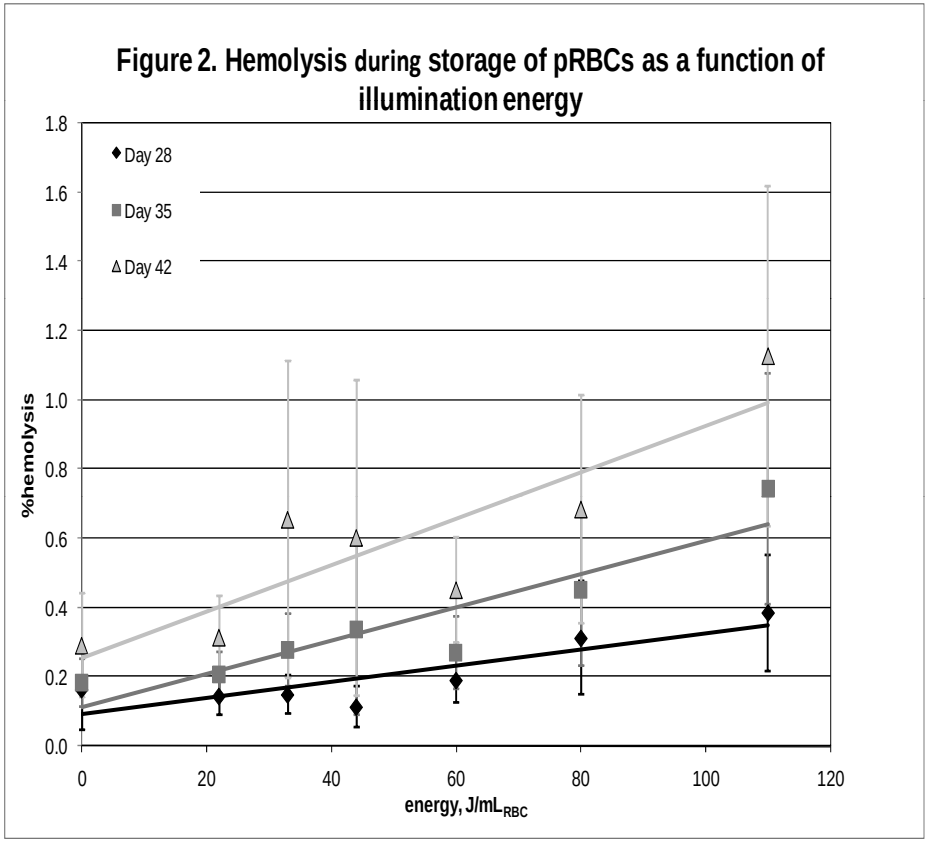


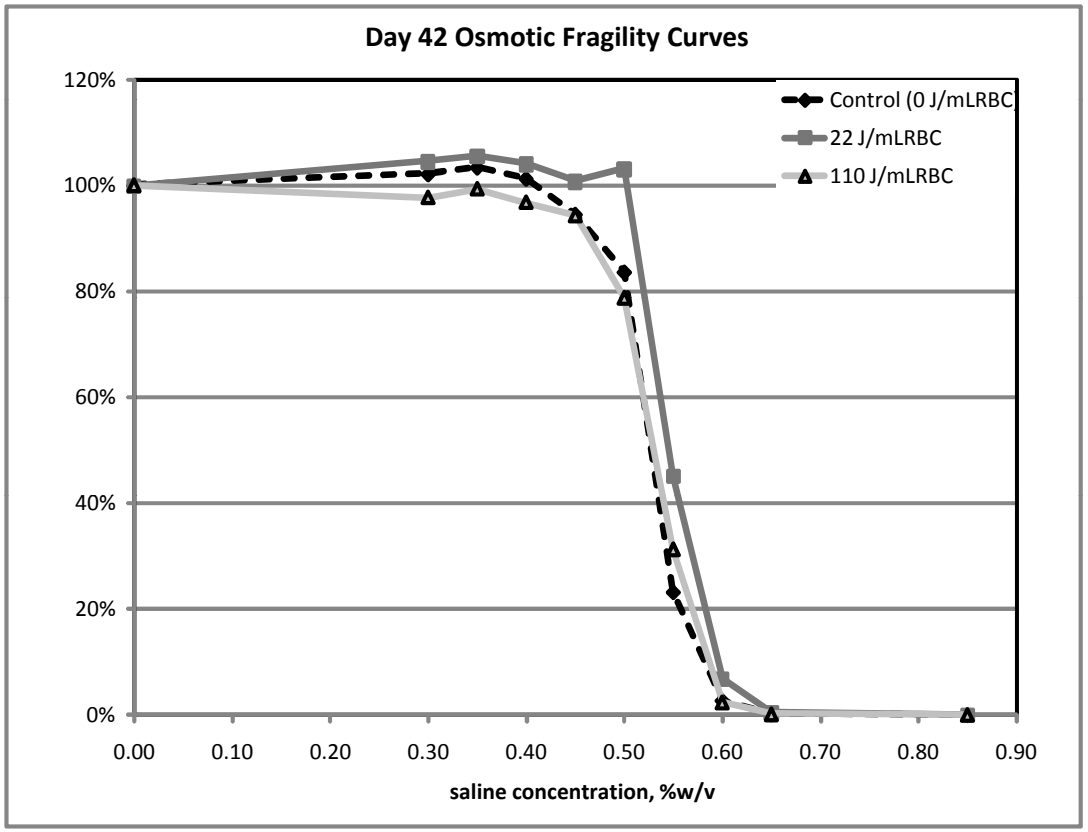


Pathogen Reduction and White Cell Inactivation Dose Ranges for Whole Blood

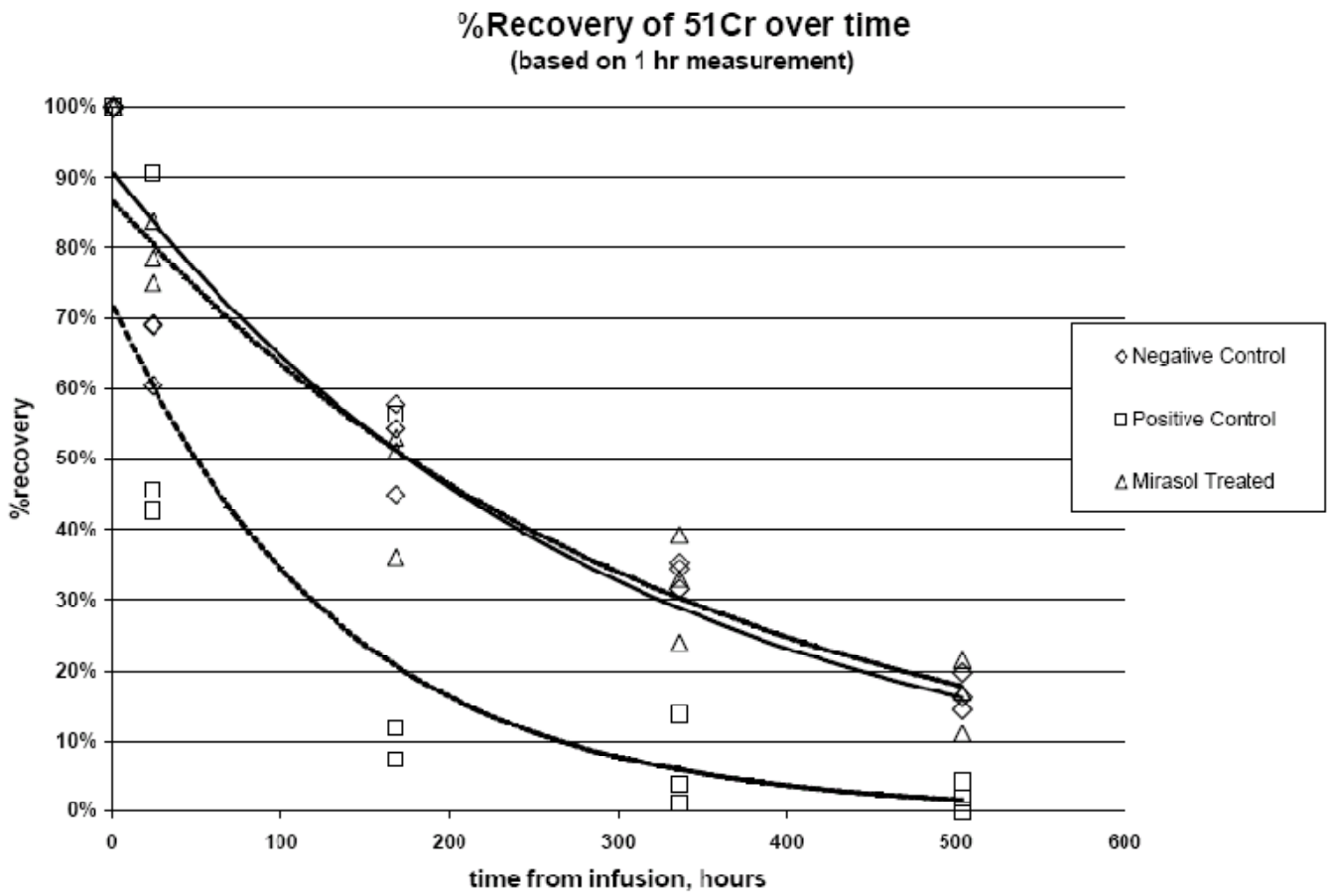


Red Cell Quality





Red Cell Quality; Baboon In Vivo Study



No evidence of neoantigen formation; Goodrich et al. *Transfusion* (2009)

Platelet Quality Data; PRP Platelets

		Control (n=4)	44 J/mL $_{RBC}$ (n=6)	110 J/mL $_{RBC}$ (n=11)
Test Parameter				
Swirl	Day 1	3+	3+	3+
	Day 5	3+	3+	3+
pH	Day 1	7.18 ± 0.08	7.27 ± 0.03	7.22 ± 0.10
	Day 5	7.07 ± 0.14	7.10 ± 0.16	7.07 ± 0.11
pO_2 mmHg	Day 1	57.7 ± 19.1	76.9 ± 10.0	85.0 ± 21.1
	Day 5	46.9 ± 36.1	76.9 ± 15.5	91.0 ±2 2.9
pCO_2 mmHg	Day 1	44.3 ± 5.6	33.7 ± 2.6	38.5 ± 9.9
	Day 5	32.9 ± 5.6	22.6 ± 2.8	20.6 ± 1.9
[Lactate] mmol/L	Day 1	4.6 ± 1.4	3.9 ± 0.6	4.7 ± 1.1
