Cold-stored and frozen platelets

Denese C Marks
Australian Red Cross Lifeblood
Sydney, Australia
dmarks@redcrossblood.org.au

Disclosures

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- Vitrafy Life Sciences Pty Ltd
- Research funds from US Department of Defense (cold-stored platelets)
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- ARCLB is participating in the CLIP-II and CHIPS trials
- Member of Scientific Advisory Board for Vitrafy Life Sciences Pty Ltd

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Platelet shortages

COMMENTARY
Transfusion Practice

TRANSFUSION

Addressing platelet insecurity – A national call to action

Eric A. Gehrie[1] | Pampee P. Young[1,2] | Sridhar V. Basavaraju[3] | Arthur W. Bracey[4] | Andrew P. Cap[5] | Liz Culler[6] | Nancy M. Dunbar[7] | Mary Homer[8] | Iris Isufi[9] | Rob Macedo[10] | Tanya Petraszko[11] | Glenn Ramsey[12] | Christopher A. Tormey[9] | Richard M. Kaufman[7] | Edward L. Snyder[9]

Short shelf-life (5-7 days) due to RT storage

→ Inventory management and supply challenges:

- Increased appointment cancellation post-pandemic
- Regional hospitals – large distances - sometimes no supply at all
- Weather events
- Shortage of specific HLA-matched donors (platelet refractoriness)
- Surge capacity during domestic crises??
- Military needs +/- surge

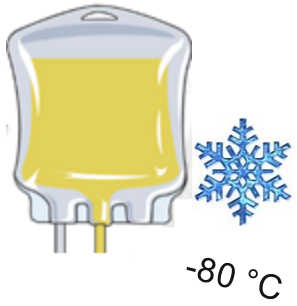
Alternative modes of platelet storage

Refrigeration

- 2 - 6 °C, no agitation
- Potential shelf-life of at least 14 days
- Approved in United States

Cryopreservation

- -80 °C (5% DMSO)
- > 2 year shelf-life



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Cold-stored platelets

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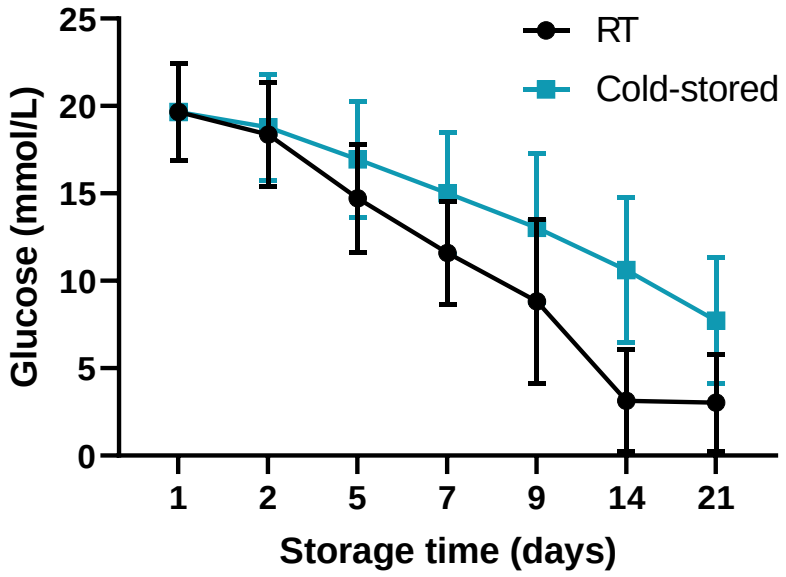
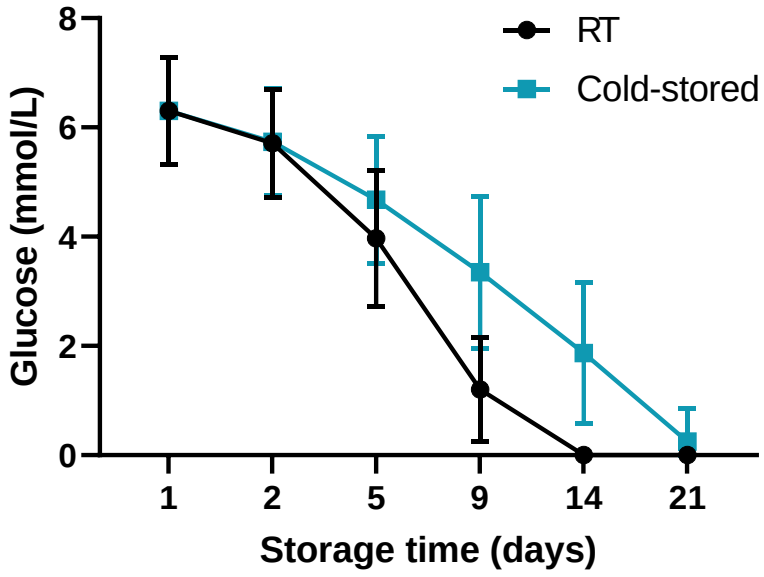
Cold storage slows metabolism

Reduced metabolism ► longer shelf-life

Apheresis platelets in 100% plasma



Apheresis platelets in 40% plasma/60% SSP+



Adapted from Johnson *et al*., Transfusion 2021

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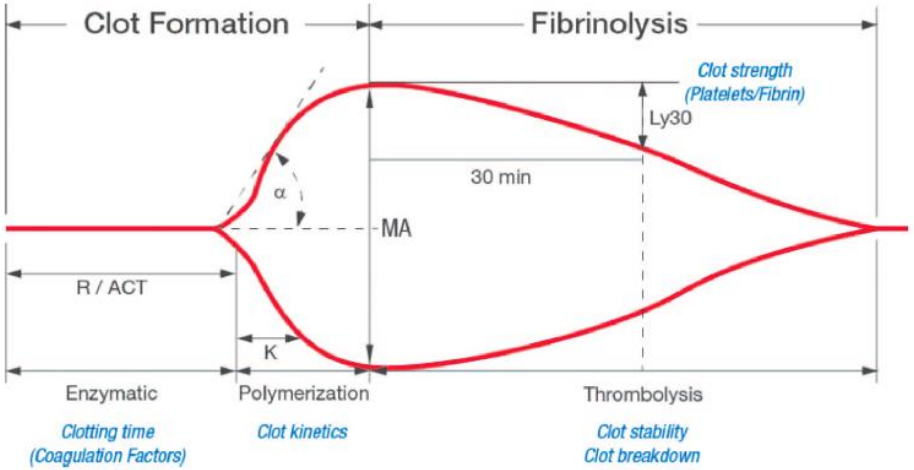
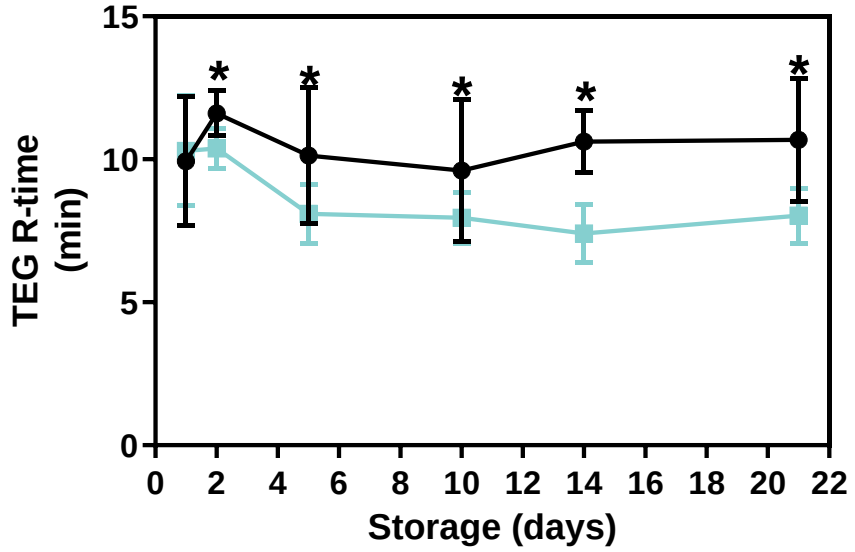
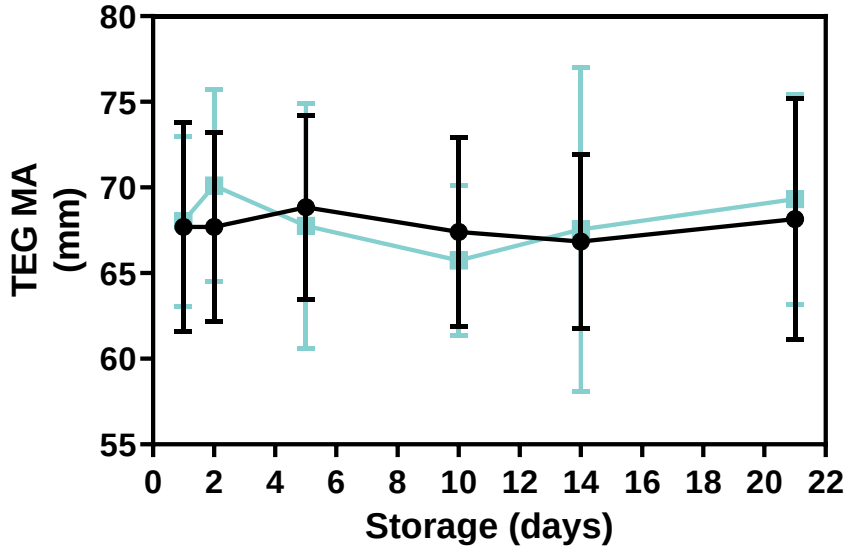
Cold storage results in faster initiation of clot formation



