Irish Blood Transfusion Service
Seirbhís Fuilaistriúcháin na hÉireann

Introducing platelet function testing to standard quality assessment

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Disclosure

No conflict of interest to declare

Scientific Support & Component Development

SSCD

Blood product Development

Validation Studies

Improve Blood Production Methods

Enhance Blood Quality

Increase Transfusion Efficacy

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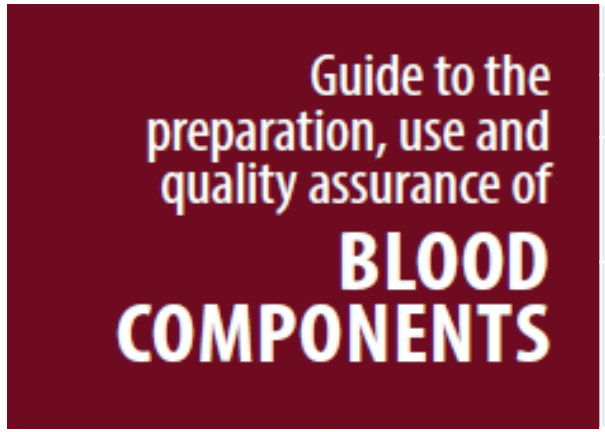
Platelets Quality And Function

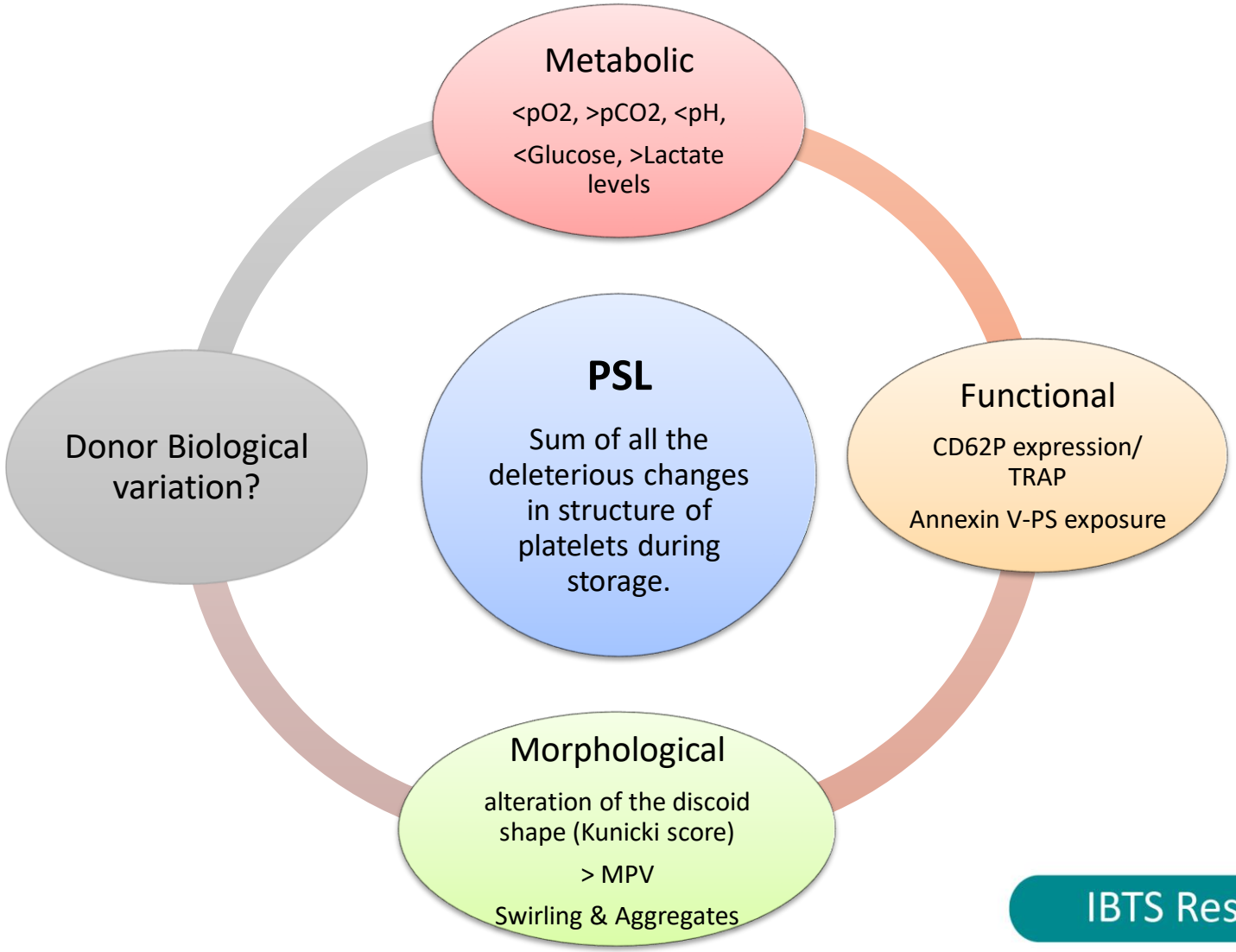
- Routine quality assessment and validation of Platelet Concentrates only includes basic parameters .
- Additional parameters are needed to properly assess the impact of the blood component preparation and storage on platelets.
- Including functional marker could improve platelets supply, functionalities and potentially predict the efficacy of a platelet transfusion.
- Consideration of donor variables may benefit platelets during storage.



