AABB Annual Meeting Education Program 2014

October 25-28, 2014 | Pennsylvania Convention Center | Philadelphia, PA

Presentation Handouts

(9127-TC-HEM) PAS the Platelets, Please: The Data Behind Platelet Additive Solution

October 25, 2014 ✧ 4:00 PM - 5:30 PM

Advancing Transfusion and Cellular Therapies Worldwide

Event Faculty List

Event Title: **(9127-TC-HEM)** PAS the Platelets, Please: The Data Behind Platelet Additive Solution
Event Date: October 25, 2014
Event Time: 4:00 PM - 5:30 PM

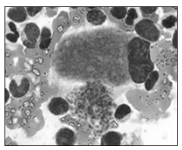
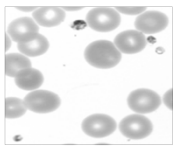
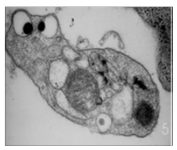
Director/Moderator
William Savage, MD, PhD
Associate Medical Director, Transfusion Medicine
Brigham and Women's Hospital
wjsavage@partners.org
Disclosure: Yes

Speaker
Andrew Heaton, MD
Senior Director, BB and Transfusion Service
North Shore-LIJ Health System
WHeaton@NSHS.edu
Disclosure: Did not disclose

Speaker
Aaron Tobian, MD, PhD
Associate Professor of Pathology
Johns Hopkins University
atobian1@jhmi.edu
Disclosure: Did not disclose

Speaker
Pieter van der Meer, PhD
Senior Scientist
Sanquin Blood Bank
p.vandermeer@sanquin.nl
Disclosure: Yes

aaBB Workshop: Session 9127-TC

PAS: for Improved PC Quality, Longevity, & Utility:
Current development directions: Implications & Options

NOVO – 7 USE IN CARDIAC SURGERY

Andrew Heaton, MD

Prof. of Pathology, Hofstra NSLIJ School of Medicine
Senior Director of Transfusion Medicine, NSLIJHS.

Saturday 4th October 2014 16:00 – 17:30

The views expressed are those of the presenter and do not represent NSLIJ Health System

North Shore LIJ
North Shore-Long Island Jewish Health System

HOFSTRA NORTH SHORE-LIJ SCHOOL of MEDICINE AT HOFSTRA UNIVERSITY

Disclosures

Andrew Heaton has:

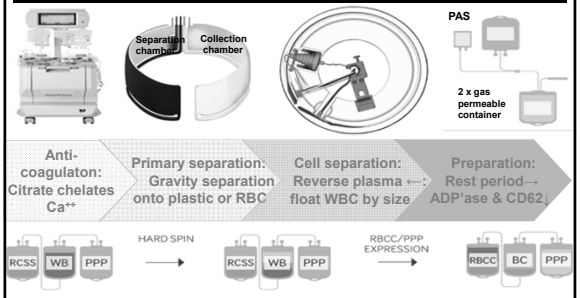
- Received research support from Verax, Haemonetics, Fresenius-Fenwal, Light Integra, & Immunetics.
- Honoraria from Verax, Haemonetics, Fresenius-Fenwal, TerumoBCT & Immunetics
- Consulted for Cerus, Verax, Novartis Diagnostics, and Beckman Coulter
- And is a member of the aaBB and ISBT Task Forces on Bacterial Assays.

PAS-PC Storage: Developments & Outcomes

Educational Objectives:

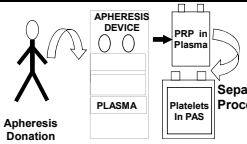
- PAS: Original goals and development history
  - Inventor's goals/critical elements → Generational Changes
- Storage Lesion: causes/offsetting effects of PAS formulation
  - Activation and Metabolic Effects
  - Membrane and ageing changes
  - Current developments leading the move → Glucose & HCO_3
- Platelet In-Vivo Outcomes: Critical Differentiators
  - Differences between first 3 generations:
    - Can we rely on in-vitro studies
    - Do ↓CCI's affect the inter-Tx interval
- PC washing need → reaction reduction
- Next steps

Automated Platelet Harvest & Implications



- Collection: Contact & tissue factor stimulation offset by ↓ pH & Ca^{++} removal
- Separation: Plastic surface impact, ↑ G force, cell/cell interaction, & pelleting
- Re-suspension: Disaggregation = rest & agitation followed by ↓surface CD62

PAS Inventors: Goals & Issues



Technology Variables :
- Buffer type = ↑ pH Vs. ↓ stimulation
- Energy need → Glucose/Acetate
- Plasma content ≈ nutrient/buffer
- Stimulation α HCO_3/buffer need