	Day 5	11.1 ± 2.8	12.0 ± 2.6	13.4 ± 2.5
[Glucose] mmol/L	Day 1	19.4 ± 1.9	18.9 ± 3.1	17.9 ± 1.3
	Day 5	15.1 ± 2.6	14.2 ± 3.7	13.4 ± 1.4

Plasma Quality; FFP

Energy (J/mL_{RBC})	Fibrinogen (mg/dL)	Factor V (%)	Factor VIIIc (%)	Factor XI (%)
0	**323 ± 97**	**77 ±13**	**108 ± 61**	**80 ± 20**
22	**264 ± 46**	**76 ± 13**	**96 ± 18**	**65 ± 9**
33	**249 ± 56**	**61 ± 21**	**71 ± 26**	**64 ± 17**
44	**251 ± 58**	**61 ± 9**	**92 ± 20**	**69 ± 13**
80	**214 ± 15**	**68 ± 13**	**85 ± 15**	**70 ± 12**
110	**165 ± 37**	**49 ± 10**	**52 ± 14**	**54 ± 10**
Normal Range *(Beeck and Hellstern, 1998)*	**145 - 385**	**54 - 145**	**45 - 168**	**42 - 144**

TEG and Impact-R Analysis of Treated Whole Blood Stored at Room Temperature

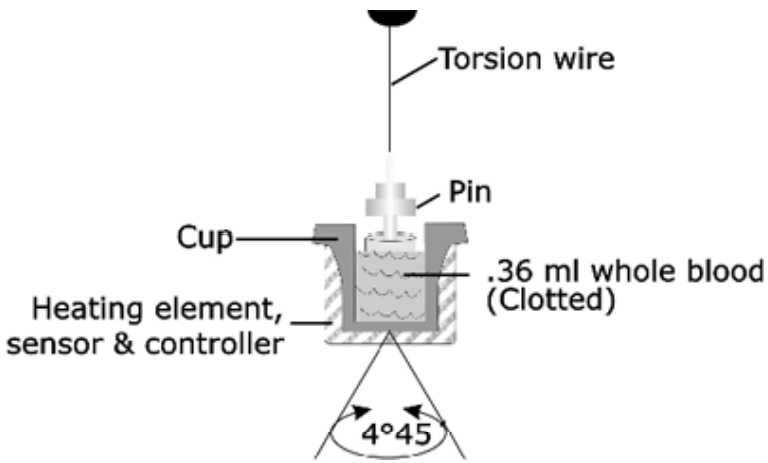
Assessing the Functionality and Quality of Whole Blood