Parameter to be checked	Requirements	Frequency of control
Platelet content per final unit	≥ 2×10^{11}	as determined by SPC
Residual leucocyte content per final unit	< 1×10^{6}	as determined by SPC
Glucose measured at the end of the recommended shelf-life b, or pH > 6.4	Above Limit of Quantification (LoQ)	as determined by SPC

EDQM
21st Edition
2023

Platelet quality and functional assessment (Platelets Storage Lesion)



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IBTS Research & Development

Storage studies assessing platelet functionality in SSCD

Cold Stored Platelets

- Renewed interest in cold stored platelets for use in actively bleeding patients
- Reduction in bacterial proliferation, reduced platelet metabolism, extended shelf life, reducing waste, and improving hemostasis in acutely bleeding patients.
- FDA licensed in the US and Norway for certain indications for 14 days
- Potential to improve logistics of clinical supply of platelets where access is currently limited (pre-hospital care; the battlefield).
- New research assessing functional and quality profiles and the impact on those of donor-related variation are needed for the widespread use of cold stored platelets and their intended use.

Cold Stored Platelets

ORIGINAL ARTICLE

Vox Sanguinis ISBT International Society of Blood Transfusion

The impact of donor biological variation on the quality and function of cold-stored platelets

Alice Lorusso[1] | Harry Croxon[1] | Sarah Faherty-O'Donnell[1] | Stephen Field[1,2] | Áine Fitzpatrick[1] | Aileen Farrelly[1] | Tor Hervig[1] | Allison Waters[1,3]

Aims

- To characterize the effect of storage at both 22°C and 4°C for up to 8 days, on the quality and function of platelets
- To investigate if the differences observed between CSP and RTP were also linked to donor biological variation.

Study Design

Paired study, 10 double apheresis doses in 100% plasma split equally in 2 groups: cold stored with no agitation and RT with agitation. Testing on day 1, 4, 6 and at expiry.

Pathogen Reduction

Background

- The INTERCEPT device uses amotosalen (a photoactive compound) and low energy ultraviolet (UVA) illumination to photo- chemically treat platelet components.

- Lowering the amount of potentially harmful agents in blood products to prevent Transfusion Transmitted Infections (TTI). This includes some known and unknown pathogens.

Aims

- Validate the preparation and storage of pooled buffy-coat platelets and apheresis platelets pathogens reduced with the INTERCEPT Blood System (IBS) at the Irish Blood Transfusion Service (IBTS).