Arch Pathol Lab Med. 141(4):569-77; 2017

- Cold-stored platelets initiate faster clot formation
- Clot strength maintained





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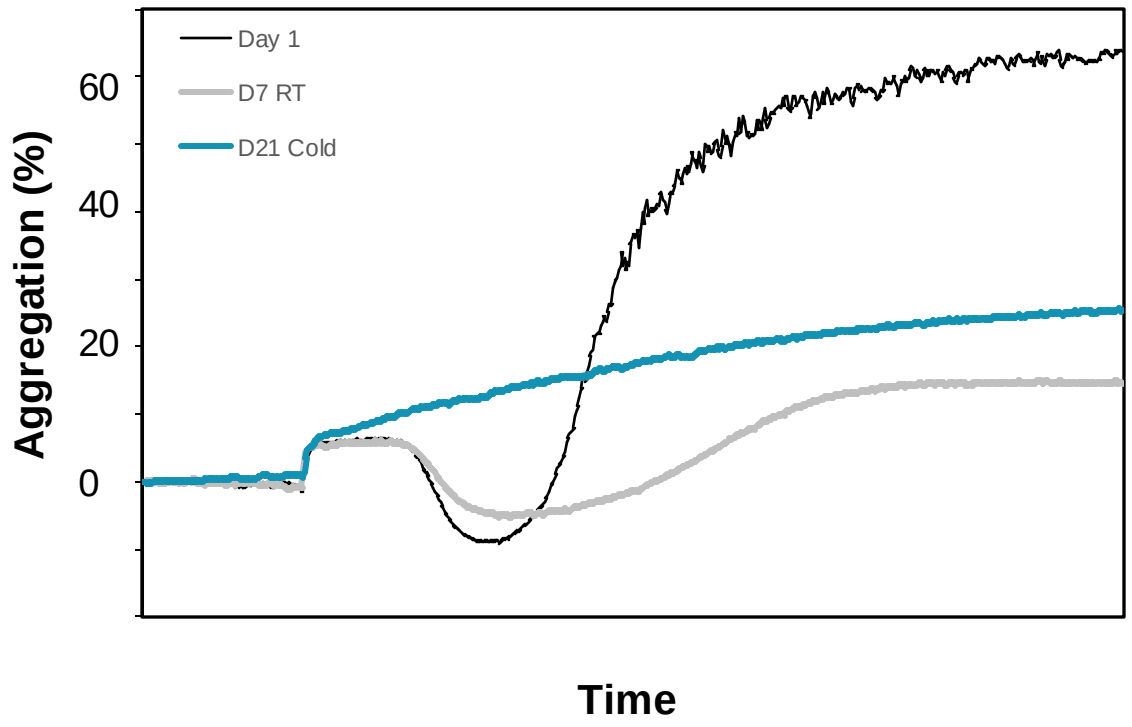
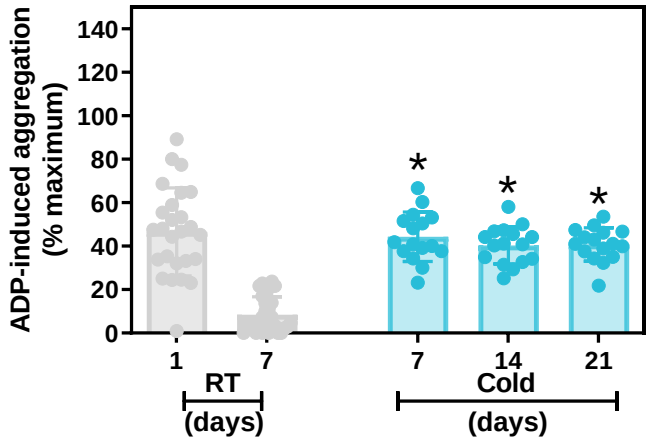
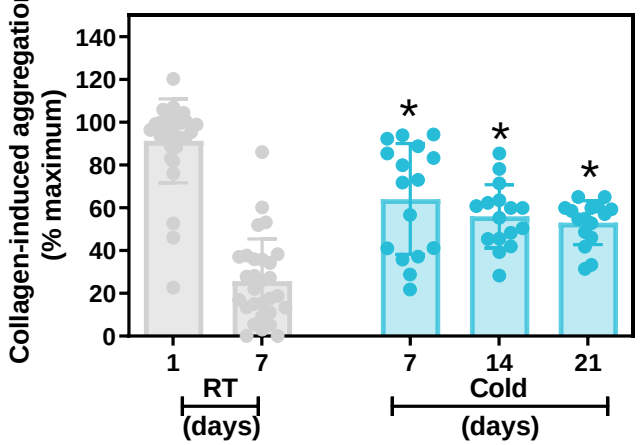
Cold storage promotes platelet aggregation

