

Pathogen Reduction: Our first years experience

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Layers of Safety

- Donor selection and deferral
- ELISA and NAT testing for infectious markers
- Skin cleansing and diversion pouch to reduce bacterial contamination
- Bacterial screening of platelets
- Leukoreduction and gamma irradiation
- Pathogen reduction

Why implement Pathogen Reduction?

- There is not a screening assay for every pathogen, especially emerging pathogens
- Despite many screening measures there is still a residual risk of infection during window period
- Potential for fewer donor deferrals
- Reduce wastage from false positives from bacterial screening assays
- Not all WBC are removed by leukoreduction
- Simpler component preparation if irradiation not required

Pathogen Reduction: Capability

Viruses

CMV

HIV

HAV, HBV, HCV

WNV,

B-19

Chikungunya

Rabies virus

Influenza A (incl. Avian flu)

Parasites

Plasmodium falciparum

Trypanosoma cruzi (Chagas' disease)

Leishmania major

Babesia microti

Bacteria

Staphylococcus aureus

Staphylococcus epidermidis

Streptococcus pyogenes

Streptococcus mitis

Escherichia coli

Bacillus cereus

Propionibacterium acnes

Acinetobacter baumannii

Enterobacter cloacae

Klebsiella pneumoniae

Yersinia enterocolitica

Pathogen Reduction: Capability

- 2 commercialised technologies – INTERCEPT and Mirasol
- Capable of treating plasma and platelets in both additive and plasma
- Technology for treating whole blood or red cells still in development

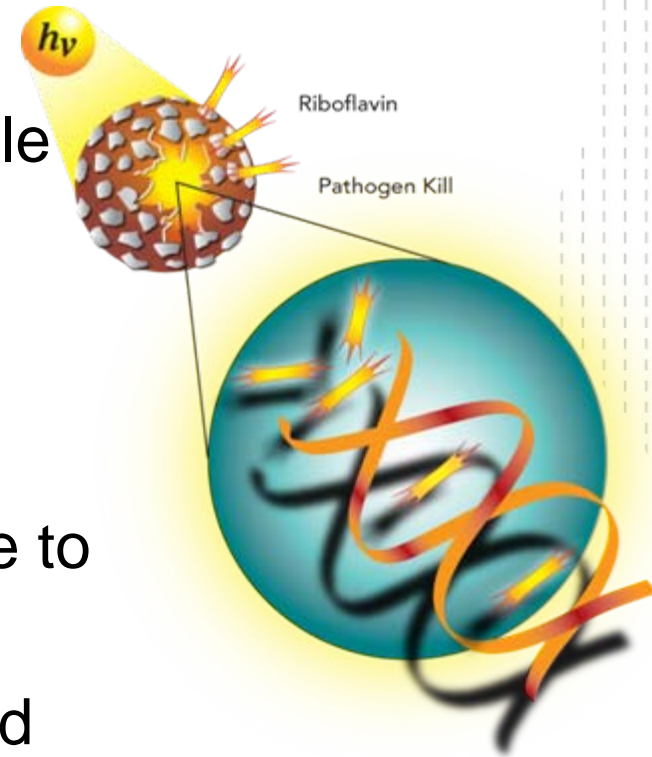
Choice of PRT system - Mirasol

- Psoralen compound used in INTERCEPT system is toxic and must be removed, riboflavin not removed
- Removal of psoralen takes up to 24h → longer processing time
- Removal of psoralen leads to 7-10 % loss platelet numbers

Mechanism of action: Mirasol system

UV light + riboflavin: irreversible inactivation

- Riboflavin molecules form complexes with nucleic acids
- UV light activates the riboflavin molecule in the complex
- Photo-activated riboflavin induces a chemical alteration to the functional groups (such as guanine bases) of nucleic acids making pathogens unable to replicate
- Riboflavin does not need to be removed



Pathogen Reduction

Is sufficient platelet quality maintained following treatment of platelets in additive solution?

Metabolic pathways in platelets

- ~ 20 % anaerobic glycolysis – favoured in glucose rich solutions:
glucose → 2 ATP molecules
→ 2 lactate molecules → pH decrease
- ~ 80 % (aerobic) oxidative phosphorylation in mitochondria → 38 ATP molecules

Platelet storage lesion

Progressive deterioration of platelet structure and function during storage, including changes in:

- metabolism
- morphology
- activation
- apoptosis

Pathogen reduction: experimental process

Pool 2 standard
BC platelets in
SSP+/plasma
(70%: 30%)