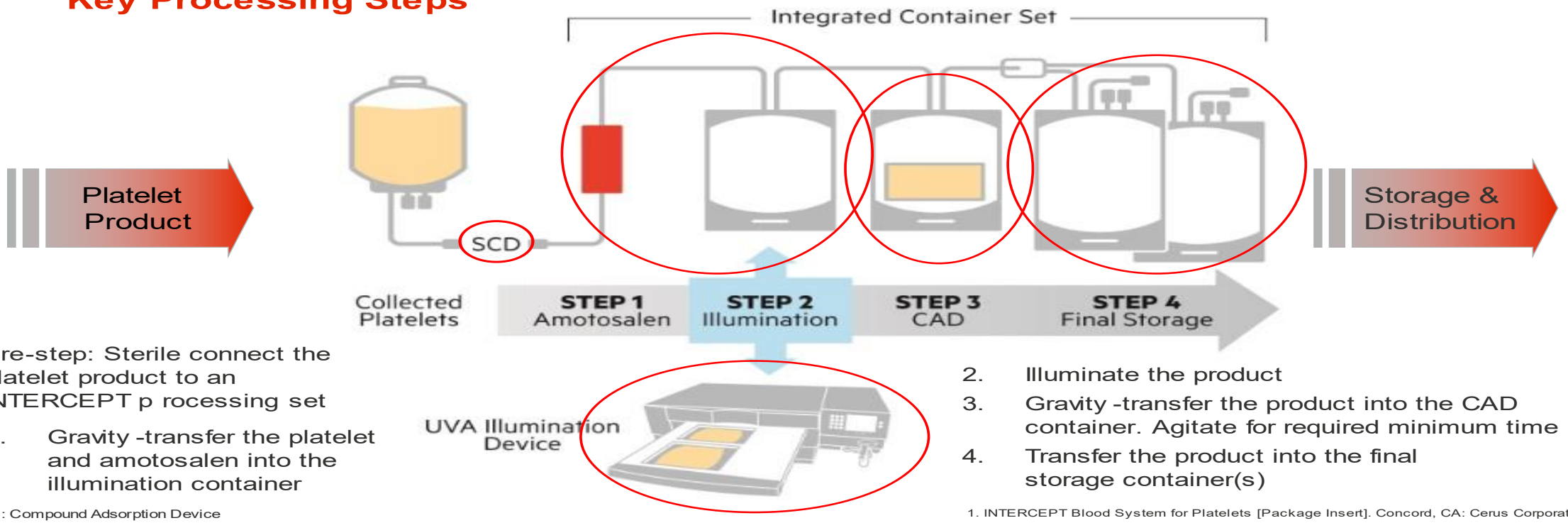
- Investigate the impact of the illumination process on the quality and function of platelets throughout 7 days of storage and at the expiry.

Study Design

- Paired Study: “Pool and Split” method , 70 buffy coats used, 5 DD pool splits to the Test group (intercept treated) and 5 DD pool split to the control group (not treated). Testing baseline, day 2, 6 and at expiry

Pathogen Reduction

INTERCEPT® Blood System Pathogen Reduction System for Platelets Key Processing Steps[1]



Pre-step: Sterile connect the platelet product to an INTERCEPT p rocessing set

1. Gravity -transfer the platelet and amotosalen into the illumination container
2. Illuminate the product
3. Gravity -transfer the product into the CAD container. Agitate for required minimum time
4. Transfer the product into the final storage container(s)