Light transmission aggregometry

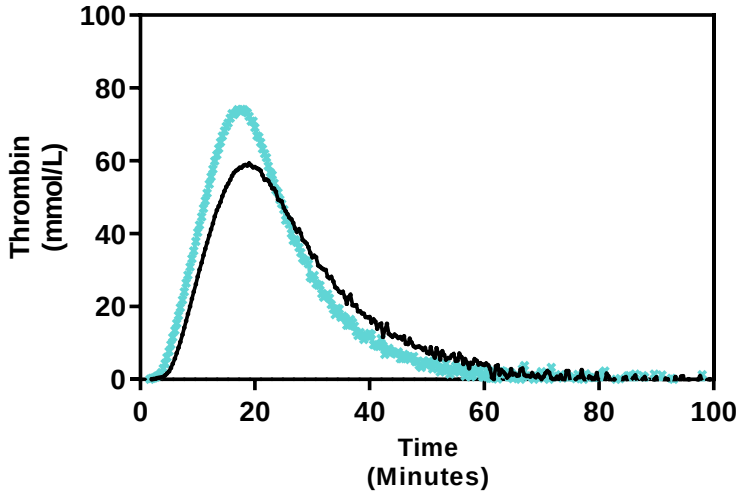






* p<0.05 between RT (day 7) and cold

Cold-stored platelets have better clotting function *in vitro*



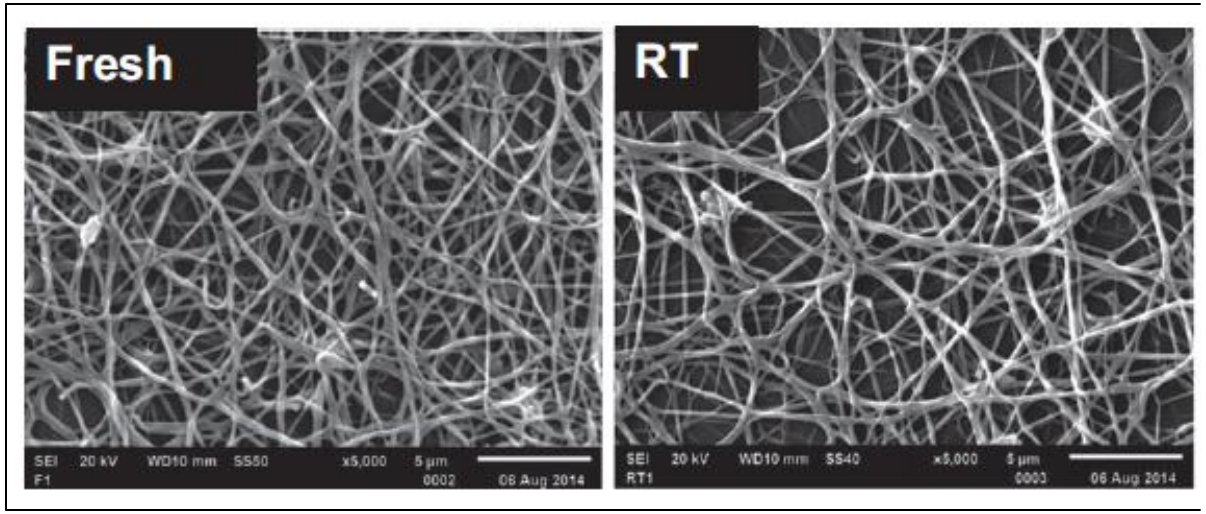


Figure from Nair *et al*., BJH 2017

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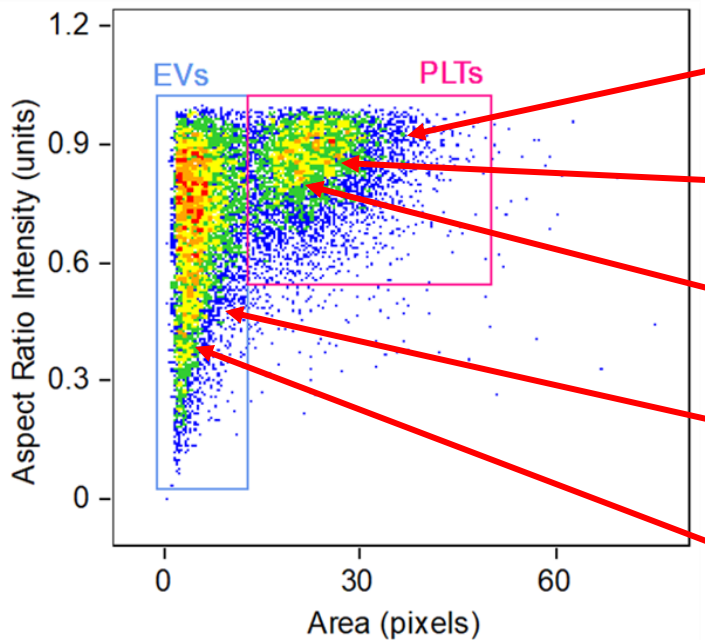
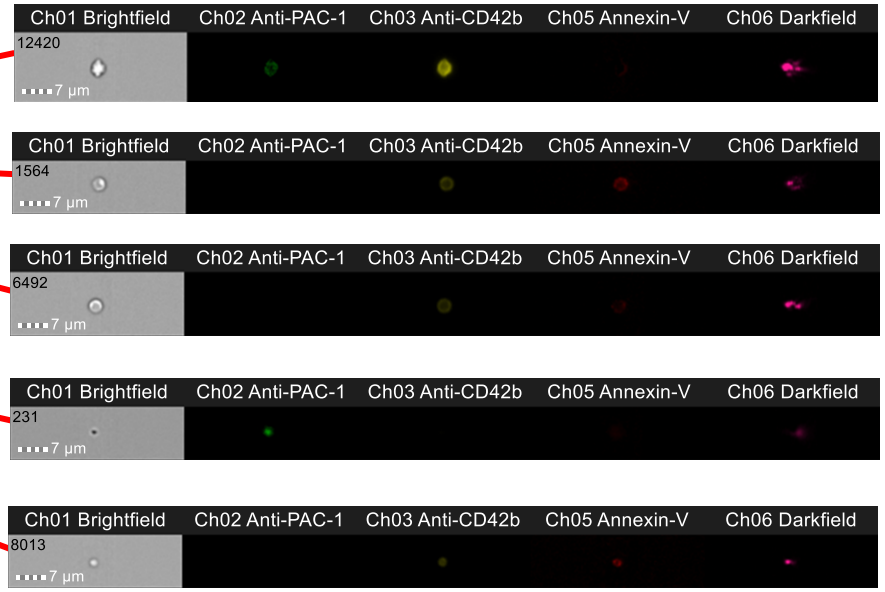
Sub-populations in cold-stored platelets

ImageStream: flow cytometry + confocal microscopy

Platelet component





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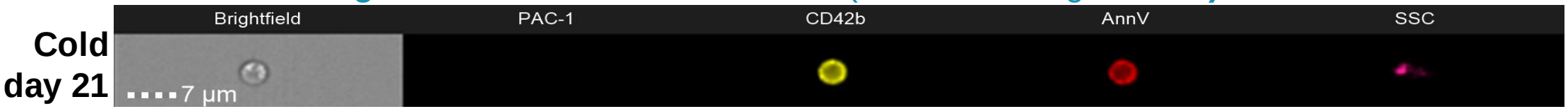
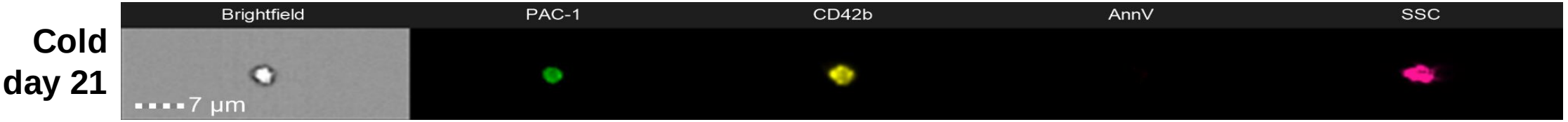
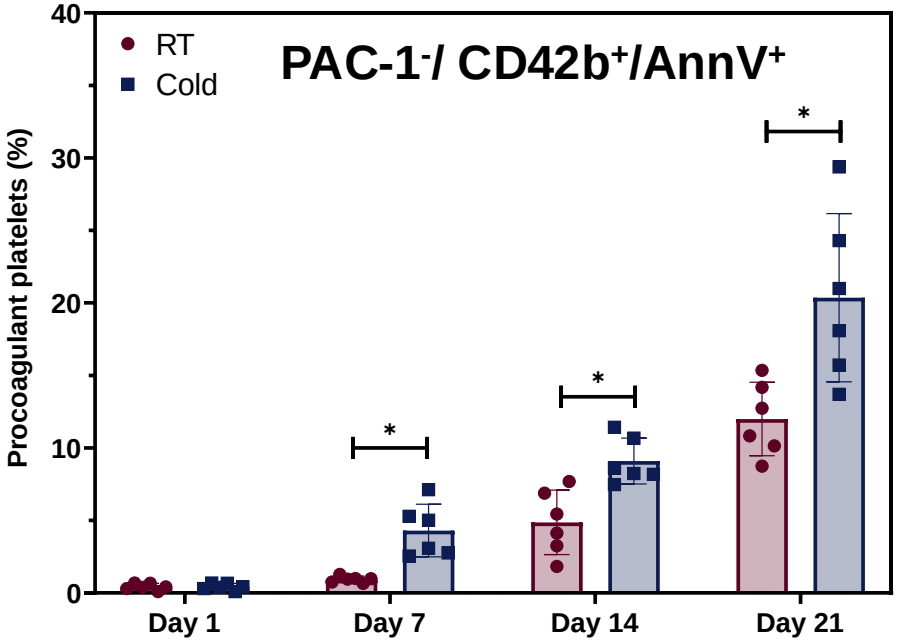
Sub-populations mediate platelet phenotype