- Goals included plasma replacement & Prolonged storage of high quality SDP

Inventor	Date	Patent #	Key development	Implications
Rock **Adams**	8th May 1984	4,447,415	Platelet storage in a plasma free medium (Plasmalyte)	• plasma-free isotonic medium • initiated acetate/gluconate • later reissued
Holme **Heaton**	22nd Sep 1987	4,695,460 4,961,928	PSM of a physiologically compatible aqueous electrolyte solution	• HCO_3 buffered physiologic • included glucose…10 days • later modified for RBC also
Murphy **Bertolini**	9th May 1989	4,828,976	22°C Storage with low plasma in O_2 permeable container & electrolytes	• included salts K, Ca, & Mg • residual plasma load focus • acetate → energy substrate

3 Generations of Platelet Additive Solutions

	PAS - B (T-Sol)	PAS- C InterSol	PAS- D CompoSol	PAS- E SSP+
NaCl	116	77	90	69
KCl	-	-	5	5
$MgCl_2$	-	-	1.5	1.5
Na_3-Citrate	10	11	11	10
NaH_2/Na_2HPO_4	-	28	-	26
Na-acetate	**30**	**33**	**27**	**30**
Na-gluconate	-	-	23	-

Holme & Heaton. 7 day viability… **seven** days in a platelet additive solution. BJH 1987;66:233.
Holme &, Heaton. Platelet storage lesion… **Correlation with ATP** levels. Vox Sang 1987;53:214.
Holme, Bode & Heaton. Improved platelet viability medium with **inhibitors**. J Lab Clin Med 119:144:1992.
Bode, Holme, Heaton. **Extended** storage with **Prostaglandin E_1** and Theophylline. Vox Sang 1991;60:105-112
Murphy, Kagen, Holme, Heaton. Platelet storage… **no glucose/bicarbonate**. Transfusion 1991;31:16-20.
Holme. **Effect** of additive solutions on platelet **biochemistry**. Blood Cells 18:421-430, 1992.
Holme. Storage of PC in **plasma-free** media. In: Sibinga et al. Klüwer Acad. Press, Boston, MA, 1990,119-127.

Where next:PAS development- direction & drivers

Manufacturing Simplicity — **Manufacturable…Platelet Quality**

Anticoagulant salt & buffer	Platelet specific & pH sparing nutrient	Membrane protection & Metabolic stimulant	↑ Storage, ↓ Activation, ↑ Function

Improved platelet quality & less plasma

Early developments	Energy substrate support	↓ NA^+/K^+ pump & ↑ enzyme potentiation	Focus on: ↓ activation & membranes
• **Plasmalyte** • Available • Safe/Simple • **PSM-1** • Physiologic • Nutrient free	• **PAS-2** • Acetate (↓H^+) • Nutrient…ATP • ↓Glycolysis • **PAS-3** • ↑PO_4 glycolysis	• **PAS-IIIM/SSP+** • K^+/Mg^{++} ≈ membrane • Glycolytic enzyme ↑ • Citrate • **Composol** • Gluconate…stabilizer	• **PAS- 5 , F & G** ▪ Less plasma carry-over ▪ ↓ plasma = activation ▪ Glucose for ↑ storage ▪ ↑HCO_3 essential ▪ Membrane stabilization ▪ Ca^{++} for cytoplasm & membrane markers

In-Vivo/Vitro Correlations ≥5 Days @ 22 C

in O_2 Permeable Containers

In-Vitro Assay Correlation (r)	RDP in Plasma @ 5-14 days*	SDP in Plasma or PlasmaLyte @ 5-8 d^0	Plasma SDP & UVB @ 5 d^+
pH	0.56	—	0.88
ESC (%)	0.62	0.5	—
HSR (%)	0.66	0.53	0.65
Lactate	0.68	—	0.91
CD62/annexin	0.28/—	—/0.51	0.84/—

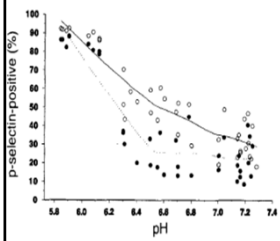
- In-vivo/vitro correlations offer insight into predictive capacity:
  - Metabolic measures are leading indicators
  - Biologic & membrane measures are trailing indicators
  - Correlation models should include PTR range from 10 – 80%
  - Inter-donor, storage, agitation, & activation affect outcomes

UVB = ultraviolet B light
ESC = extent of shape change
HSR = hypotonic shock response

* Holme. Vox Sang 59:12:1990
^{0} Slichter. Transf 50:2199:2010
\+ Goodrich. Vox Sang 90:279:2006

* RDP & PAS-G, ^{0}PAS-F , and $^+$UVB & Mirasol & UVB only.