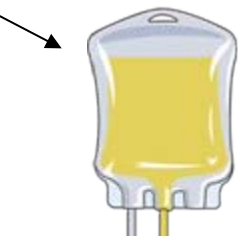
control



saline



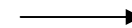
→ No further treatment



Mirasol treated



35 ml riboflavin
solution

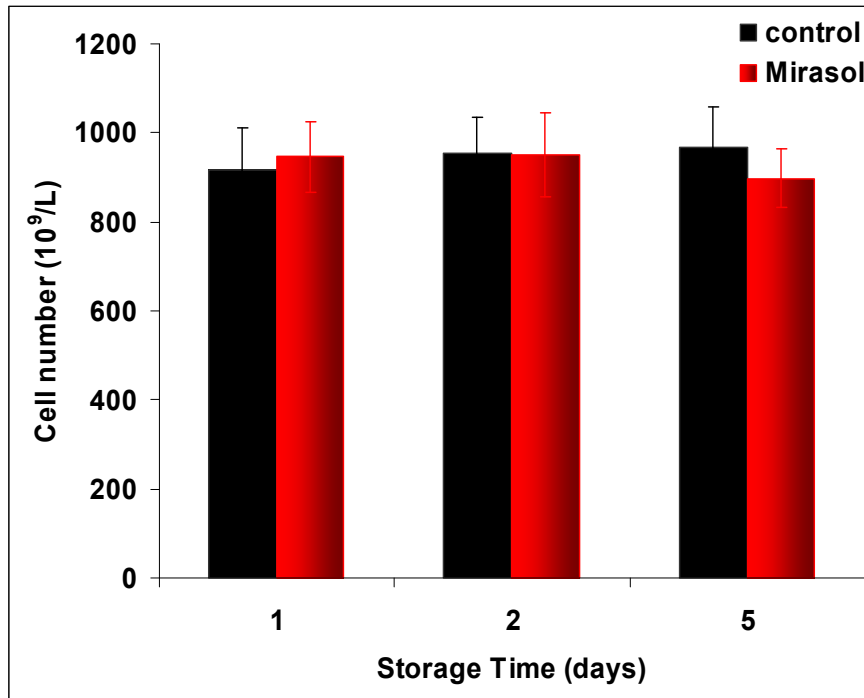


Mirasol
Illumination