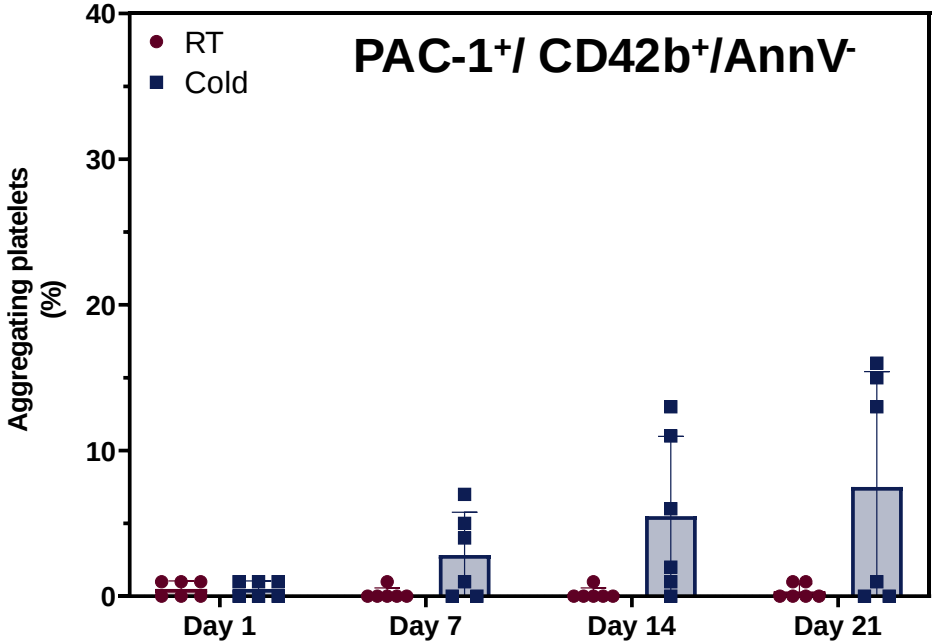
Procoagulant: PAC-1^{-}/ CD42b^{+}/AnnV^{+} (identified using A23187)



Aggregating: PAC-1^{+}/ CD42b^{+} /AnnV^{-} (identified using thrombin and collagen as controls)







Day 21: 9% were apoptotic: PAC-1^{-}/CD42b^{-}/AnnV^{+}

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Johnson *et al.*, Transfusion Medicine 2022: 32 S3: 1-40

Plasma or PAS for storage?

Cold storage of platelets in platelet additive solution maintains mitochondrial integrity by limiting initiation of apoptosis-mediated pathways

Kristin M. Reddoch-Cardenas[1] | Grant C. Peltier[1] | Tiffani C. Chance[2] | Prajeeda M. Nair[1] | Michael A. Meledeo[1] | Anand K. Ramasubramanian[3] | Andrew P. Cap[1] | James A. Bynum[1]

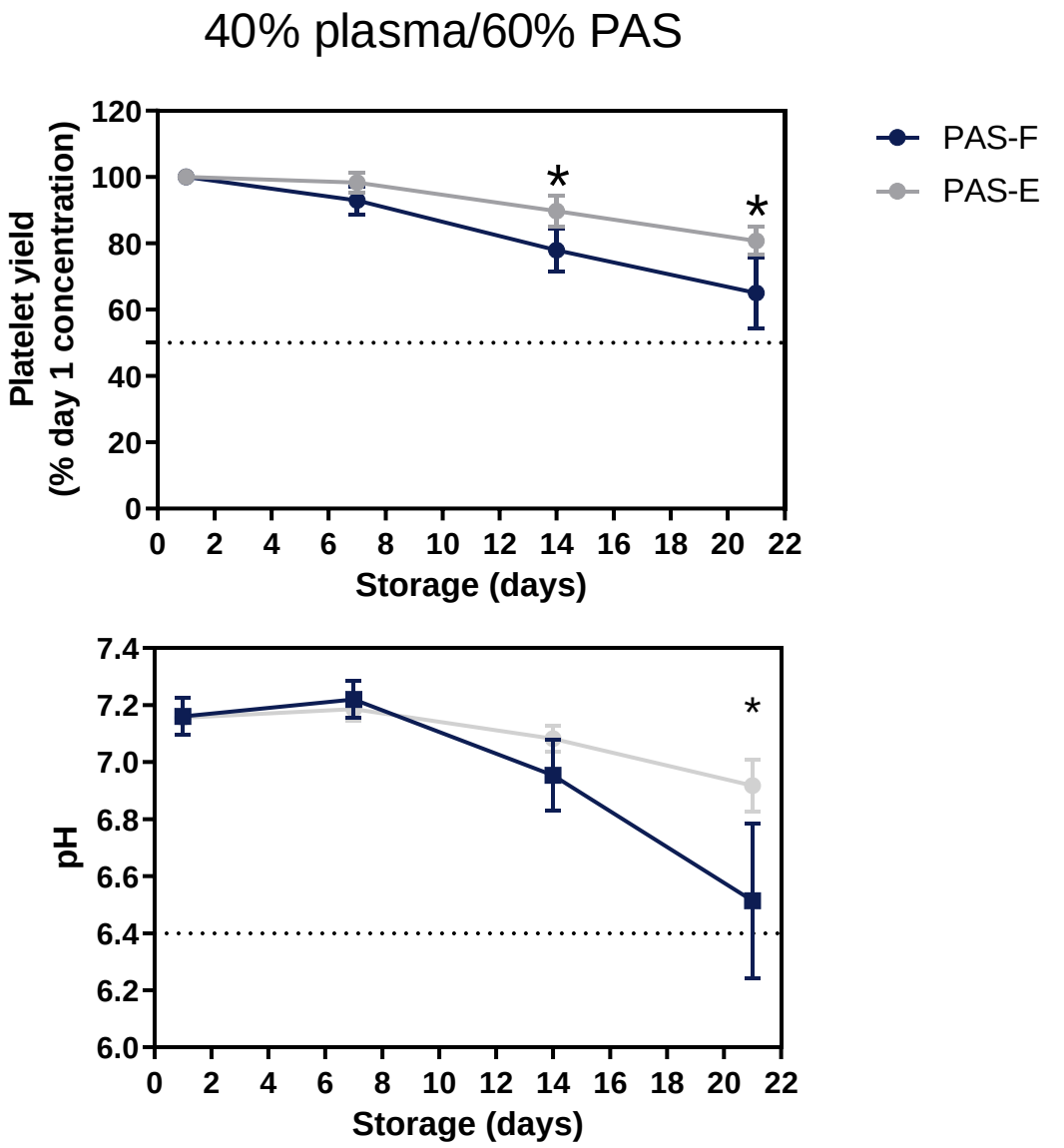
	RTP-PLASMA	RTP-PAS	CSP-PLASMA	CSP-PAS
1	1234 ± 552	1372 ± 614	1234 ± 552	1372 ± 614
5	1278 ± 572	1301 ± 582	993 ± 444*	1025 ± 458
10	1278 ± 544	1303 ± 583	745 ± 333*	1150 ± 514
15	1215 ± 543	1370 ± 613	807 ± 361*	1112 ± 497