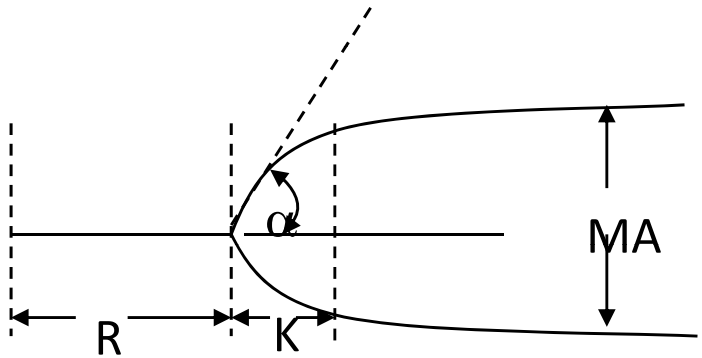
Preliminary Results

Experimental Design

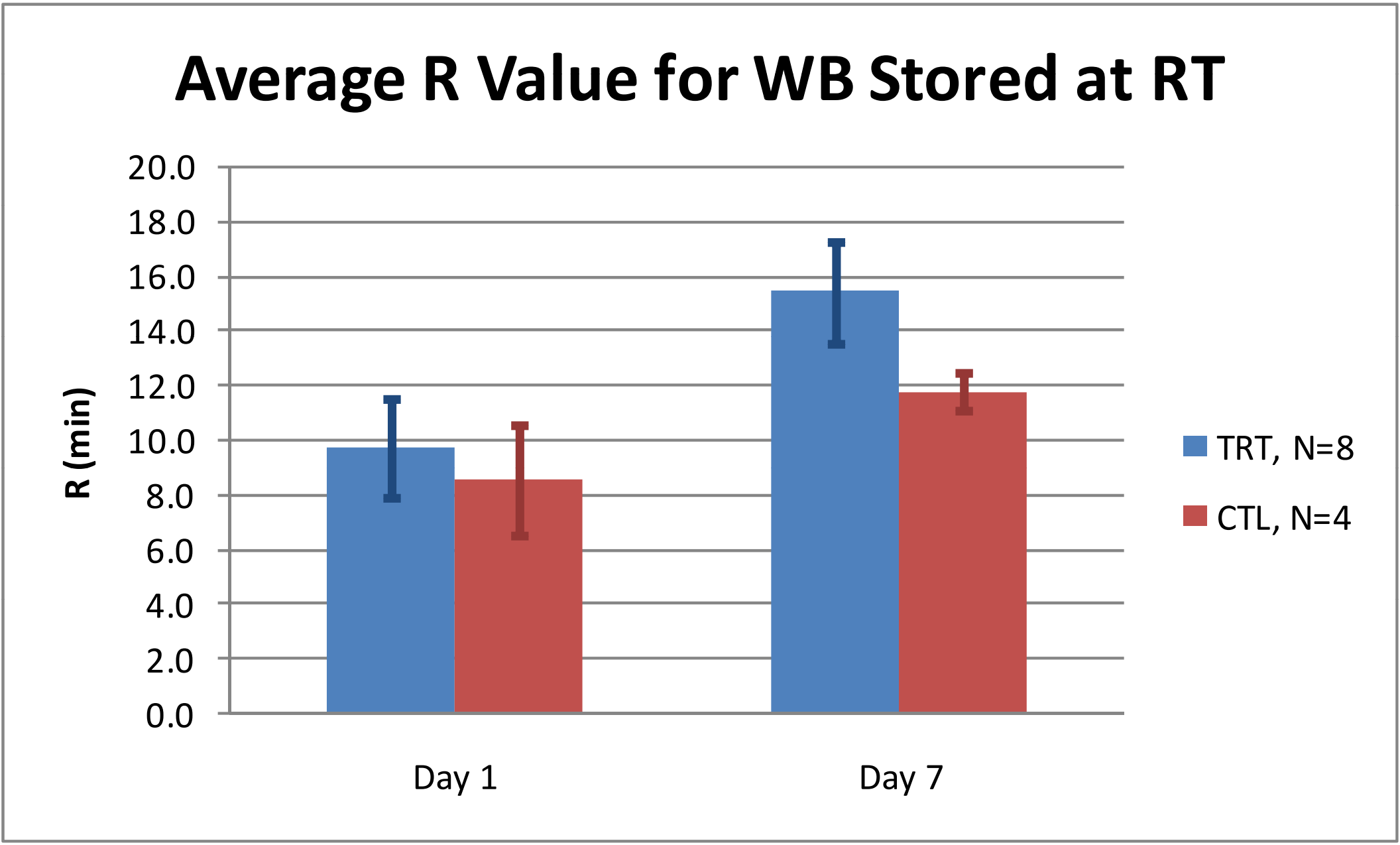
- Units of whole blood were treated with the PRT Process
  - Collected and stored in CPDA
  - Energy delivered = 80 and 90 J/mL_{RBC}
- Units were then stored in standard blood bags at room temperature (No agitation)
- Samples were analyzed after treatment by TEG and Impact-R analysis

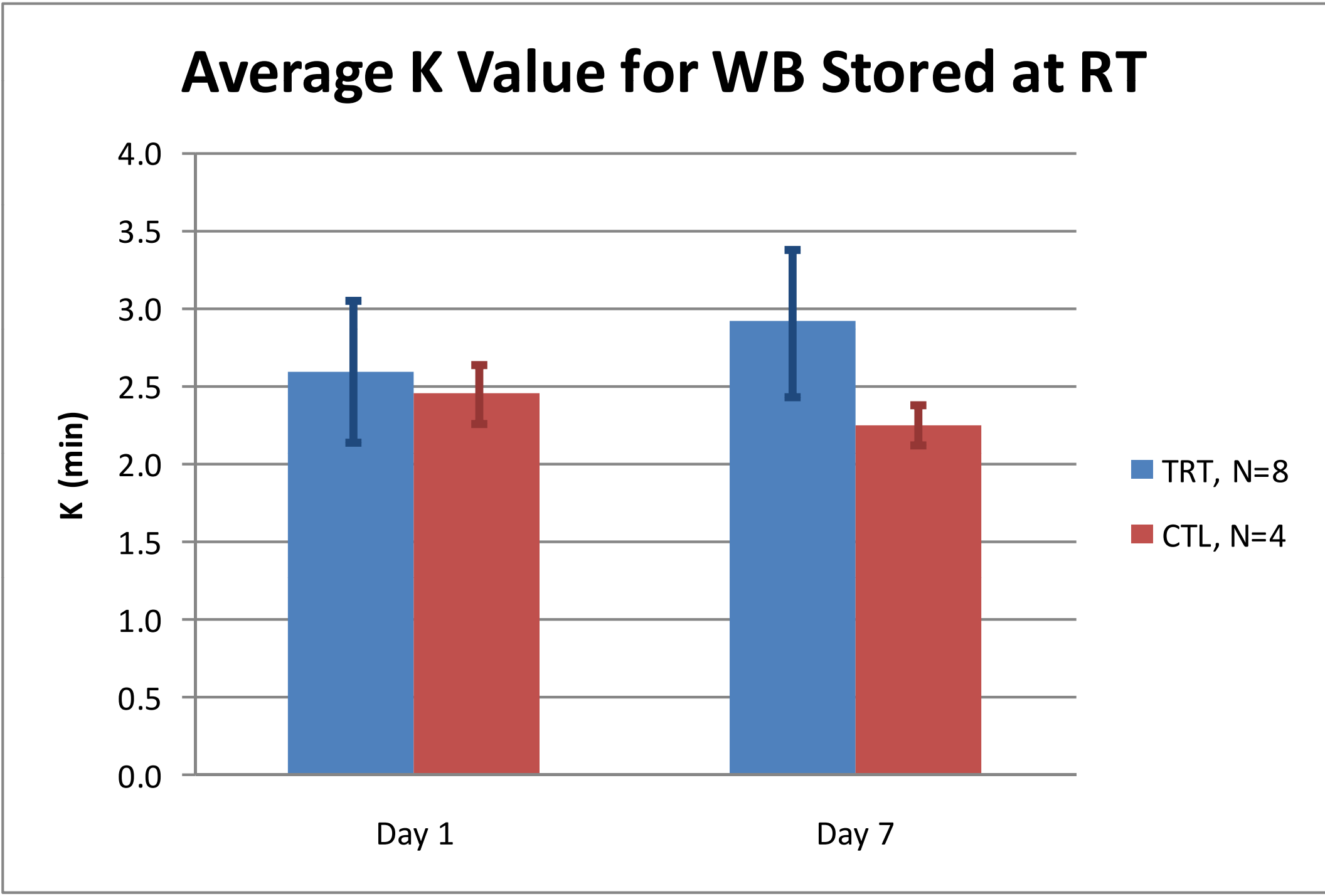
Functional Analysis Thromboelastogram (TEG)