*CAD: Compound Adsorption Device

1. INTERCEPT Blood System for Platelets [Package Insert]. Concord, CA: Cerus Corporation; 2

MKT-EN 0050501 v1.0

cerus

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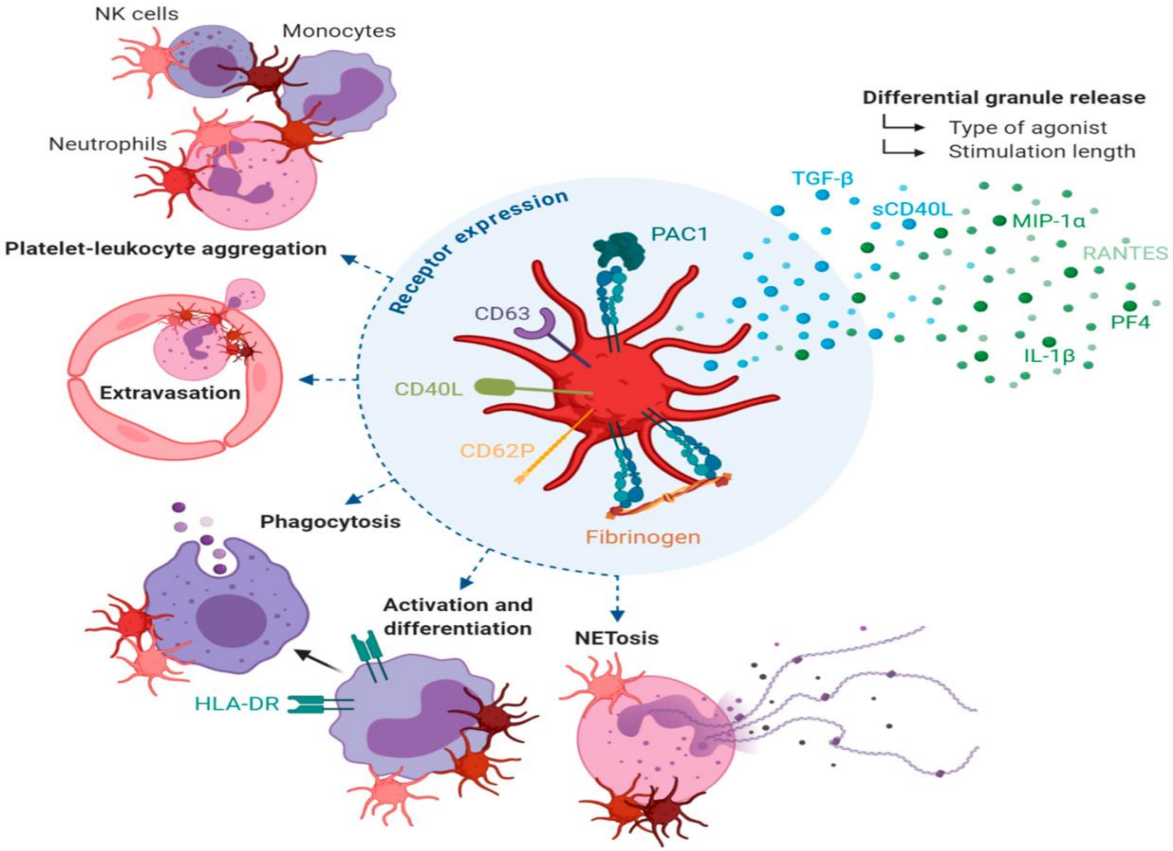
IBTS Research & Development

Functional assessment by flow cytometry
TRAP Agonist-induced CD62P expression

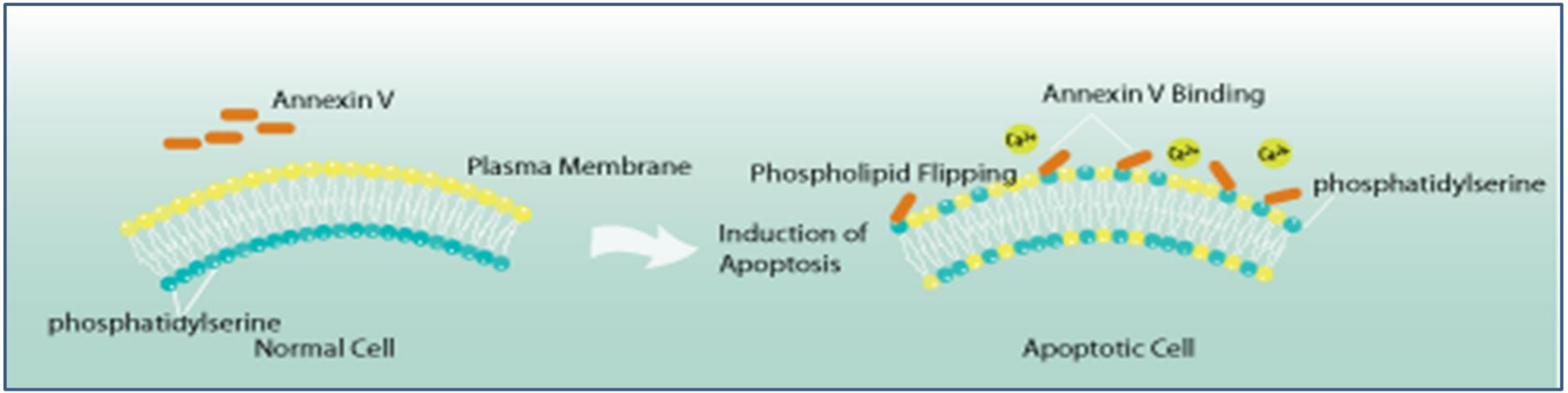
- P-selectin CD62P most abundant platelets activation marker release of the alpha-granule content.
- Advanced flow cytometry targeting PLTs CD62P expression without-stimulation to investigate the impact of storage temperature/ Pathogen reduction on the activation response.
- Thrombin key enzyme in the maintenance of normal hemostatic function, and important Platelets agonist.
- *In-vitro h*emostatic functionality estimated based on the proportion of platelets expressing CD62P in the presence of TRAP(Thrombin-receptor Activated Protein) agonist.