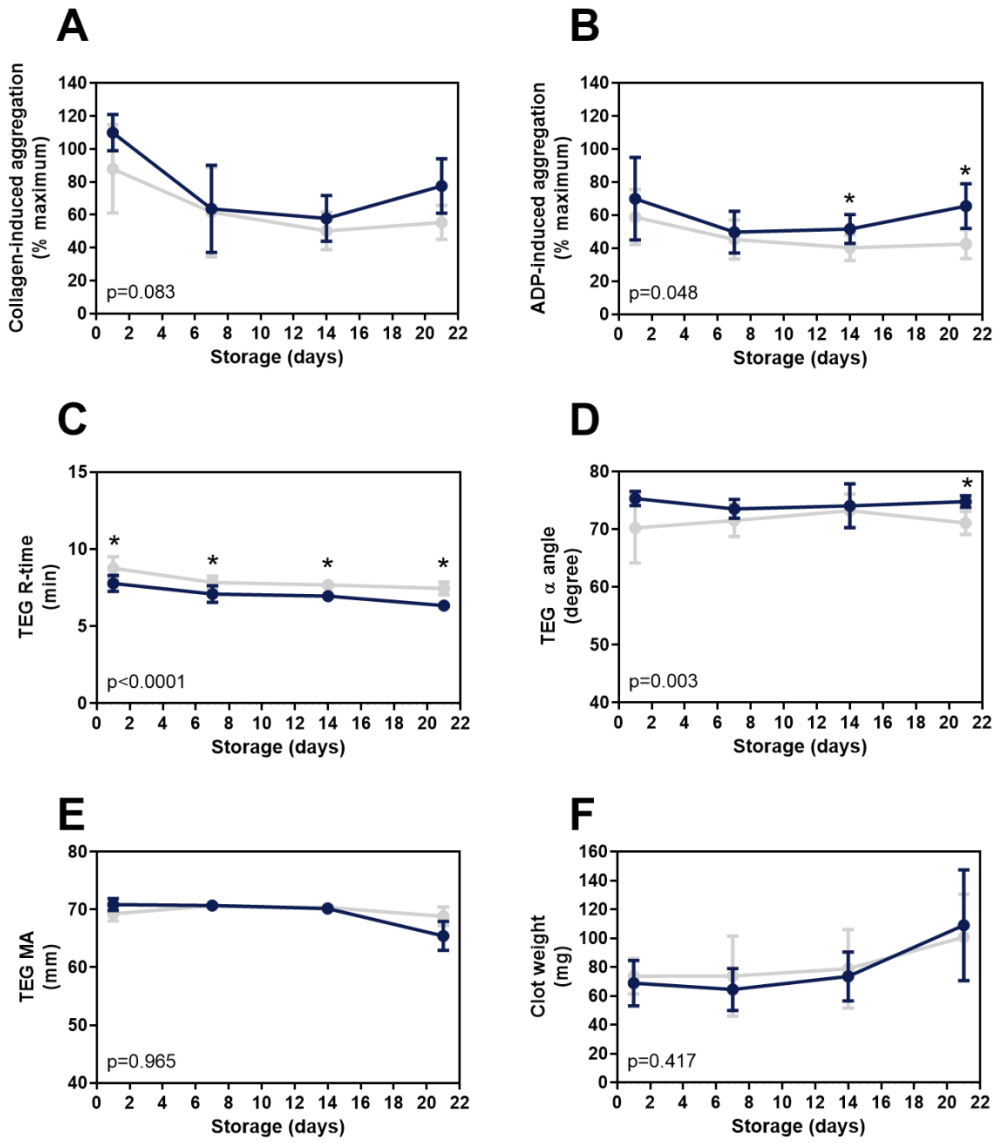
Storage of platelets at 4°C in platelet additive solutions prevents aggregate formation and preserves platelet functional responses

Todd M. Getz, Robbie K. Montgomery, James A. Bynum, James K. Aden, Heather F. Pidcoke, and Andrew P. Cap

Reddoch-Cardenas *et al*, Transfusion 2021; Getz *et al*., Transfusion 2016

Cold-storage in PAS-E (SSP+) vs. PAS-F (Isoplate)





Johnson *et al*., Transfusion 2023

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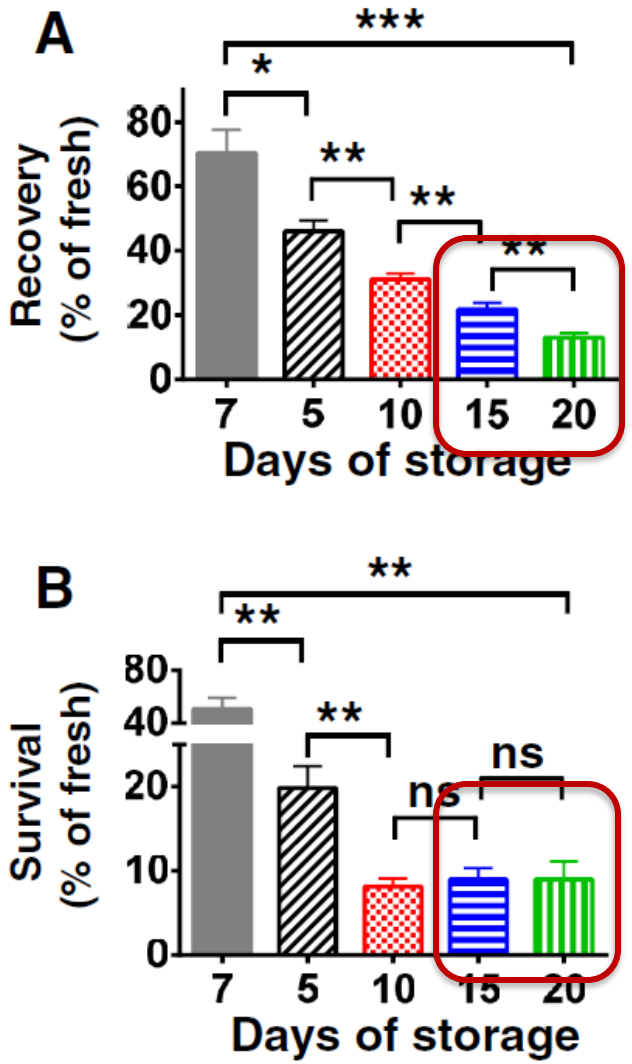
Knowledge gaps: what is the optimum shelf-life?



No internationally recognised quality control specifications
No recommended shelf-life

What is the maximum shelf-life that provides haemostatic efficacy?

Clinical trials to provide this evidence

Are they still clinically effective?