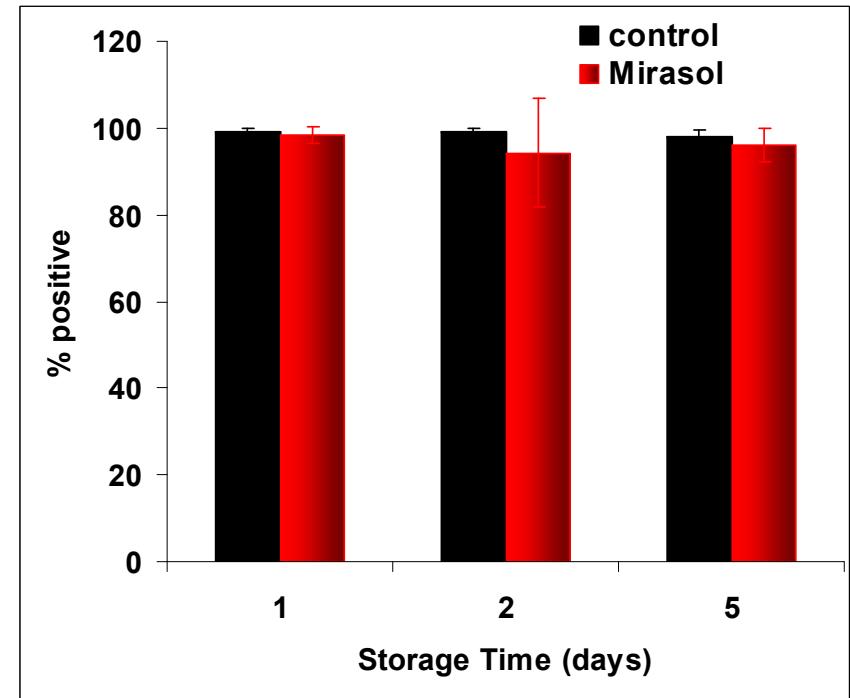


Platelet numbers and viability

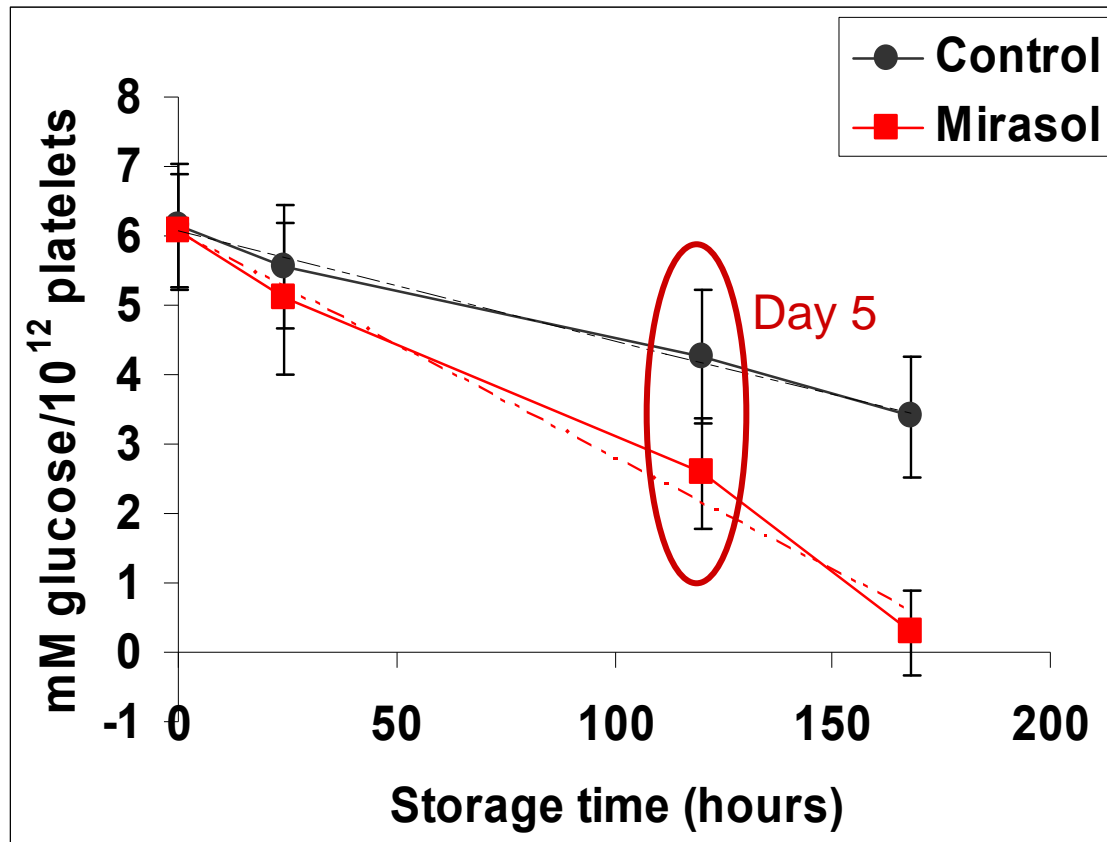
Platelet count



Viability

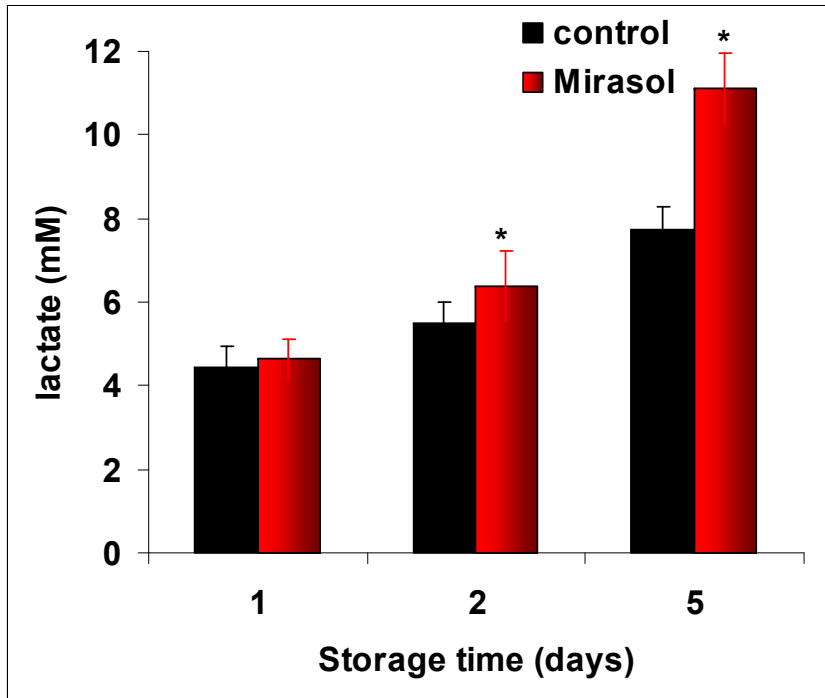


Increased glucose consumption

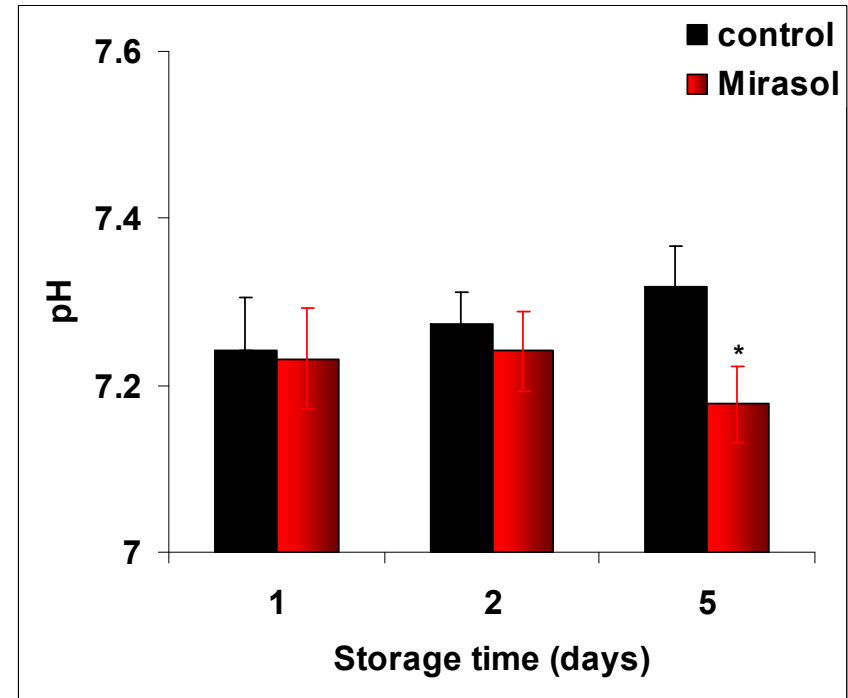