Flowcytometry

Annexin V and Phosphatidylserine (PS) exposure analysis

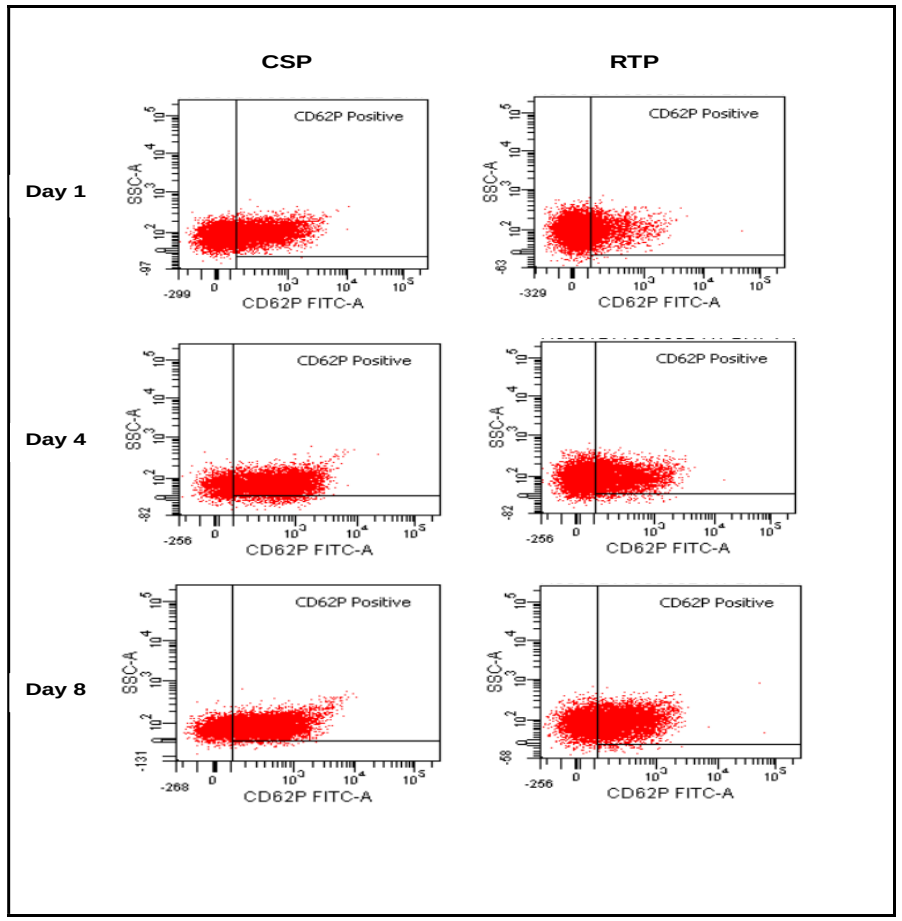
- PS exposure is believed to contribute to the development of inflammatory processes and regulates clearance of platelet from circulation and is marker of pre-apoptosis and pro-coagulant activity.
- In activated PLT elevated cytosolic Ca2+ causes the redistribution of phospholipids and PS exposure.
- In the presence of calcium, intracellular calcium-binding proteins Annexin V binds to PS.
- Apoptosis quantified by flow-cytometry detecting PS-Annexin V binding.
- Pro-coagulant activity estimated based on the proportion of platelets expressing PS-Annexin V binding of the non-physiological agonist Ca2+ ionophores.