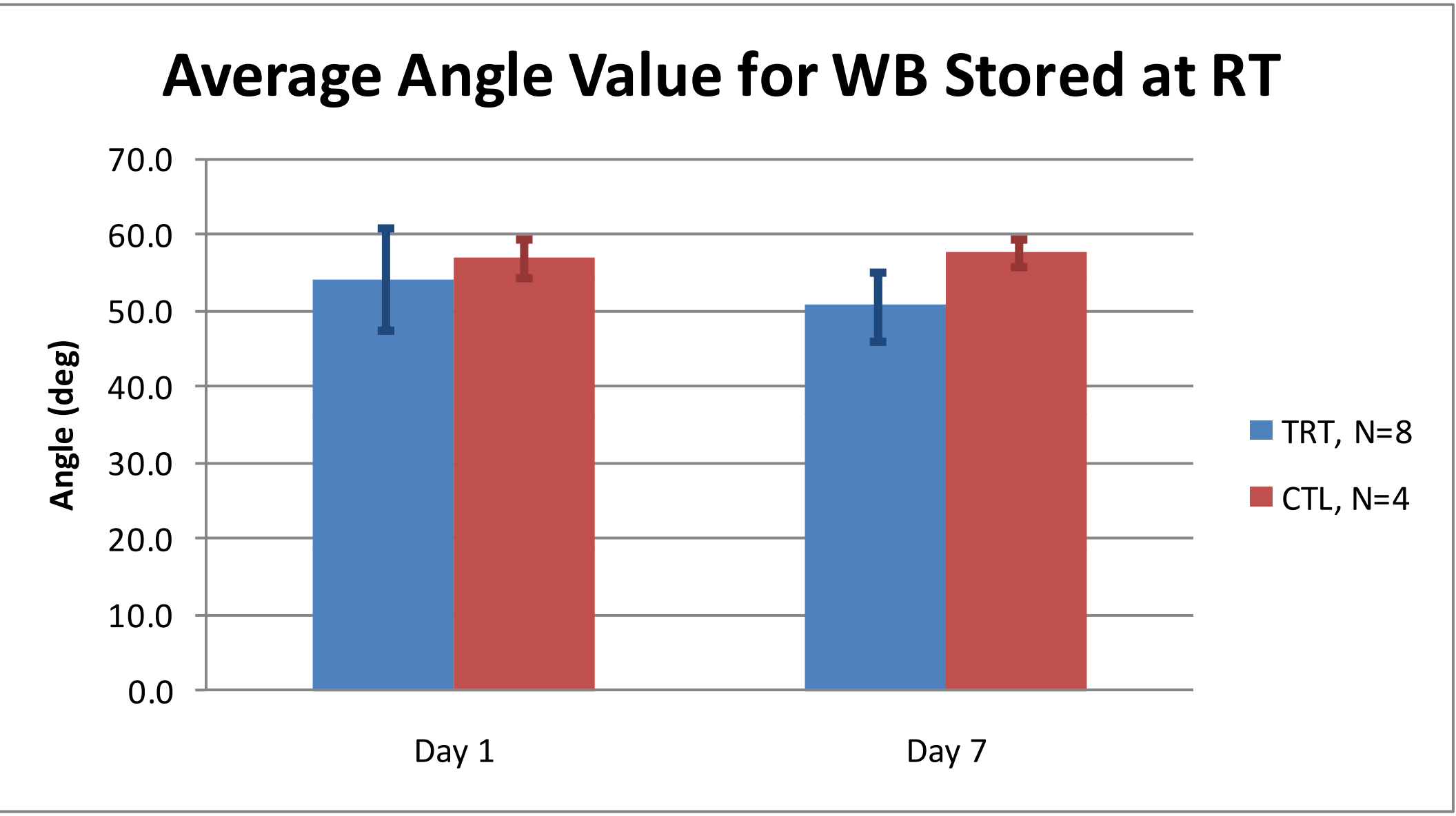


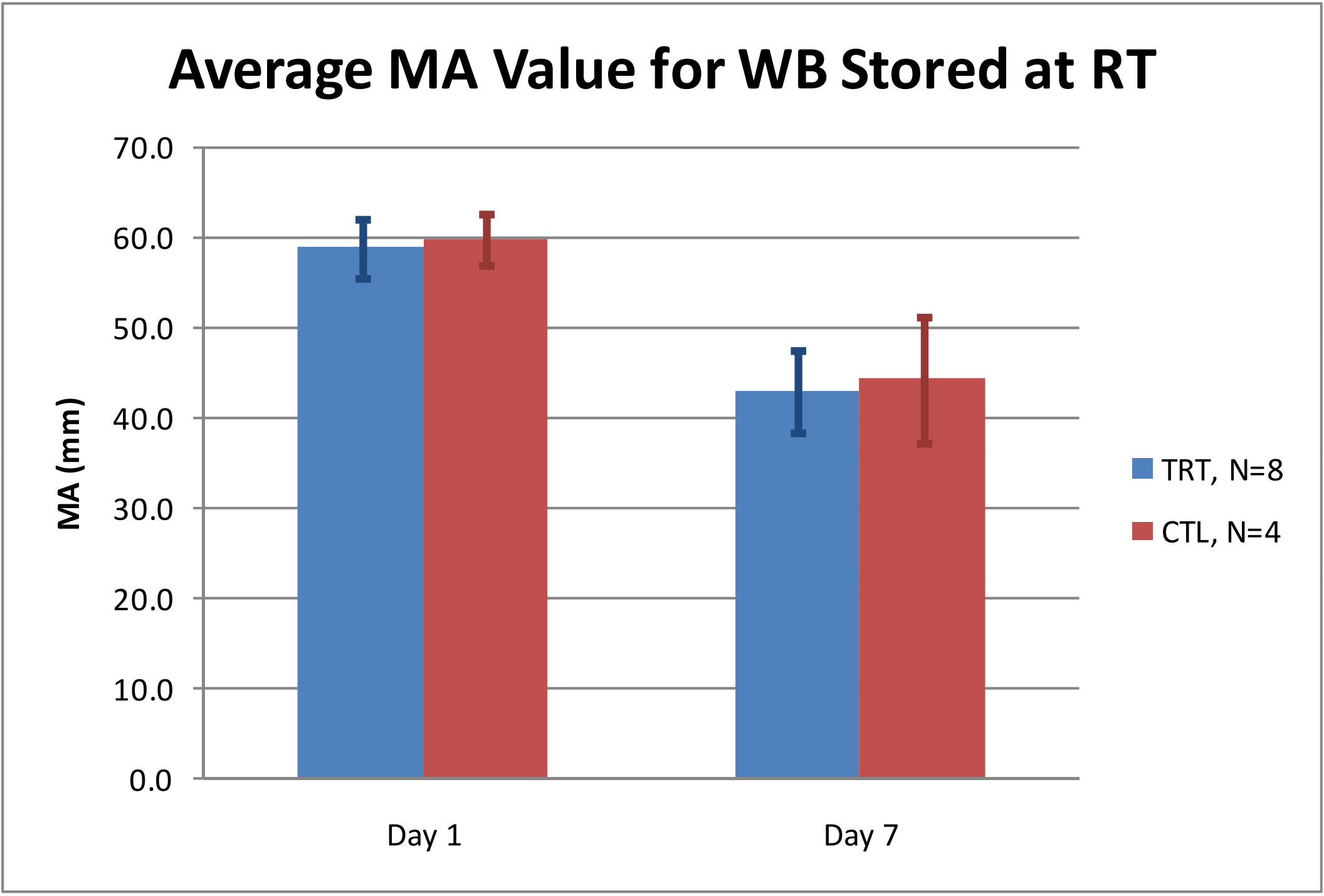


Clotting time	R	The period of time of latency from the time that the blood was placed in the TEG® analyzer until the initial fibrin formation.
Clot kinetics	K	A measure of the speed to reach a specific level of clot strength.
	alpha	Measures the rapidity of fibrin build-up and cross-linking (clot strengthening)
Clot strength	MA	A direct function of the maximum dynamic properties of fibrin and platelet bonding via GPIIb/IIIa and represents the ultimate strength of the fibrin clot.