Increased lactate production and pH decrease

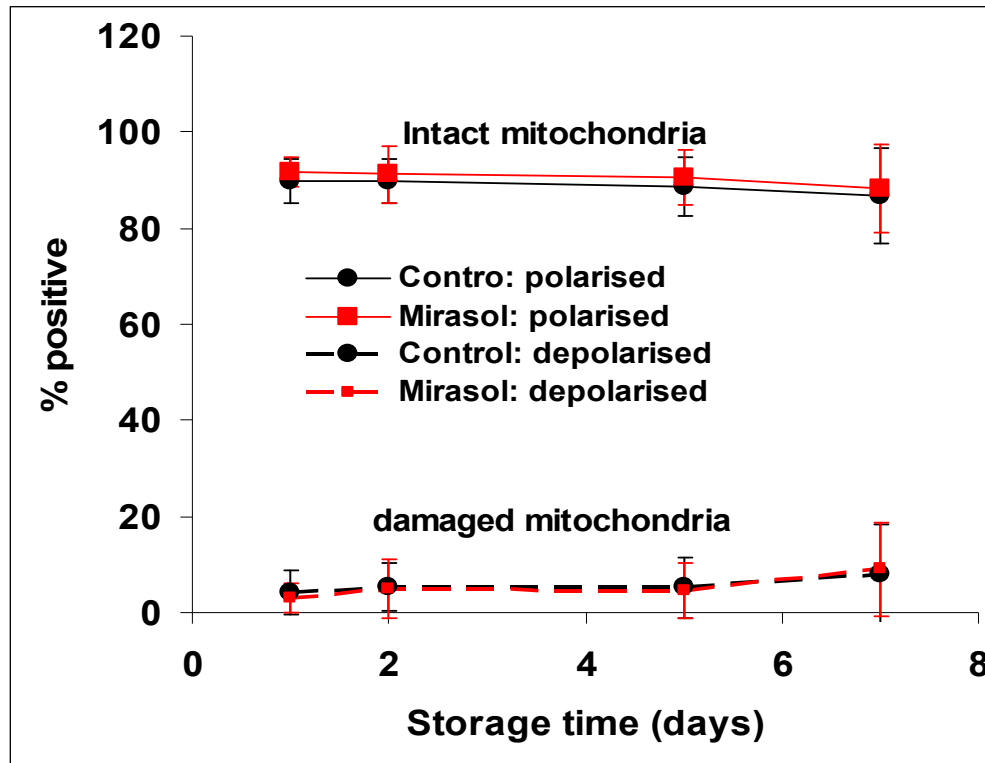
Lactate



pH

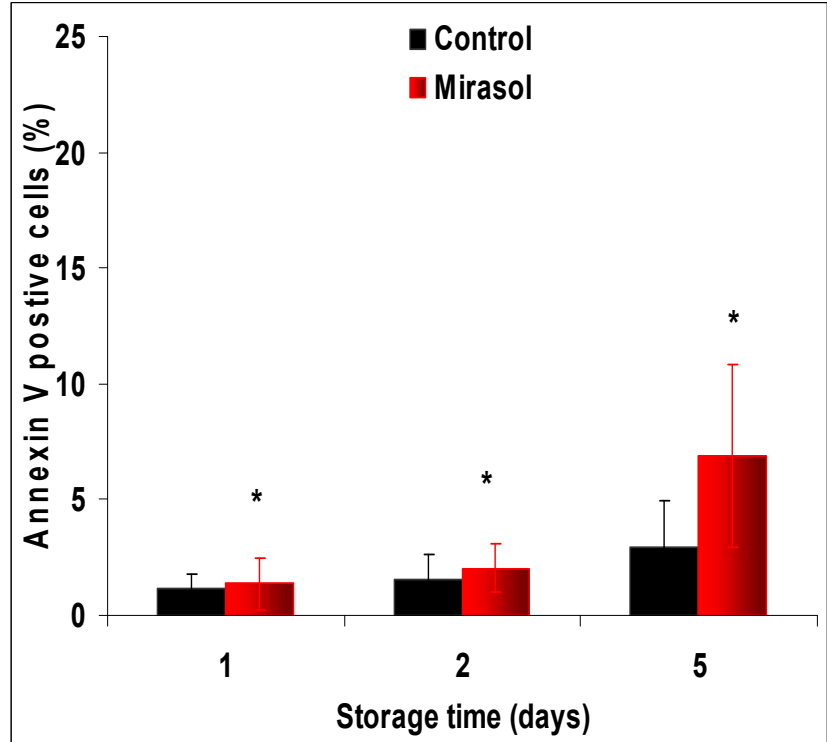


Mitochondria intact: no membrane depolarisation

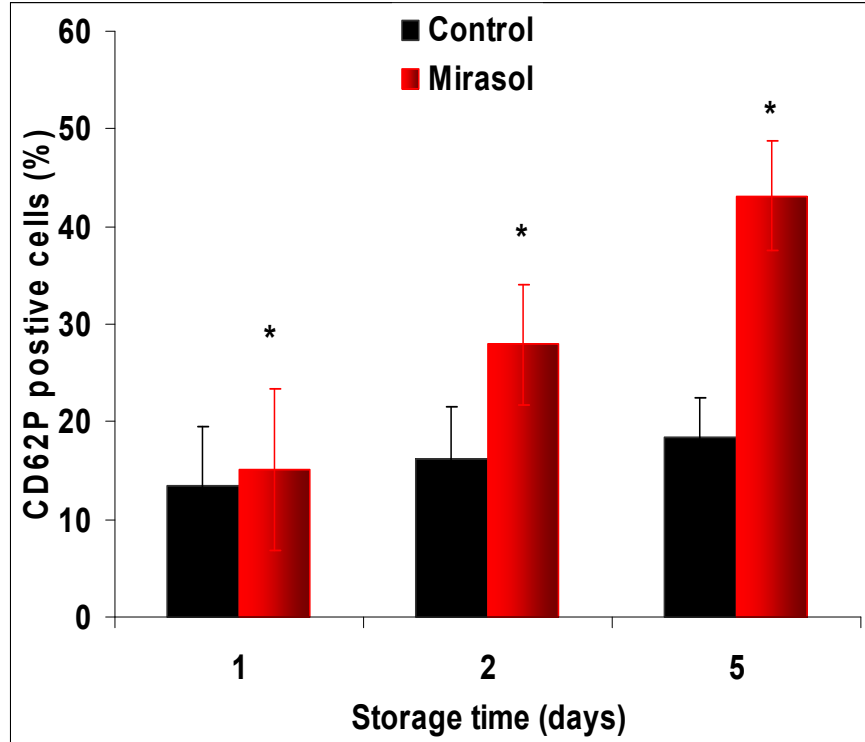