Cold Stored Platelets
Results |QUALITY

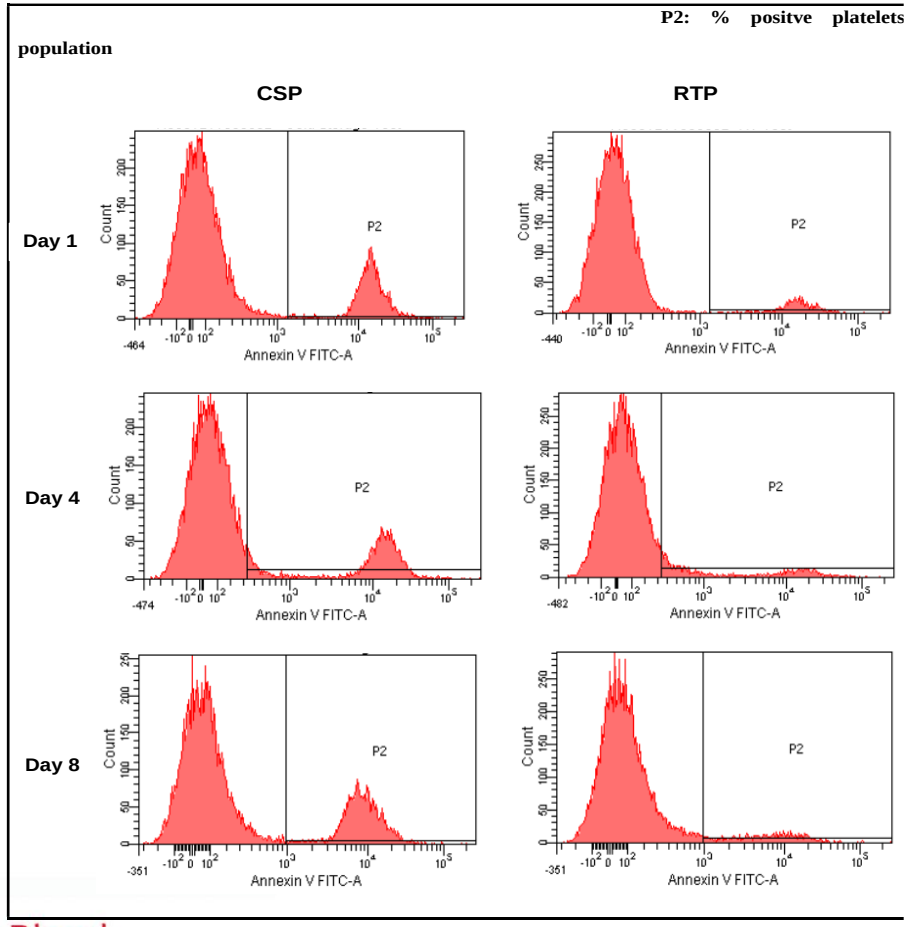
- Significant drop in platelet concentration in CSP days 4-8 of storage
- Comparable oxygen levels indicating adequate gas exchange in both agitated RTP and non-agitated CSP
- pH levels >7.0
- Significantly higher Glucose levels in CSP ($p<0.05$), indicating decreased metabolic activity reflecting platelet shelf-life extension beyond 7 days at 4°C.
- Negative bacteria growth investigations for both RTP and CSP.

Cold Stored Platelets
Results | Haemostatic Response



- Significantly higher (50% VS 25%) CD62P expression in unstimulated CSP, indicating greater activation and PSL compared to RTP.
- Despite their increased activation marker levels, CSP have been shown to be responsive to TRAP and thus retain the ability for hemostatic control.
- Comparable responsiveness to TRAP, indicating both CSP and RTP are capable of haemostatic response beyond 7 days of storage.

Cold Stored Platelets
Results | PS exposure



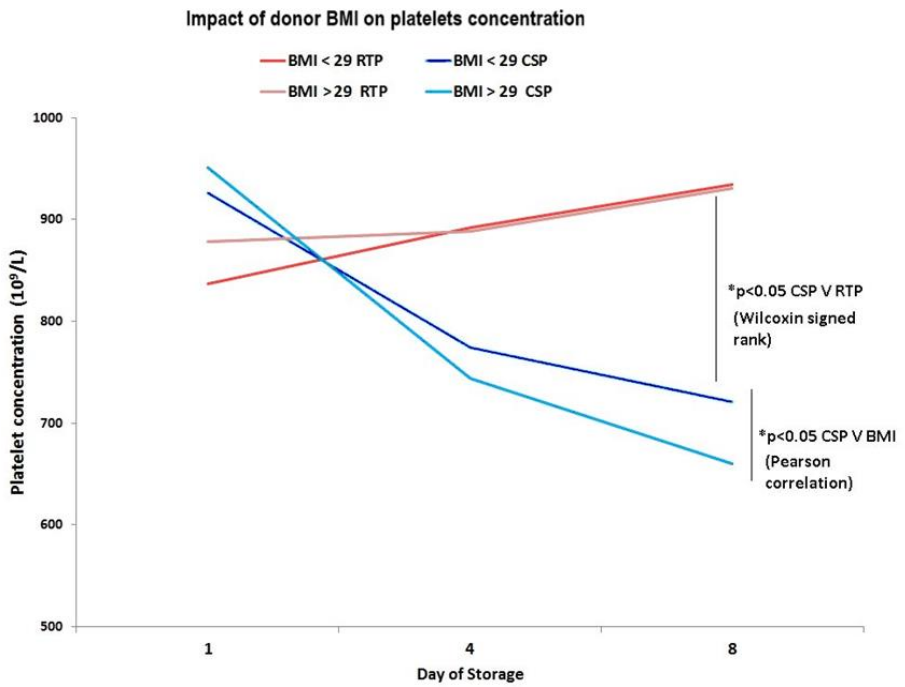
- The (%) of platelets with Annexin V-PS was calculated as an indicator of platelet apoptosis and activation
- Greater PS exposure detected in CSP indicating platelet apoptosis and activation, as a results of stronger cold-induced PSL.
- Stimulation with agonist (calcium) resulted in comparable levels of PS exposure in this study and a high level of responsiveness.