Average R Value for WB Stored at RT
R (min)
20.0
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Day 1
Day 7
TRT, N=8
CTL, N=4


Average K Value for WB Stored at RT
K (min)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Day 1
Day 7
TRT, N=8
CTL, N=4

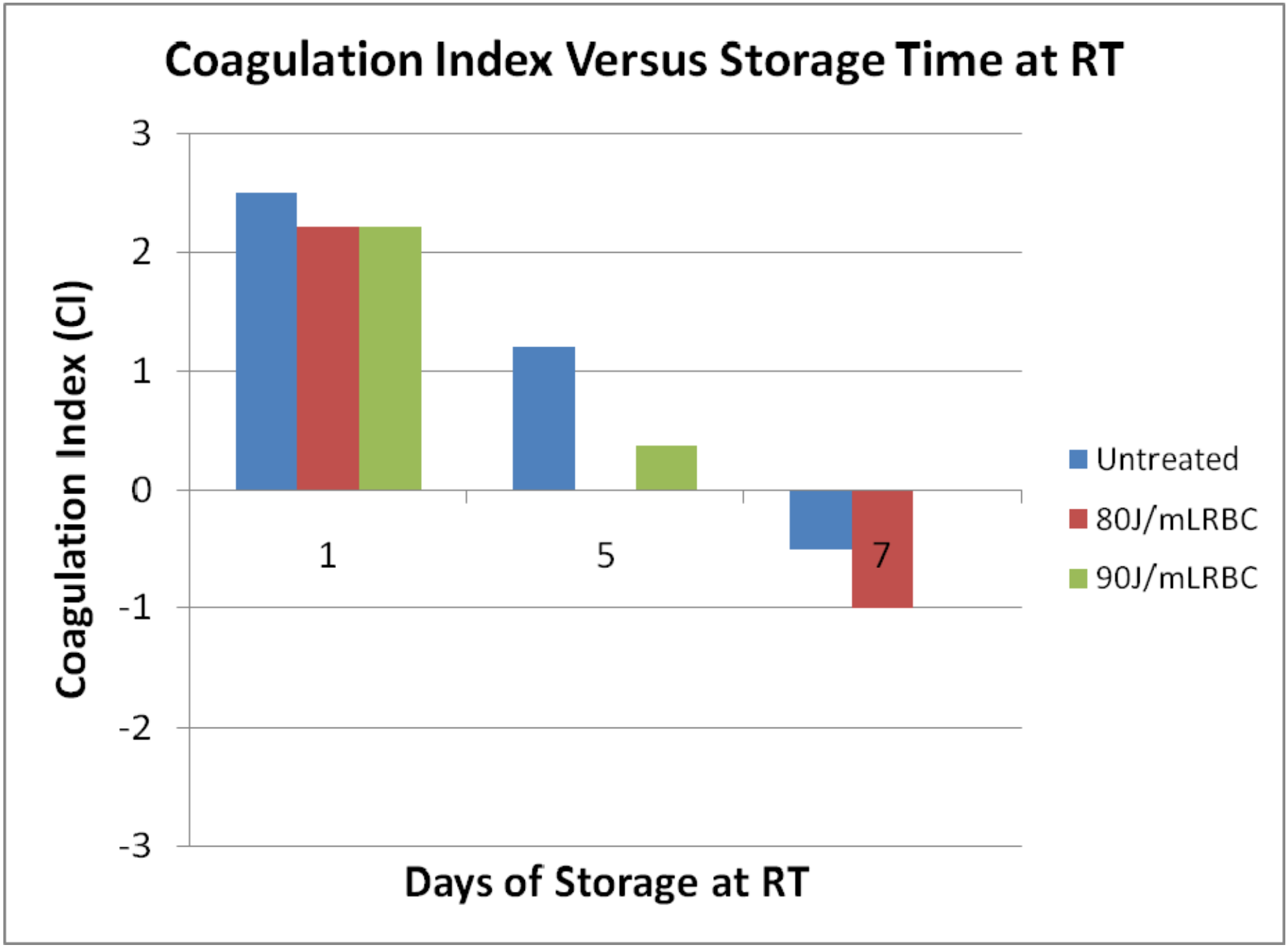

Average Angle Value for WB Stored at RT
Angle (deg)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Day 1
Day 7
TRT, N=8
CTL, N=4


Average MA Value for WB Stored at RT
MA (mm)
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
Day 1
Day 7
TRT, N=8
CTL, N=4

Coagulation Index

Sample ID	TEG D1 Coagulation Index	TEG D7 Coagulation Index
2995 TRT	2.1	-0.8
2996 TRT	1.1	-2.5
2997 TRT	1.8	-1.8
2998 TRT	2.5	-1.1
3009 TRT	2.4	-0.5
3010 TRT	2.9	-0.7
3011 TRT	2.6	-0.3
3012 TRT	2.5	-0.2
2999 CTL	2.1	-1.8
3000 CTL	2.6	0.8
3013 CTL	2.0	-1.2
3014 CTL	3.2	0.2
TRT Avg	**2.2**	**-1.0**
TRT Stdev	**0.6**	**0.8**
CTL Avg	**2.5**	**-0.5**
CTL Stdev	**0.5**	**1.2**

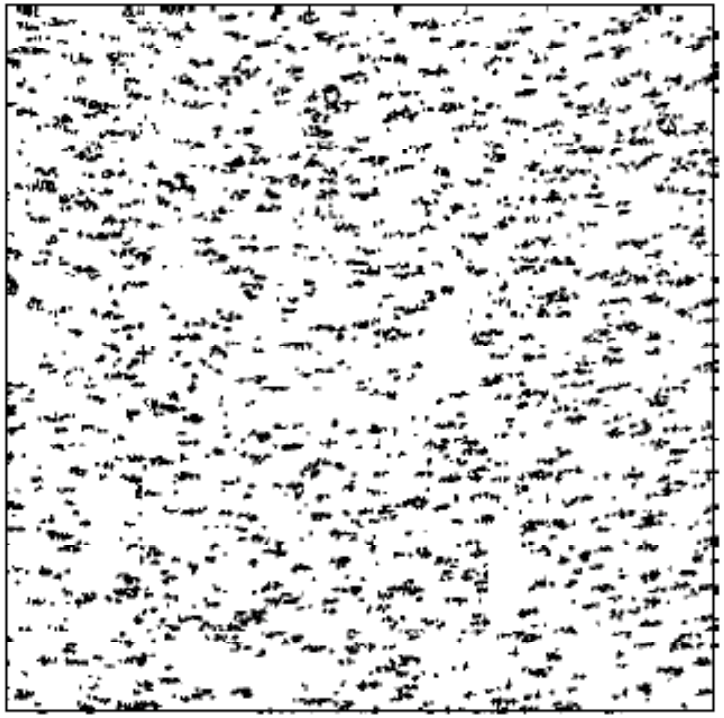
Coagulation Index = –0.1227R + 0.0092K + 0.1655MA – 0.0241alpha – 5.0220 and reflects overall coagulation status. Normal values range from –3.0 to +3.0,and reflect the number of standard deviations from the mean of zero.


Coagulation Index Versus Storage Time at RT
Coagulation Index (CI)
3
2
1
0
-1
-2
-3
1
5
7
Days of Storage at RT
Untreated
80J/mLRBC
90J/mLRBC