Other changes: activation

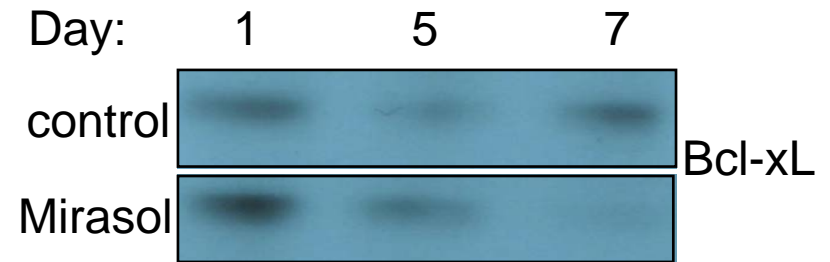
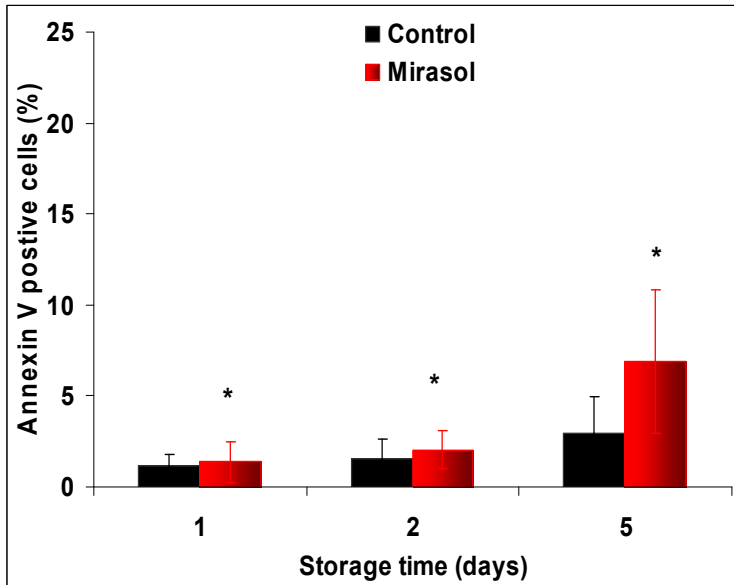
apoptosis



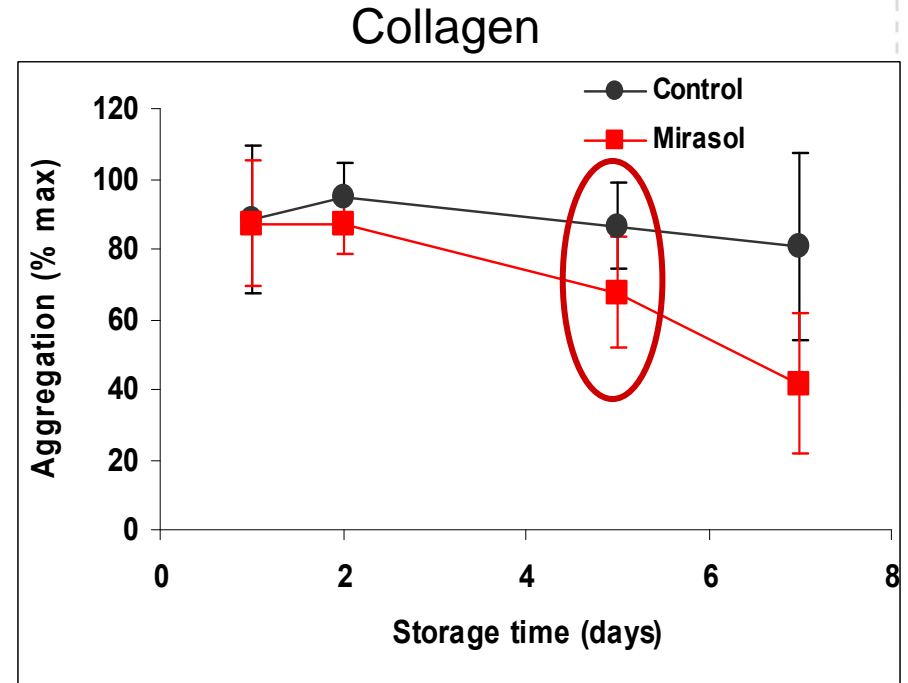
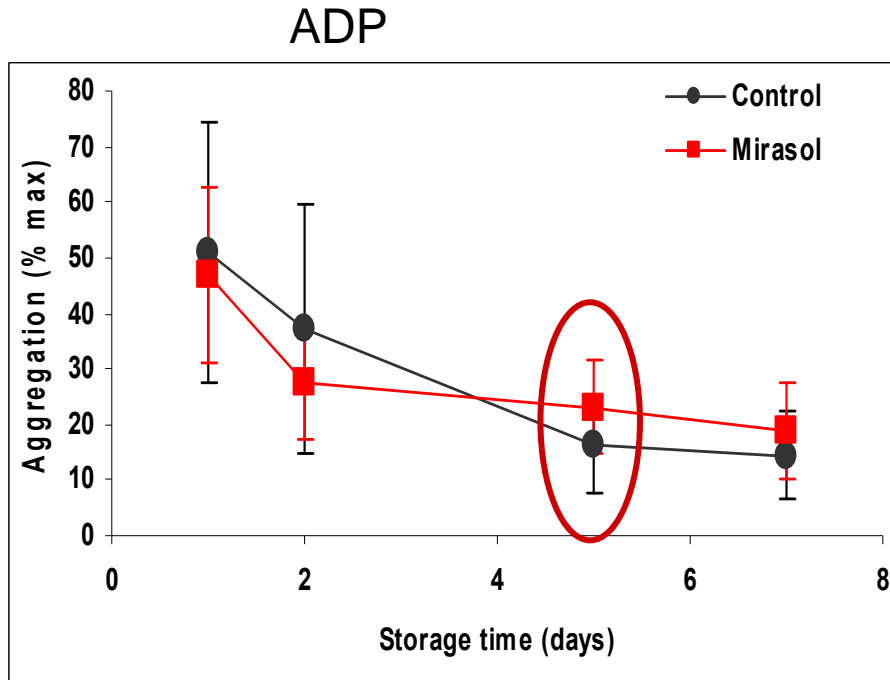
CD62P expression - degranulation