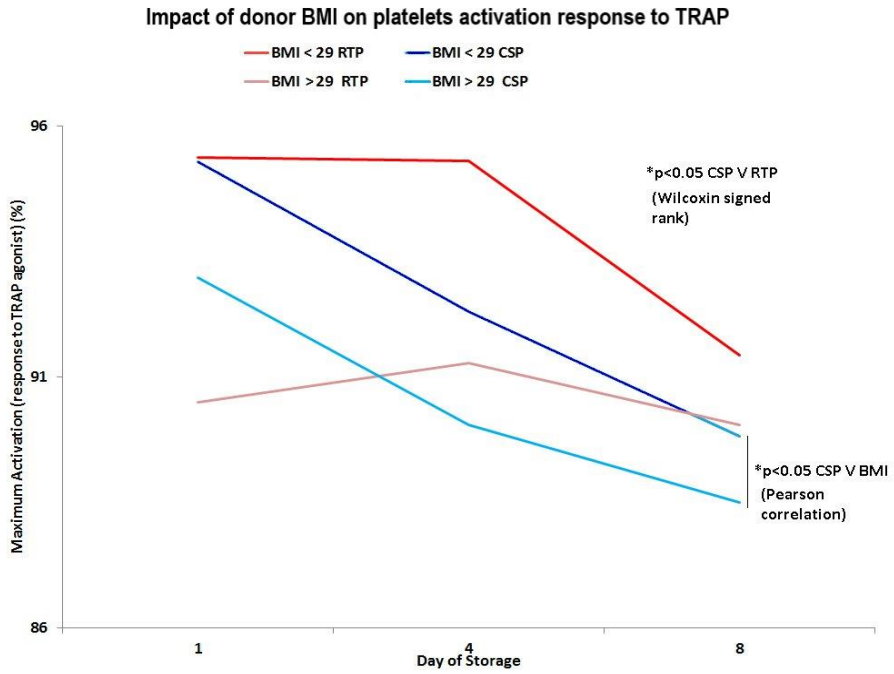
RESULTS | DONOR BMI

Donor demographic	N	Mean	Median	Maximum	Minimum
Gender—male	10				
Blood group—O Rh+	10				
Age (years)		49.0 ± 9	49.0	63.0	37.0
Body mass index (kg/m^2)		29.5 ± 3.8	29.0	37.1	24.5
Underweight <18.5	0				
Normal weight <18.5–24.9	1				
Overweight 25–29	5				
Obese ≥30	4				
Platelet count (>200 × 10^9 cells)		253.0 ± 39	253.0	324.0	197.0
Mean platelet volume (fL)		10.0 ± 0.6	10.0	10.8	9.0

Donors had an average BMI of 29.5 ± 3.8 and were categorized in BMI≤29 (n=6) and BMI > 29 (n=4).

RESULTS | DONOR BMI





Donor BMI differently, and significantly, impacted platelet concentration and Responsiveness to TRAP, depending on storage temperature.

Pathogen Reduced Platelets
Results |QUALITY

- Lower Platelet concentration in PR platelets from day 2 of storage onwards, both PR and control arm met the required specification for platelet concentration (≥ 35 ml per $60x10^9$ of platelets) on all days tested.
- Both groups of platelets presented, stable MPV, no aggregates and visible swirling throughout 8 days of storage, with better swirling in PR platelets.
- Partial Pressure: There is minimal difference between the control arm and the PR platelets for pCO_2 and pO_2.
- Glucose was detectable on day 8 in both groups and slightly higher in pathogen reduced platelets.
- The pH was very stable throughout the shelf life.