Analysis of Whole Blood Stored at RT by Impact-R Analysis

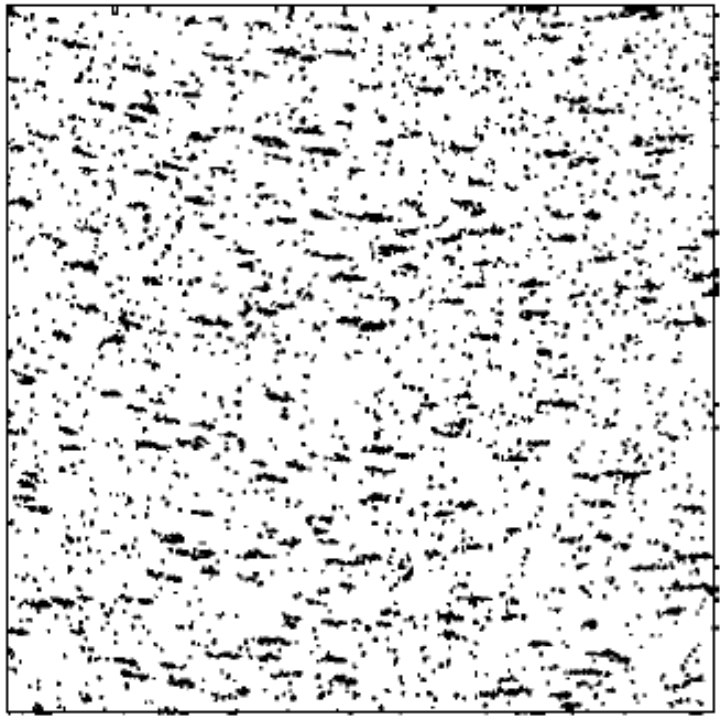
Treated Sample

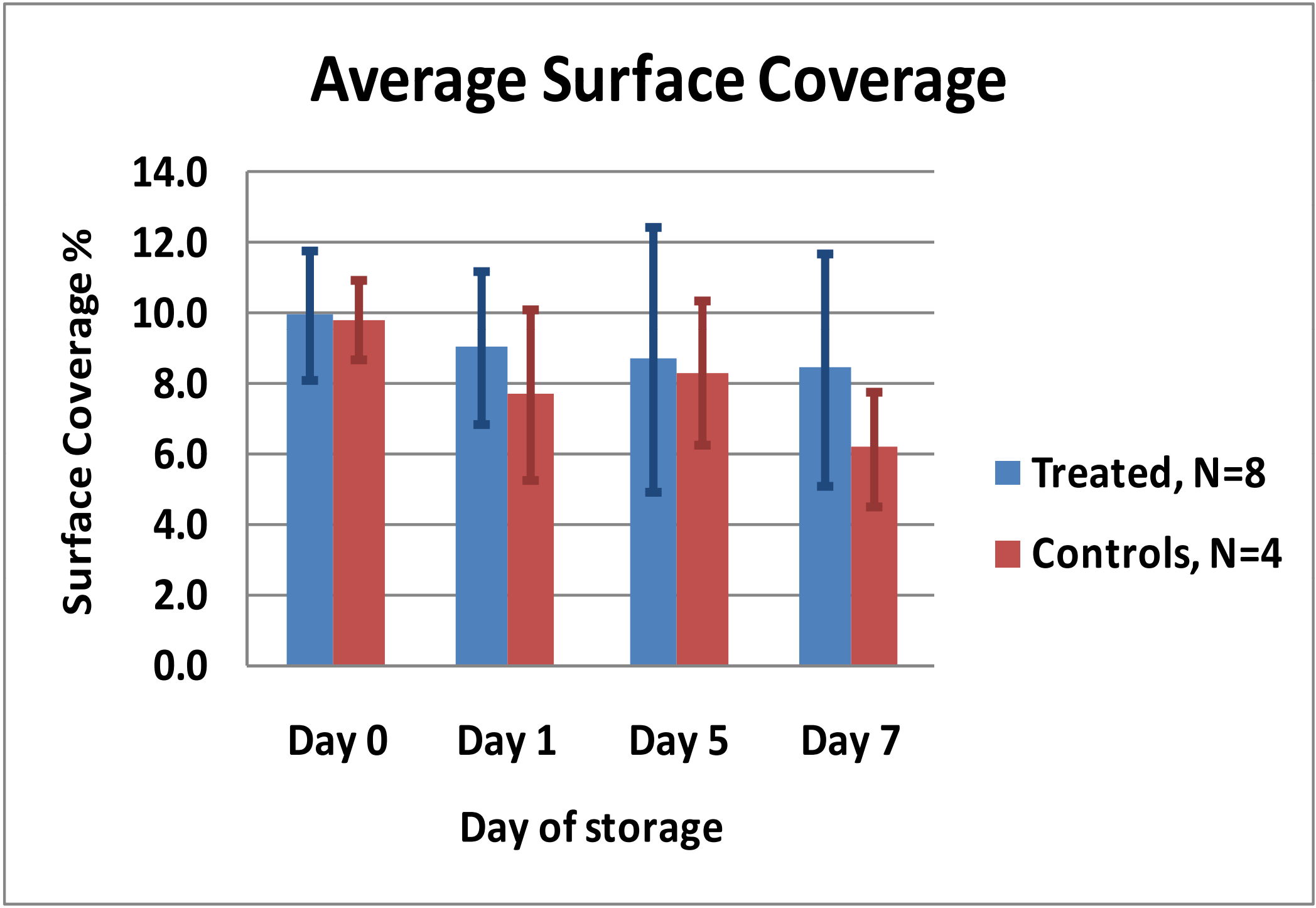
SC: 13 %
AS: 58 μm²
Ob: 1290
Test Image

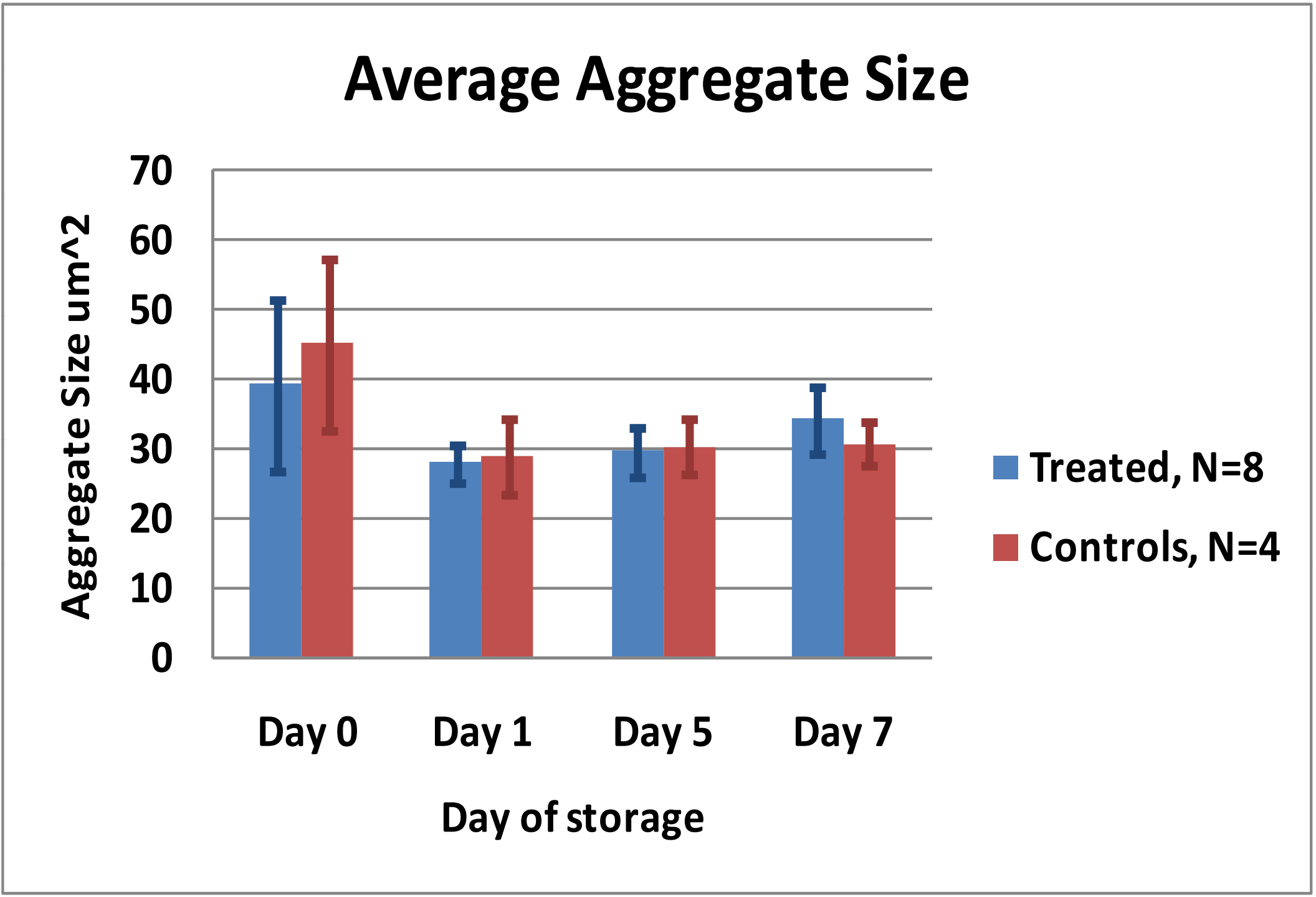


Untreated Sample

SC: 11 %
AS: 42 μm²
Ob: 1495
Test Image




Average Surface Coverage
Surface Coverage %
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Day 0
Day 1
Day 5
Day 7
Day of storage
Treated, N=8
Controls, N=4