Apoptosis



Differential aggregation response



Summary

- Increased metabolism and activation
- Decreased response to collagen
- Product quality maintained – glucose, pH

Does Mirasol treatment inactivate
leukocytes in platelet additive solution?

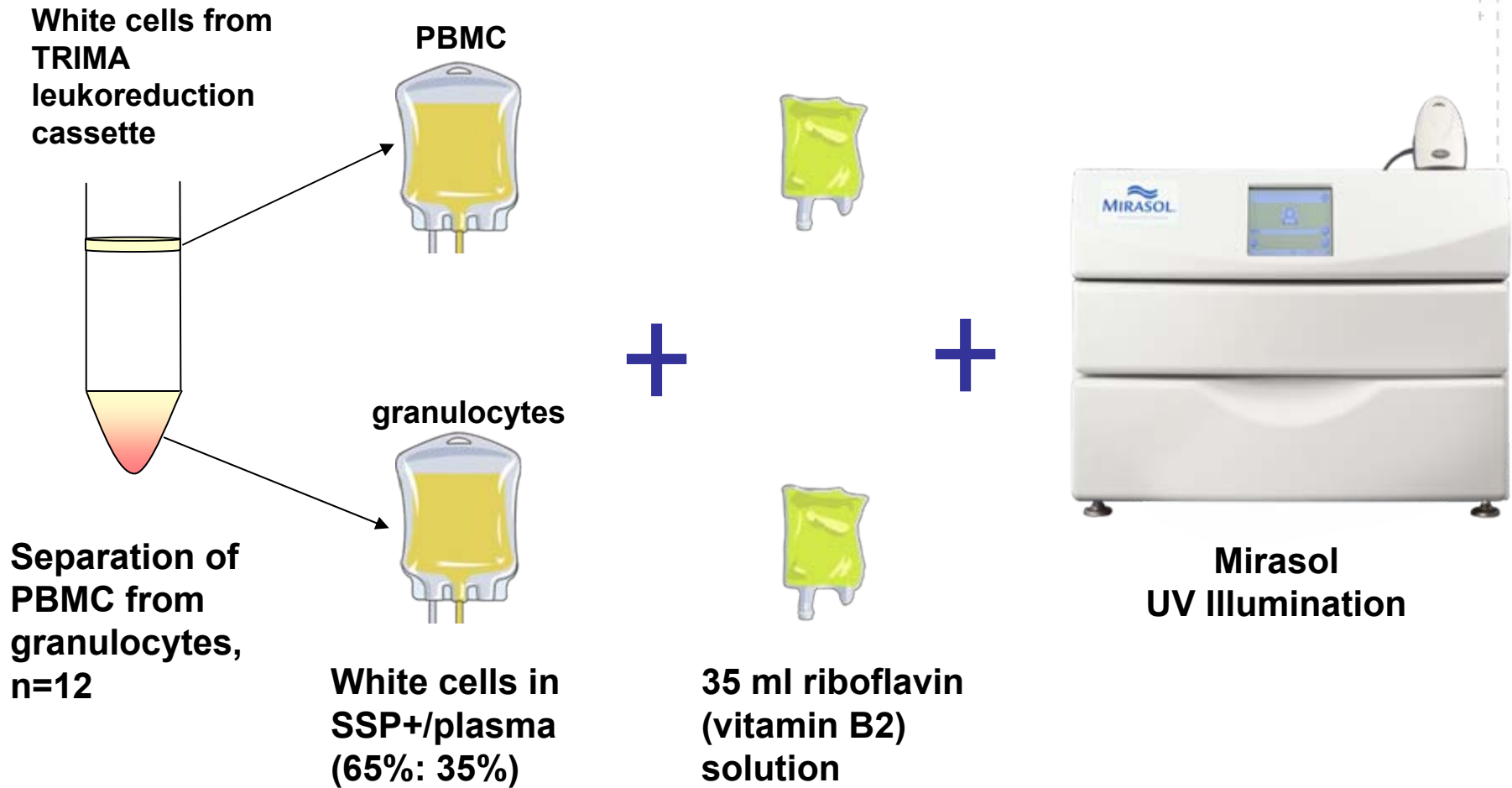
Leukocyte contamination of blood products

- TA-GVHD
- Allo-immunisation
- Cytokine production
- Transfusion Related Immunomodulation (TRIM)