Figures from Stolla *et al*., Transfusion 2020

Clinical evidence is mounting

- Norway - Pilot trial in bleeding cardiac surgery patients
  - Cold stored platelets prevented bleeding following 7 and 14 days of cold-storage
  - No adverse events[1]
- Cold-stored platelets (delayed transfer to cold) used at Mayo Clinic during COVID-19 pandemic
  - Safe and provided effective haemostasis[2]
- CriSP-HS: cold-stored platelets in haemorrhagic shock[3]
  - Safe – no difference in VTE or AE
  - No difference in 24 hr mortality; no effect due to storage age
- CriSP-TBI: cold-stored platelets in traumatic brain injury[4]
- CHIPS trial: conventional versus cold-stored platelets
  - Will recruit 1000 adult and paediatric cardiac surgery patients
  - Storage up to 21 days[4,]

- + FDA guidance – 14 day shelf-life in USA[5]

1. Strandenes *et al*., Anesthesiology 2020; 2. Warner *et al*., Transfusion 2020; 3. Sperry *et al*., Ann Surg 2024; 280: 212-221; 4. www.clinicaltrials.gov 5. Zantek *et al*., Clin Trials 2023; 5.. US FDA Guidance for Industry release June 2023.

Cryopreserved platelets

Not new technology

- Technology developed in 1970s for US Navy – Dr Robert Valeri[1,2]

- Used in civilian or military settings in Netherlands, Australia, Czech Republic, France, Singapore and others[3,4, 5,6]

Advantages

- Shelf-life of up to 12 years at - 80 °C[7]

Disadvantages

- More expensive – DMSO cost
- Thawing time

1. Valeri *et al*., Blood 1974; 2. Valeri *et al*., Tranfusion 2005; 3.; 4. Lelkens *et al*., 2006; 5. Noorman *et al.,* Plos ONE 2016;
5. Bondar *et al*., Transfusion 2014 (AABB abstract); 6. Cohn *et al.*, Vox Sanguinis 2017; 7. Noorman *et al*., Transfusion 2013

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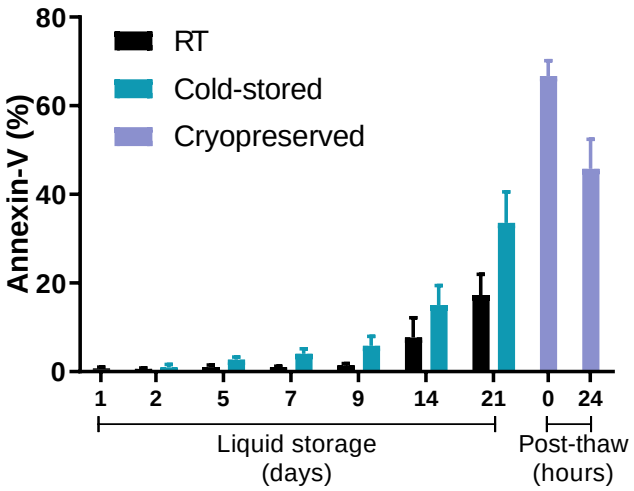
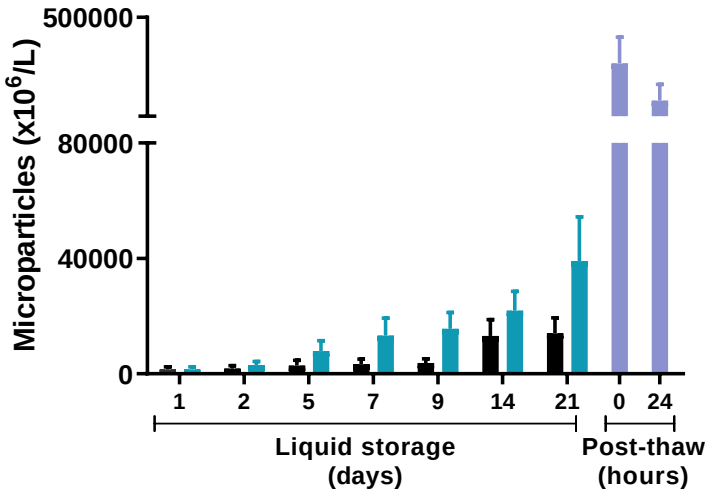
Increased haemostatic potential

Multiple changes due to freeze-thaw damage

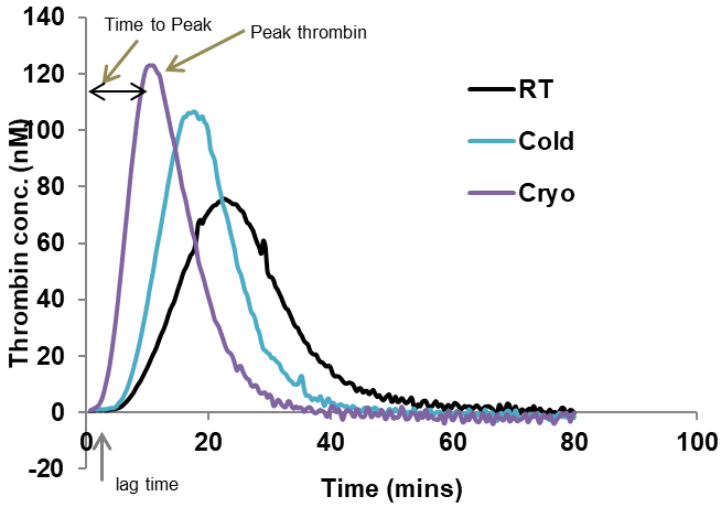
↑ PS externalisation



↑ Microparticle generation



↑ Thrombin generation



Adapted from Wood *et al*., Transfusion 2016; Johnson *et al*, Transfusion 2018; Tohidi-Esfahani *et al*., Vox Sanguinis 2017 (ISBT abstract)

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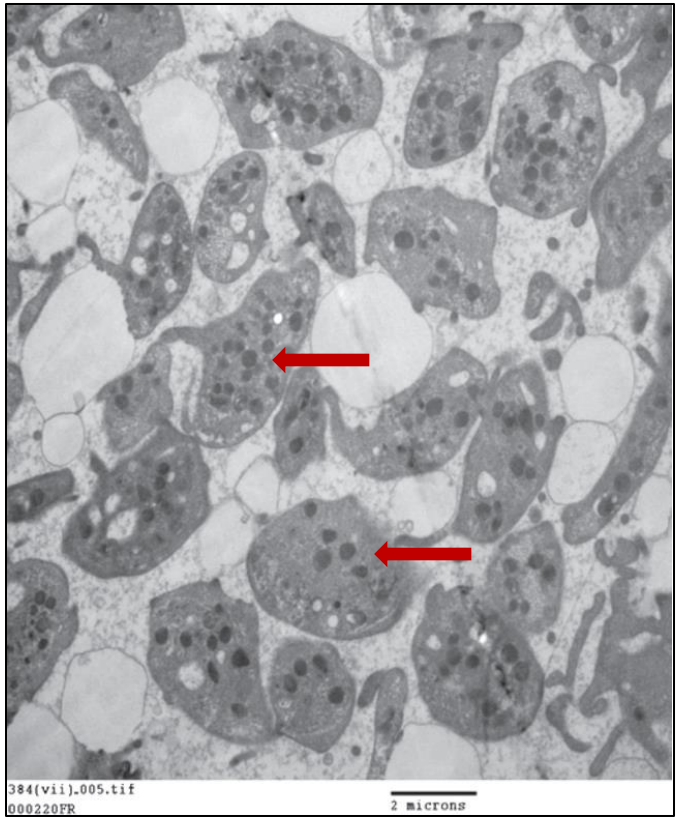
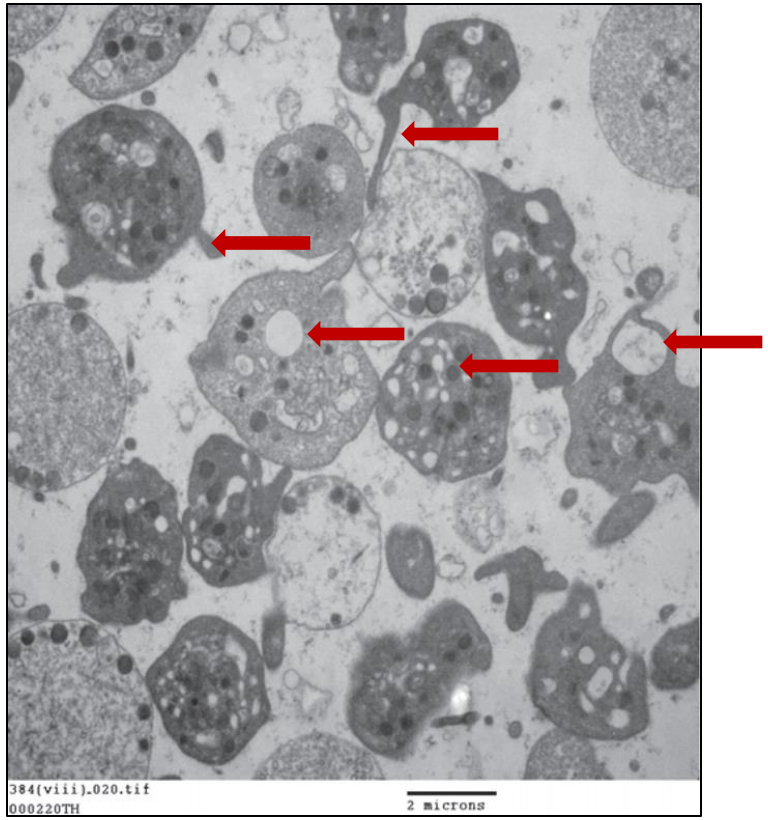
Platelet damage following thawing