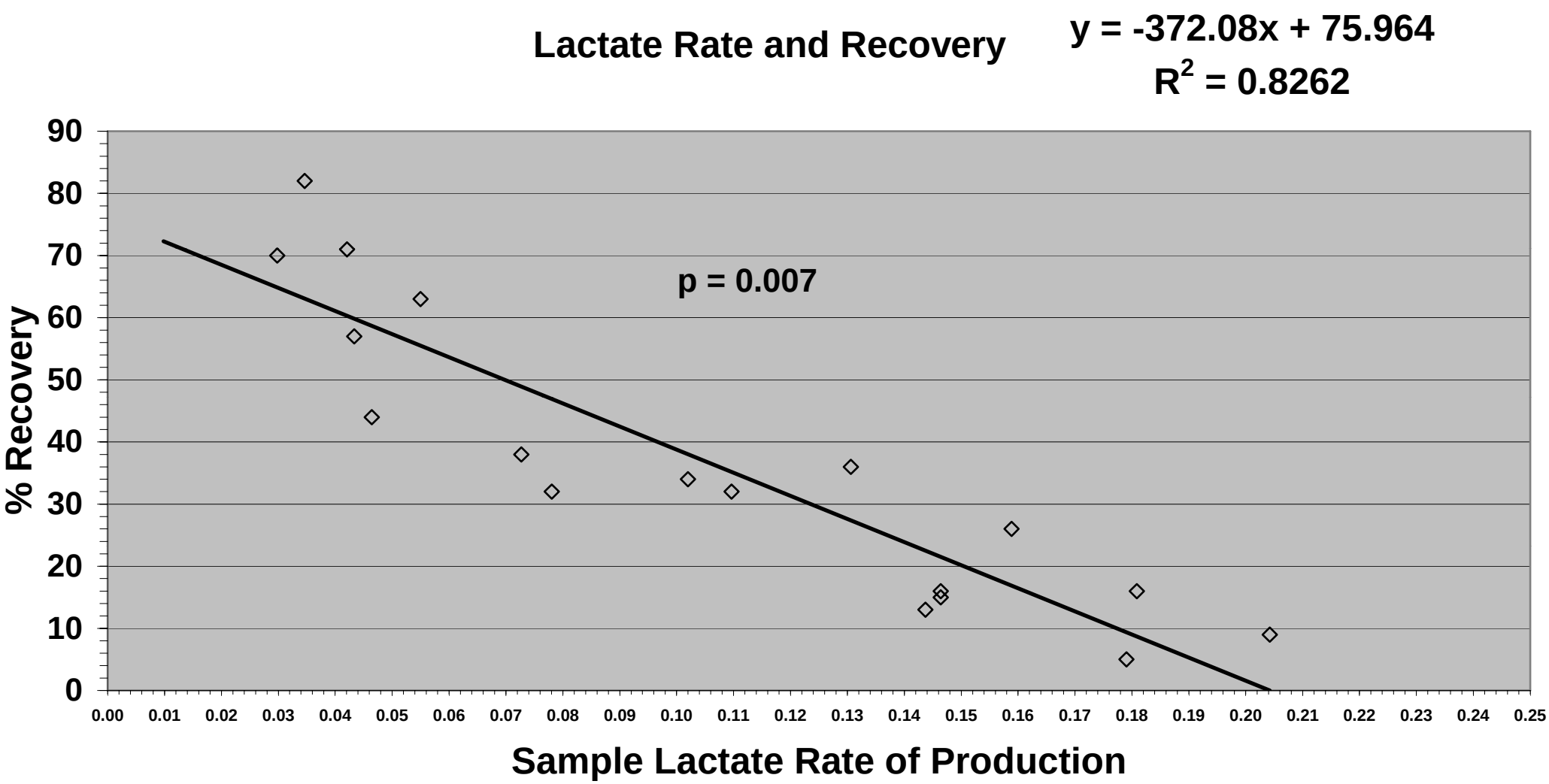

Average Aggregate Size
Aggregate Size um^2
70
60
50
40
30
20
10
0
Day 0
Day 1
Day 5
Day 7
Day of storage
Treated, N=8
Controls, N=4

Safety Profile of the System for Whole Blood

- The toxicology program performed for the PRT System for Platelets supports the use of the System for whole blood
  - Photoproduct profile is identical in whole blood and in platelet and plasma products

- Neoantigenicity test of treated RBCs was performed in baboons
  - No treatment-related neoantigenicity was observed- No antibody production following immunization
  - Recovery and Survival of treated red cells was the same as that observed for untreated control products
    - Goodrich et al. (2009) ***Transfusion***; 49: 64-74.

Platelet Study Correlating in vitro and in vivo Performance



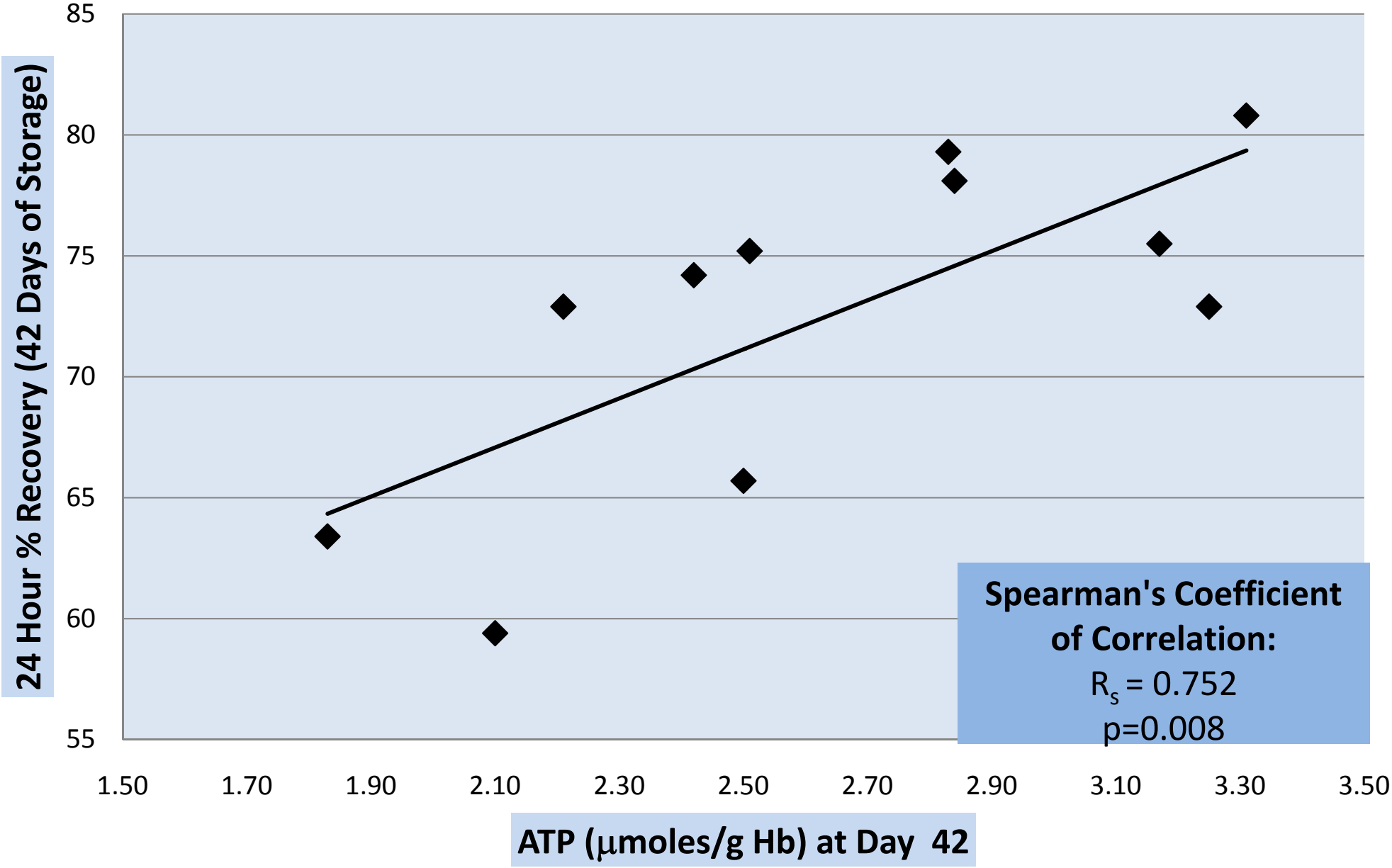
Goodrich et al. ***Vox Sanguinis*** (2006); 90: 279-285