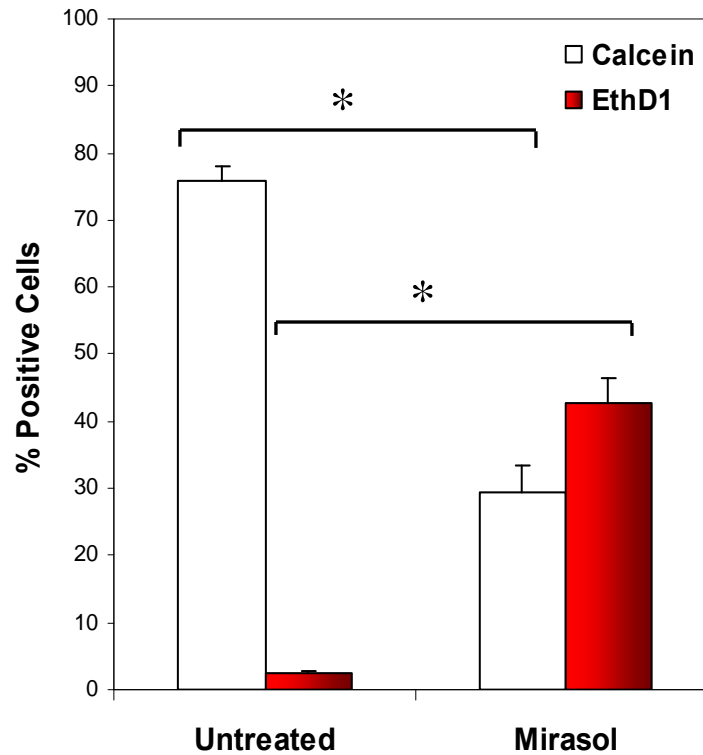
Leukocytes currently reduced by:

- Leukoreduction
- Gamma irradiation

Leukocyte inactivation process

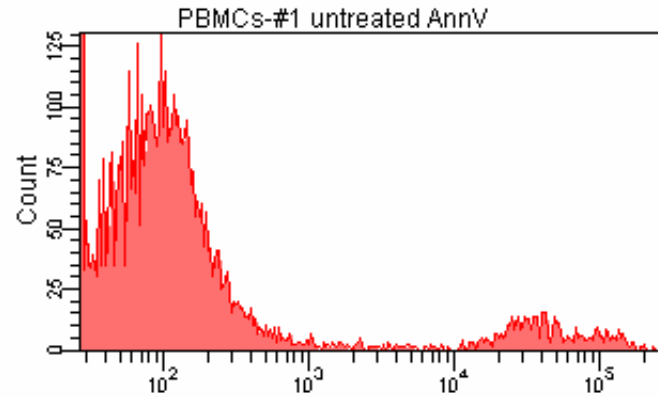


PRT treatment decreases PBMC viability

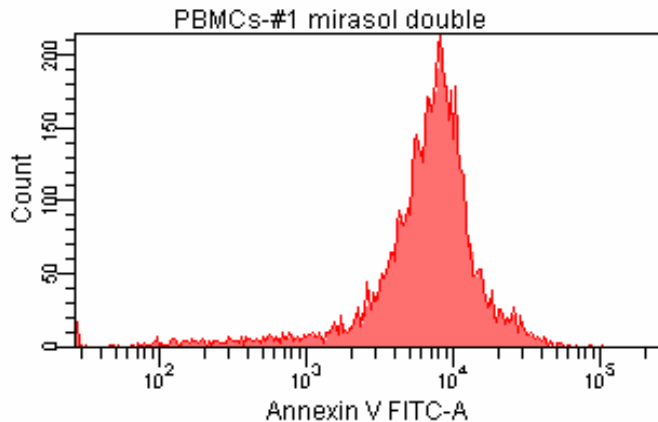


Calcein-AM binds to live cells
Ethidium dimer binds dead cells

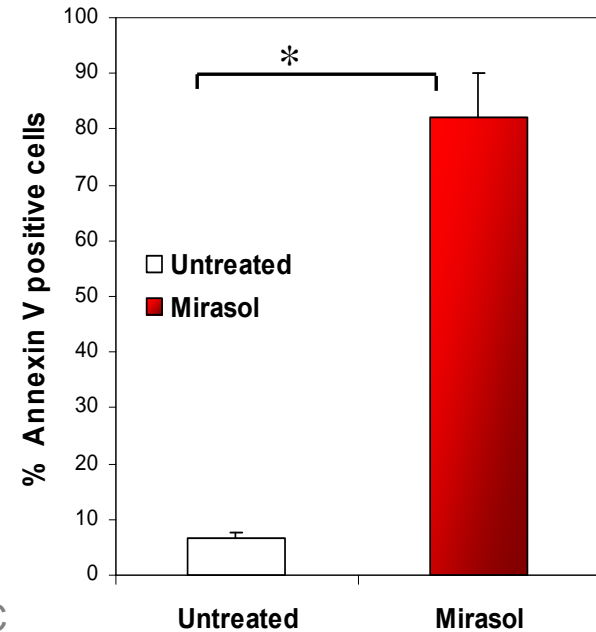
PRT treatment induces apoptosis



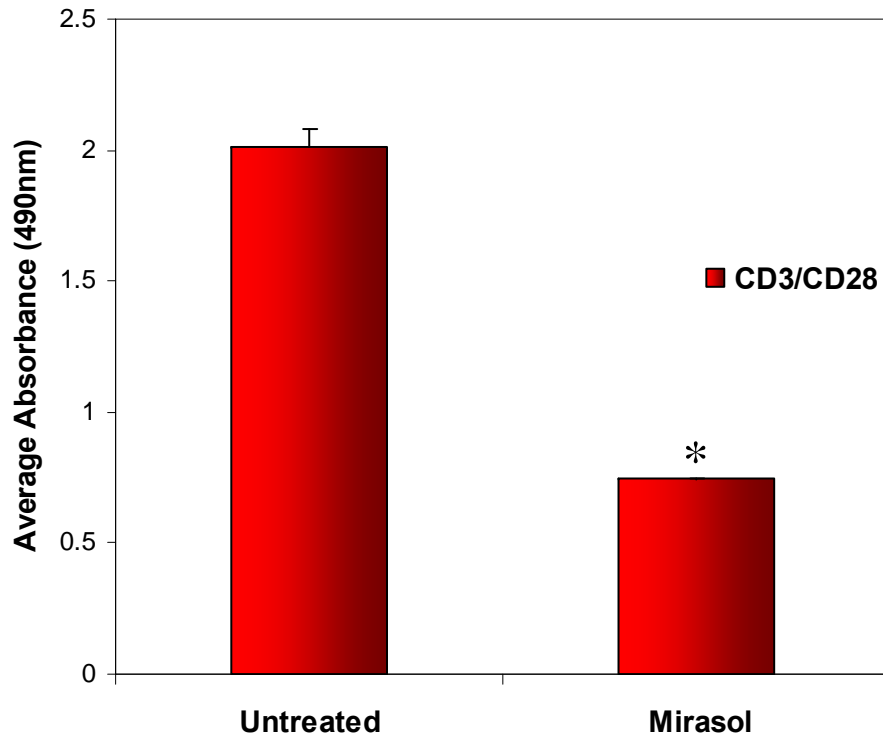
Untreated PBMC



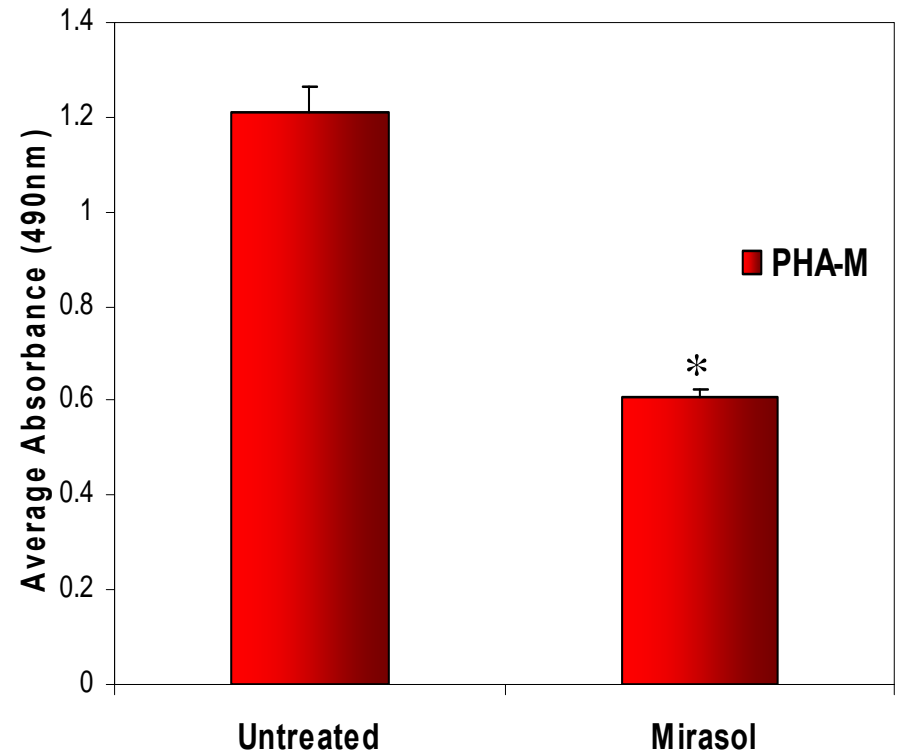
Mirasol treated PBMC



PRT treatment inhibits PBMC proliferation



CD3/CD28 induced proliferation



PHA induced proliferation

Reduced cytokine production by PRT- treated PBMC

Cytokine	Untreated Cells (pg/mL)		Mirasol-treated Cells (pg/mL)	
	without LPS	with LPS	without LPS	with LPS
IL-1 β	15 \pm 22*	10650 \pm 9040*	<0.5	<0.5
IL-2	<4	<4	<4	<4
IL-4	<4	<4	<4	<4
IL-5	<4	<4	<4	<4
IL-6	25 \pm 60	59337 \pm 18034*	<4	<4
IL-8	133 \pm 236	60914 \pm 18347*	872 \pm 425	879 \pm 320
IL-10	1 \pm 4	980 \pm 443*	<2	<2
IL-12 (p70)	28 \pm 55	<4	<4	<4
IFN- γ	<8	12931 \pm 26695	<8	<8
TNF- α	<8	485 \pm 686	8 \pm 20	114 \pm 84

* Indicates significantly higher cytokine production than untreated cells, p<0.05

Summary

PRT treatment effectively inactivates leukocytes:

- Induces apoptosis and reduced viability
- Inhibits PBMC proliferation
- Reduces cytokine production

Acknowledgements

- Lacey Johnson, Kelly Winter, Samantha Reid, Tanja Hartkopf-Theis
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- Catherine Trester, Ray Goodrich

Where to next?

- Apheresis platelets in additive solution
- Ongoing studies to understand mechanisms behind these findings
- Consultation with wider transfusion medicine community and other blood services: **what are the concerns clinicians would have with transfusing a unit of PRT-platelets?**