

Storage of platelets: PAS or plasma?

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Blood and Beyond



I have nothing to disclose.



Platelet production in NL, 2016

Donations:

420,163 whole blood donations 306,402 apheresis donations (mainly plasma)

Platelet concentrates:

- 56,000 buffy coat pools
- About 40% in PAS, 60% in plasma
- 4,000 apheresis units (7%)
 - HLA matched for refractory patients
 - HPA 1a-neg for pediatric use
 - in case of shortage of pooled PC

Sanquin Blood Supply Platelet additive solution

A balanced electrolyte solution that sustains platelet storage

Originally developed to

- remove plasma as source of proteolytic and glycolytic enzymes;
- prevent platelet storage lesion;
- supplement buffering capacity of plasma; maintain pH>6.0.



Sanquin Blood Supply Platelet additive solution

Additional benefits

- More plasma for transfusion/fractionation
- Standardized composition
- Sterile, pathogen-free
- Ability to control storage environment
- Lower ABO titer
- Allow for photochemical inactivation of pathogens
- Less protein fewer allergic reactions



International Council for Commonality in Blood Banking Automation

	PAS-A	PAS-B	PAS-C	PAS-D	PAS-E	PAS-F	PAS-G
			Trade name				
_	"PAS"	PAS-2	PAS-3	Composo	PAS-IIIM	Plasma-	M-Sol
		PAS-II	PAS-III		SSP+	Lyte A	
		T-Sol	InterSol			Isoplate	
		SSP					
Citrate	Х	Х	Х	Х	Х		Х
Phosphate	e X		Х		Х		Х
Acetate		Х	Х	Х	Х	Х	Х
Magnesiur	n			Х	Х	Х	Х
Potassium	Х		\ /	Х	X	Х	Х
Gluconate				Х	\land	Х	
Glucose							Х



• Stored at room temperature, shaking (60 rpm)



Platelet storage lesion

(elevated) glucose consumption

lowering of pH

lactate production

platelet activation

Sanquin Blood Supply Platelet storage lesion

All things "bad" happening to a platelet

- increased activation and metabolism
- increased signals for removal from circulation
- poorer response to stimuli
- reduced adhesion
- ...

In the patient:

- lower recovery, shorter survival
- less able to stop or prevent bleeding



Composition of PASs used to better maintain platelet quality during storage



Citrate Acetate Potassium Magnesium Phosphate Bicarbonate Calcium Glucose

Sanquin Blood Supply Platelet metabolism

Platelets are extremely metabolically active cells:

- Oxygen consumption rate = $3 \mu mol/10^{10}$ cells/h
- 6 times as fast as resting muscle; 30% as fast as mammalian brain

Platelets (inefficiently) use glucose to make ATP

Glucose consumption

- <2%: full oxidation 0.05±0.01 µmol/10¹⁰ platelets/h (net yield ~30 ATP)
- >98%: into lactate 3.1±0.4 µmol/10¹⁰ platelets/h (net yield 2 ATP)

• Challenge for PAS: lactate production, maintain neutral pH Guppy Vox Sang 1990;59:146



Modulation of PLT metabolism: Acetate

PASs need to provide an alternative substrate that can readily be metabolized by platelets

Serendipity: Acetate was present in infusion fluids that were used in the early PAS studies

Lactate production

- No acetate: 2.4±0.5 µmol/10⁹ platelets/day
- 23 mM acetate (+K/Mg): 1.3±0.3 µmol/10⁹ platelets/day
- All modern PASs contain acetate

Shimizu& Murphy Transfusion 1993;33:304



Modulation of PLT metabolism: K⁺ and Mg²⁺

Day 7	рН	%CD62P+
PAS-B	6.98±0.07	49±10
PAS-B + Mg ²⁺	7.10±0.07*	41±14
PAS-B PAS-B + K ⁺	6.93±0.04 7.19±0.03*	55±6 35±8*
Plasma PAS-B PAS-B + Mg ²⁺ + K ⁺	7.03±0.06 6.94±0.05* 7.15±0.10*	35±8 50±8* 23±6*