Fresh platelets



Thawed platelets



From Dumont *et al.*, Transfusion 2013; 53: 128-137.

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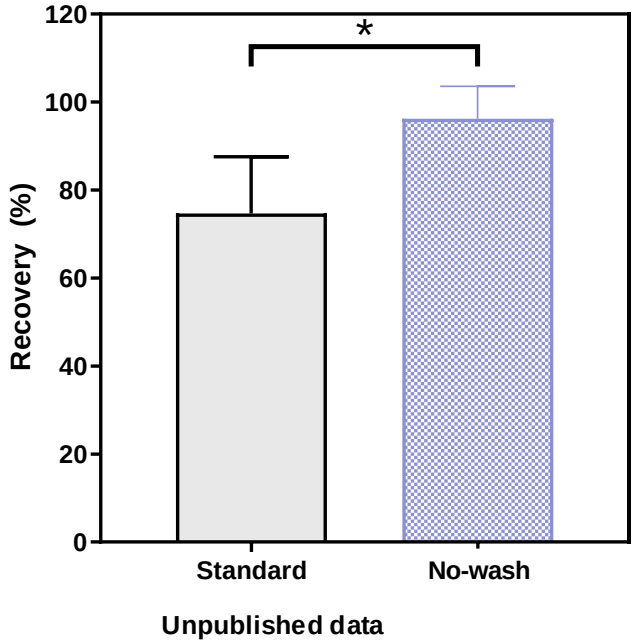
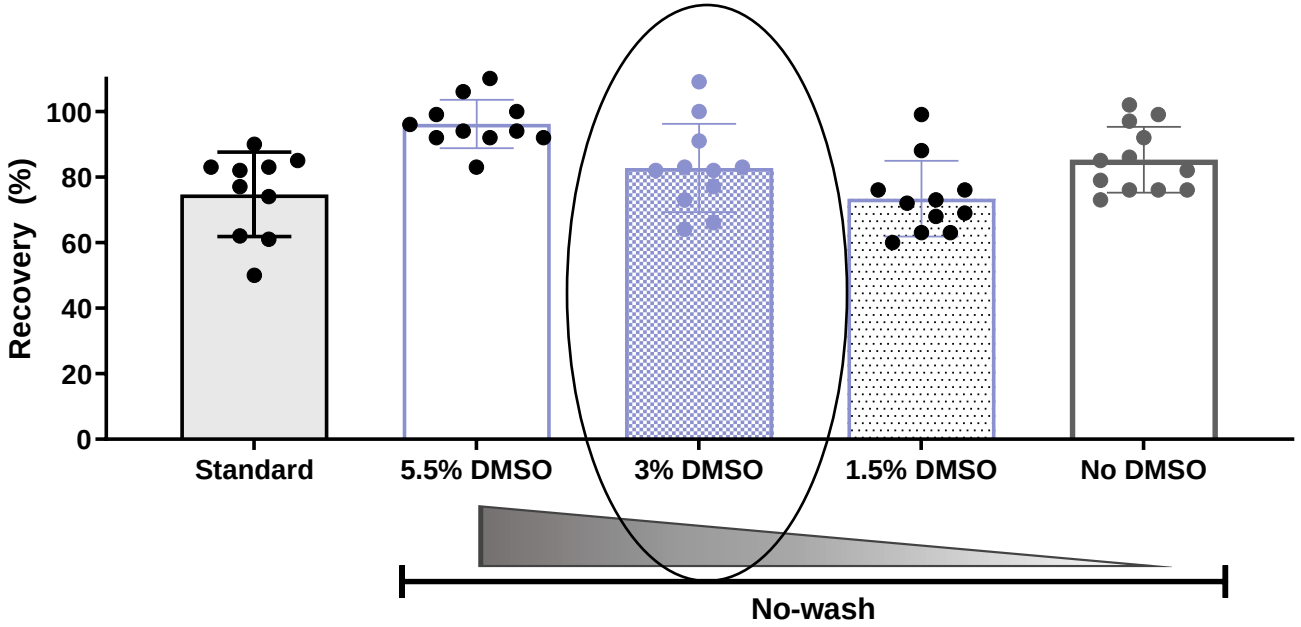
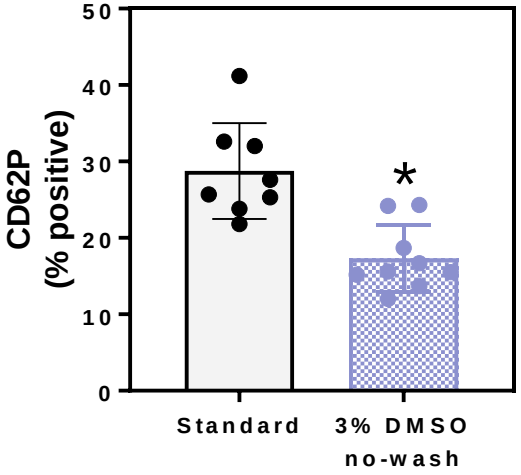
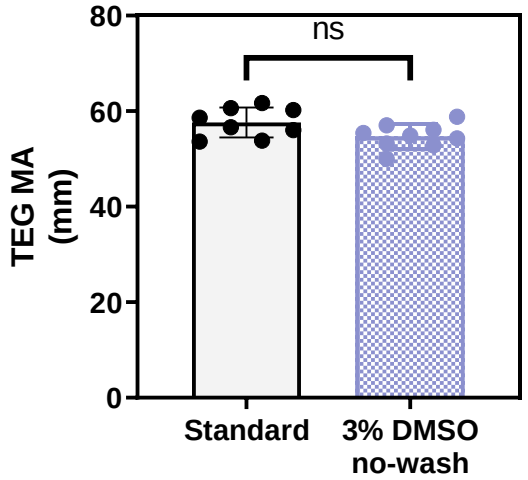
Preventing platelet damage