Table 1. Analysis between INTERCEPT treated and untreated platelet quality parameters throughout 8 days of storage

	Variables	Days of storage	PR-Pools Intercept Treated Mean Value	Control Pools Untreated Mean Value
Quality Parameters	PLTs Concentration (10^9/L)	Baseline	1278	1272
		Day 2	1230	1272
		Day 6	1176	1285
		Day 8	1143	1238
	MPV	Baseline	10.1	10.2
		Day 2	10.2	10.1
		Day 6	10.1	10.1
		Day 8	10.1	10.0
Metabolic	Glucose	Baseline	9.0	9.0
		Day 2	8.1	7.2
		Day 6	4.7	4.0
		Day 8	2.2	1.7
Biochemical	PH (37°C) (kPa)	Baseline	7.1	7.1
		Day 2	7.1	7.1
		Day 6	7.1	7.2
		Day 8	7.1	7.1
	pCO2 (37°C) (kPa)	Baseline	4.3	4.1
		Day 2	4.0	3.9
		Day 6	2.1	2.2
		Day 8	1.9	2.1
	pO2	Baseline	15.6	16.4
		Day 2	17.3	21.6
		Day 6	21.8	21.2
		Day 8	19.9	19.0

Pathogen Reduced Platelets
Results | Haemostatic Response

- Cell surface levels of CD62P expression are comparable for both treated and untreated samples (47% vs 44.3% at expiry)
- Comparable responsiveness to TRAP, indicating that PR platelets are capable of haemostatic response beyond 7 days of storage.
- Overall, only a low to moderate loss of PLT in vitro function compared to conventional PLTs, occurred gradually during 7 days of PLT storage.

		Pathogen Reduced Mean value	Control Arm (Not Pathogen Reduced) Mean Value
CD62P expression (%)	Baseline	27.1	29.1
	Day 2	30.5	32.9
	Day 6	42.2	39.4
	Day 8	47.1	44.3
Responsiveness to Trap (%)	Baseline	83.5	87.9
	Day 2	83.9	89.2
	Day 6	83.1	87.9
	Day 8	86.2	89.1

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Pathogen Reduced Platelets
Results | PS exposure

- PS exposure levels are comparable in this study, but gradually increased toward the end of storage in PR PLT.
- Stimulation with agonist (calcium) resulted in comparable levels of PS exposure in this study and a high level of responsiveness.

		Pathogen Reduced Mean value	Control Arm (Not Pathogen Reduced) Mean Value
PS exposure (%)	Baseline	2.2	2.5
	Day 2	2.6	2.5
	Day 6	8.3	6.7
	Day 8	11.1	8.4
Agonist induced-PS exposure (%)	Baseline	99.4	99.3
	Day 2	99.5	99.5
	Day 6	99.7	99.8
	Day 8	99.6	99.8

Conclusions

- CSP demonstrated viability and haemostatic superiority in vitro, despite the greater PSL compared to RTP.
- New set of quality markers are required to effectively assess CSP products.
- High BMI ranges in western male donors might reduce platelet concentration and activation response in CSP.
- Potential inclusion of BMI as a novel quality assessment measure may improve and standardize the quality and function of CSP.
- A larger and diverse donor cohort study is required to corroborate these findings.
- Pathogen Reduction with INTERCEPT did not have a greater impact on platelet quality and functional parameters when compared to non-treated platelets. But further statistical analysis is required from a research/publication perspective.
- Limited established parameters for comparison when assessing platelet functionality in storage study results.
- Functional Markers should be included as part of standard platelets quality assessment. Functional specification will help to provide platelets of better quality with lower levels of lesions.

Further Work

- Use of the functional assays in platelet-derived extracellular vesicles PhD Project
- Pathogen Reduction paper publication
- More data to enable guideline development to inform in vitro platelet function specification
- Are cold-stored platelets desired in Ireland?
- Is there a mechanism to introduce a product like this in Ireland, in the absence of an EDQM product monograph?

Thank you!

'We are continuously grateful for the contribution of our donors, and their donations, to our services, and the lives of the patients they directly benefit.'

I would also like to express my appreciation to Harry Croxon, the apheresis clinic, the SSCD team, Barry Doyle, Allison Waters, and the QC team for their help during the planning, performing and testing of this research work.

WE IMPROVE PATIENTS LIVES, TOGETHER

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