1.5 mM Mg²⁺, 4.5 mM K⁺; *p<0.05



Comparison of current PASs

- Paired study design: pool and split buffy coats
- Add: plasma or PAS-C (citrate) or PAS-E (citrate, K⁺, Mg²⁺)
- Centrifuge, make platelet concentrate
- Storage in the same storage containers for 8 days
- In vitro analysis

Lower glycolytic activity and better maintenance of pH in PAS-E



Sanquin Blood Supply Sanquin Blood Supply Lower PLT activation in PAS-E





- PC in PAS: 65% PAS / 35% plasma (carryover from BC)
- Plasma only source for glucose
- With "old" PAS at least ±30% plasma needed to preserve structural integrity



- 1. PLT day 1: typical resting spindle form without any signs of damage or activation
- 2. Day 8 days in plasma: 20% PLT damaged, α -granules in contact with plasma membrane
- 3. Day 8 in 80% PAS-B: nearly all PLT swollen or lysed

Klinger, Vox Sang 1996;71:13

Sanquin Blood Supply Protein or glucose needed?

- Pool and split 2 platelet concentrates
- Add 10% ACD, centrifuge, remove all supernatant
- Unit A: PAS-E +12 mM glucose

16.1±2.7 mM

14.6±3.3 mM

- Unit B: 35% plasma/65% PAS-E
- Store for 8 days
- Various in vitro measures
- n=3

Sanquin Blood Supply Percent residual plasma





- No difference between 100% PAS with added glucose versus 65% PAS/35% plasma
- Therefore, the plasma carryover was necessitated by the glucose requirement; protein is not essential
- Work in progress: use of "dry" BCs to make PCs with 15-20% plasma



	Tx, n	Total, %	Allergic	Others (incl FNTR)	Solution
Oksanen, 1994	23	78%	52%	26%	plasma
	86	23%	19%	5	PAS-A
Bertolini, 1989	448	1.4%	0.9%	0.5%	plasma
	129	0%	0	0	PAS-F
De Wildt-Eggen, 2000	192	12%	5.2%	7.2%	plasma
	132	5.3%	0	5.3%	PAS-B
Kerkhoffs, 2006	311	5.5%	n.r.	n.r.	plasma
	373	2.3%	n.r.	n.r.	PAS-B
Andreu, 2007 BC	1275	n.r.	0.16%	0.08%	plasma
	8206	n.r.	0.02%	0.15%	PAS-B
Apheresis	25698	n.r.	0.60%	0.16%	plasma
	3525	n.r.	0.31%	0.14%	PAS-B



Good recovery and survival of PAS-stored platelets

n	Source	Solution	Storage	Recovery	Survival,
			days	%	days
6	Apheresis	plasma	5	59±7	6.5±0.6
10	Apheresis	plasma	7	44±5	4.9±0.7
6	Apheresis	PAS-F	5	59±5	6.3±0.8
10	Apheresis	PAS-F	7	52±3	6.0±0.3
4	Apheresis	PAS-F	9	55±5	6.6±0.6
10	Apheresis	PAS-F	13	49±3	4.6±0.3
10	Apheresis	PAS-F	14	43±3	4.2±0.5



Over the past decades, numerous PASs have been developed. Some were good, some not so good.

Acetate partially replaces glucose consumption, limiting lactate formation and thereby the platelet storage lesion.

Various modifications have been done to further optimize platelet quality, notably the addition of **potassium** and **magnesium**.

With the 'newer' PASs, *in vitro* quality of platelets is not worse than when stored in plasma, probably even better.



Lower protein content gives fewer allergic transfusion reactions; further optimization possible?

Recovery, survival as well as CCI are good if not better for platelets in PAS than in plasma.



Storage of platelets: PAS or plasma?

With the current generation of PAS, because

- storage quality (judged *in vitro* and *in vivo*) is at least as good;
- allergic reactions are fewer

the use of PAS for the storage of platelet concentrates should be preferred.

Sanquin 2018: all platelet concentrates in PAS-E



Product and process development

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Thank you for your attention



Effect of K⁺/Mg²⁺

Potassium

maintaining membrane potential

Magnesium

- decreases the PLT activation
- activates potassium pumps
- influences influx of calcium, thereby intracellular potassium concentration
- inhibits agonist-induced PLT aggregation, by changing membrane fluidity and/or by triggering of cAMP