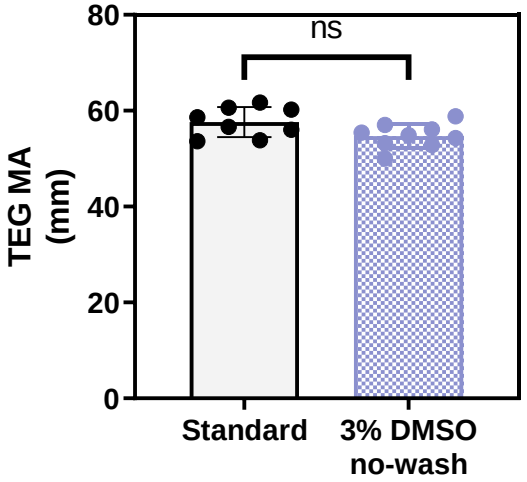


Unpublished data









Other approaches

Deep eutectic solvents

- e.g. proline-glycerol[1]
- Non-toxic

DMSO-free cryopreservation[2]

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1. Johnson *et al.*, Cryobiology 2024; 116:104913; 2. Ehn *et al.,* Int J Mol Sci 2023; 24:13097

Clinical evidence – in practice

University of Maryland

- > 1600 autologous cryopreserved platelets supported thrombocytopenic chemotherapy patients over 10 year period for times of shortage[1]
- Platelet increments achieved
- An effective way to create an inventory of frozen platelets

Dutch military

- Platelets prepared in advance to meet future transfusion needs
- 1043 cryopreserved platelets transfused in Afghanistan with no adverse effects[2]

Transfusion: -80˚C Frozen Blood Products Are Safe and Effective in Military Casualty Care

Femke Noorman[1☯‡*], Thijs T. C. F. van Dongen[2☯‡*], Marie-Christine J. Plat[3], John F. Badloe[1], John R. Hess[4], Rigo Hoencamp[5]

- Fewer patients died after introduction of a massive transfusion protocol - more patients received frozen platelets[2]

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1. Schiffer *et al*., Prog Clin Biol Res 1982; 2. Noorman *et al.,* Plos ONE 2016

Randomised clinical trials

Cryopreserved platelets effective for bleeding thrombocytopaenic patients

TABLE 3. Improvements in posttransfusion bleeding

	Pretransfusion WHO bleeding grade			
Transfusion dose	Grade 2*	Grade 3*	Grade 4*	Total
0.5-unit CPP	1/1 (100%)	2/4 (50%)		3/5 (60%)
1-unit CPP	2/4 (50%)	1/1 (100%)	1/2 (50%)	4/7 (57%)
2-unit CPP	2/3 (66%)	1/1 (100%)	1/2 (50%)	4/6 (66%)
3-unit CPP	2/3 (66%)		1/3 (33%)	3/6 (50%)
Total	7/11 (64%)	4/6 (66%)	3/7 (43%)	14/24 (58%)
1-unit LSP	1/3 (33%)	1/1 (100%)		2/4 (50%)

* Overall, in 14 of 24 (58.3%) CPP-transfused patients, bleeding improved including three of seven (43%) patients with CNS bleeding.

Shlicter *et al.*, Transfusion 2018

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Randomised clinical trials

- CLIP: Cryopreserved vs. conventional platelets in bleeding cardiac surgery patients
  - Pilot RCT, 41 patients
  - Safe and effective[1]
- CLIP-NZ: Similar outcomes[2]

TABLE 3. Efficacy outcomes: hemostasis

Outcome	Cryopreserved (n = 23)*	Liquid (n = 18)*	Difference (95% CI)	p value
Estimated OR blood loss (mL)	925 (650–1175)	900 (675–1037)	25 (–323.3 to 373.3)	0.82
Blood in drains on ICU admission (mL)	140 (65–180)	110 (40–170)	30 (–46.5 to 106.5)	0.56
Blood in drains at 24 hr (mL)	715 (540–915)	805 (591–1080)	–90 (–343.8 to 163.8)	0.41
Blood in drains at 48 hr (mL)	980 (680–1215)	1075 (810–1540)	–95 (–476.0 to 286.0)	0.45
Requirement to return to OR for bleeding on Day 1	2 (8.7)	3 (16.7)	–7.97 (–28.68 to 12.74)	0.64
Requirement to return to OR for bleeding on Days 1-3	5 (21.7)	8 (44.4)	–22.71 (–51.18 to 5.77)	0.18
BARC4 bleeding[24]	7 (30.4)	10 (55.6)	–25.12 (–54.80 to 4.55)	0.10
Day postoperative aspirin commenced	2 (2-3)	2 (2-3)	0 (–0.71 to 0.71)	0.76
Day postoperative prophylactic heparin commenced	2 (2-2)	2 (2-3)	0 (–0.5 to 0.5)	0.41

* Data are expressed as median (IQR) or number (%).

BARC4 = Bleeding Academic Research Consortium criteria for significant postoperative hemorrhage in cardiac surgery, which requires one or more of: intracranial hemorrhage <48 hours postoperatively; reoperation after sternotomy closure for the purpose of controlling bleeding; transfusion of ≥5 units whole blood or RBCs within 48 hours postoperatively; or chest tube output ≥2 L within 24 hours postoperatively.[24]

1. Reade *et al.*, Transfusion 2018; 2. McGuinness *et al.*, Vox Sang 2022

Randomised clinical trials

Larger trials

CLIP-II:

- Phase III: Cryopreserved vs. conventional platelets in bleeding cardiac surgery patients
- Australia and New Zealand[1]
- Australian arm completed (202 patients)
- Results will be presented at AABB on Saturday 19 October: AM24-ST-22-L; 1.45-2.45pm

- CRYPTICS: Phase II/III cardiac bypass: USA[2]
- MAFOD: Massive transfusion: Netherlands[3]

1. Reade *et al*., BMJ Open 2022; 2. Whitman *et al*., JTCVS Open 2023; 3. Rijnhout *et al*., Trials 2023

Summary

- Cold stored and cryopreserved platelets:
  - Extend platelet shelf-life
  - Enhanced haemostatic efficacy that suggests they may be better at stemming bleeding
  - Clinical trials will provide evidence of efficacy and shelf-life

→ The right product at the right time for bleeding patients

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Acknowledgements

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Our cool-platelet team

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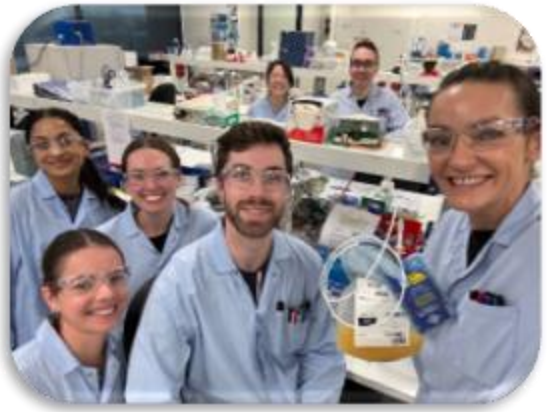
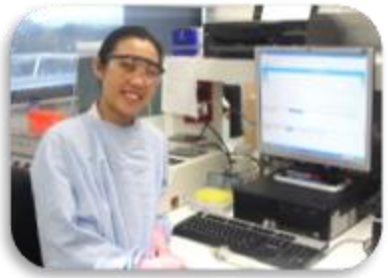
Chris Roan



CLIP – Cryopreserved vs. Liquid Platelets for surgical bleeding

Professor Michael Reade



Shereen Tan
Clinical trial co-ordinator

CHIPS

Noemi Bondar

Dr Anastazia Keegan

Prof Phil Spinella

CLIP-II is funded by the National Health and Medical Research Council of Australia;
Australian governments fund Australian Red Cross Lifeblood to provide blood, blood products and services to the Australian community.

Australian Government
National Health and Medical Research Council

NHMRC