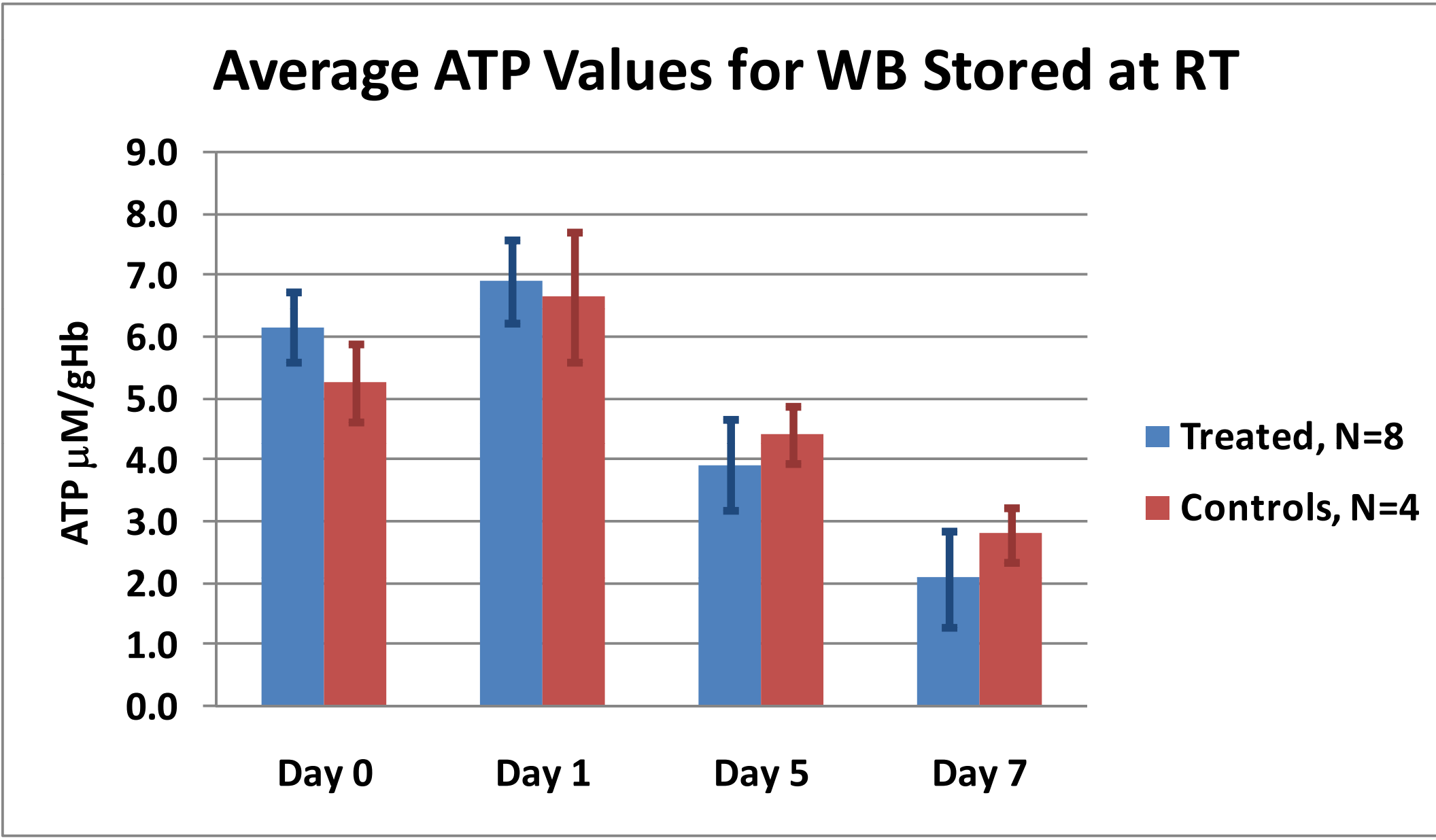
IMPROVE Study

- Treated Whole Blood; Separated into components
- Non-Leukoreduced; White Cell inactivation monitored
- In vitro Cell Quality Evaluation
  - Red Cells; 42 Days storage
  - Platelets (PRP); 5 Days storage
  - Plasma (FFP); 28 Days storage
- Human Clinical Evaluation
  - Radiolabel re-infusion of red cells; volunteer subjects
  - Correlation of in vitro parameters with in vivo recovery and survival of treated red cells

Study Initiated in US in September; FDA approved IDE; US Department of Defense Support

ATP vs 24 Hour % Recovery of 42 Day Stored RBCs in the IMPROVE Study




Average ATP Values for WB Stored at RT
ATP µM/gHb
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
Day 0
Day 1
Day 5
Day 7
Treated, N=8
Controls, N=4

Predicted 24 Hour Recovery of Red Cells From Whole Blood Products

	Untreated Products		Treated Products	
Day of Storage at RT	ATP μMole/ gHb	Predicted 24 Hour Recovery	ATP μMole/ gHb	Predicted 24 Hour Recovery
Day 0	5.27	100%	6.18	100%
Day 1	6.66	100%	6.91	100%
Day 5	4.43	91%	3.95	86%
Day 7	2.82	74%	2.10	67%

PLATELET INCUBATOR
HELMER

Acknowledgements

- The Ohio State University (Columbus, OH)
- TATRC
- WRAIR
- University of Cincinnati (Hoxworth Blood Center; Cincinnati, OH)
- Centers for Disease Control (Atlanta, GA)
- American Red Cross (Holland Labs; Rockville, MD)
- American Red Cross (Los Angeles, CA)
- Brown University, Rhode Island Hospital (Providence, RI)
- Blood Systems Research Institute (S. Francisco, CA)
- University of Texas, Medical Branch (Galveston, TX)
- Southwest Foundation for Biomedical Research (San Antonio, TX)
- CellPhire, Inc. (Rockville, MD)
- BioReliance, Inc. (Rockville, MD)

Thank You !