P-selectin decrease & recovery post PC Storage



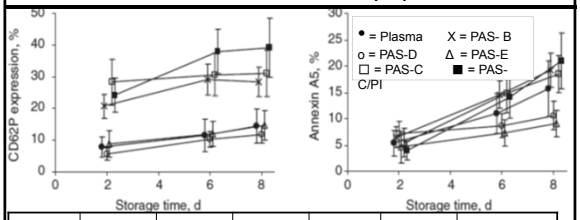
Condition	Number of studies	Percentage of platelets positive for p-selectin (mean ± SD): Direct measurement	After 1-hour incubation	After thrombin activation
Thrombin				
ACD	9	11 ± 5	3 ± 2	95 ± 5
EDTA	7	61 ± 8*	50 ± 17*	80 ± 3*
4°C	5	73 ± 9*	65 ± 18*	87 ± 2
22°C	5	32 ± 13	23 ± 12	88 ± 18
pH				
6.8-7.2	11	29 ± 10	21 ± 9	87 ± 3
6.2-6.8	16	51 ± 5†	25 ± 10	80 ± 10
5.8-6.2	9	90 ± 2†	84 ± 3†	90 ± 3

* $p<0.05$ by paired t test (EDTA vs. ACD, and 4°C vs. 22°C).
† $p<0.05$ by ANOVA and Duncan multiple range test (pH 6.2-6.8 and pH 5.8-6.2 vs. pH 6.8-7.2).

- CD62P increases during PC storage…mostly reversible
- Stimulation (as in traumatic collection or PI) increases this:
  - Partially reversible with pre-Tx incubation…but not if pH is < 6.2
  - Reduced if platelets are inhibited (PGE_1)
- P-selectin & recovery correlation: r = 0.28 & Survival r = 0.4

Holme. Transf 37:12:1997

Other Membrane Activation & Apoptotic Markers



	Plasma	PAS-B	PAS-D	PAS-E	PAS-C	PAS-C/PI
LacRate	0.13 ±.04	0.14 ±.02	0.10 ±.02	0.11 ±.03	0.17 ±.03	0.18 ±.04

- Annexin increased least with Plasma & low PO_4 PAS (D & E)
- Stimulation (as measured by CD62, and Lactate) ↑most:
  - In PAS-C and PAS-C plus Pathogen Inactivation
  - K^+ & Mg^{++} are associated with less activation

Van Der Meer. Vox Sang 98:517:2010

PAS Vs Reactions: Residual plasma effect

PAS & Study Size Study Design	Reactions RR: Cont Vs Test	Reference
• **PAS-B: N= 168 or 765 Tx** • **RCT: CCI & Reaction study**	5.5% Vs 2.4%	Kerkhoffs Blood.108:3210
• **PAS-C/PI: N = 278 or 1129 Tx** • **RCT: Reaction & CCI study**	11% Vs 9% Vs 7%	Kerkhoffs Brit J Haem 2010:May
• **PAS- C: N = 2605 or 14,005 Tx** • **Observational Non-inferiority**	1.37 Vs 0.55%	Cohn Transfusion 2014:54:epub
• **PAS- M-Sol: N = 12 or 432 Tx** • **Obs: PC 96% wash out study**	42% Vs 0.6%	Azuma Transfusion 2009: 49: 214

- PAS-B associated reactions were reduced ~ 50%
  - 24 hour PAS CI's = 16 ± 14... Vs 21 ± 16 for Plasma SDP
  - Inter Tx intervals = 2.1 ± 1 day Vs 2 ± 1 days for Plasma
- M-Sol resuspended PC reduced reactions > 90%
- Plasma ↓ to < 100mL decreased reactions from 5.5 to 1.7%*
- Observational report suggested < 20 mL plasma was desirable

Tobian. Transf 51: 676:2011

PAS Studies: learnings from earlier developments

Development Conclusions:

- Metabolic Storage Lesion: Multi-factorial
  - Platelet energy needs → Nutrient, PO_4, pH ↑ with↓glucose
  - Additives to support membranes (K^+) & metabolism (Mg^{++})
- In-vivo/vitro correlations:
  - Biologic measures correlated across ↑ ↑ changes in values
  - Membrane and apopototic markers → trailing indicators
  - Increasing pressure for ↓ plasma ≈ Glucose & HCO_3
- Early Studies offer insight into:
  - Residual plasma & relationship to ↓ transfusion reactions
  - Recognition that ↓plasma ↑ metabolic support burden

Future implications of the less obvious studies

Interactive Effect of Plasticizers & Separation Method:
- Acceleration of glycolysis…amelioration of stimulation ?
- Smaller cells or activation…effects beyond 5 day ?
- Platelet Inhibition Effect….potential for long term storage ?

Agitation effect on Content: Container Surface Areas
- PAS-PC interaction with container & agitation

Washing & Post Pathogen Inactivation implications:
- PI activation…offset by ↑ buffer, ↑ nutrient ?
- Plasma ↓ < 5%...requires glucose, acetate & HCO_3

Improved PAS Storage Options: Future Questions
- How much HCO_3 is needed
- Relationship of Acetate: Glucose ratio
- Potental for Ca^{++} to reduce apoptosis
- Effect of elutriation method, plasticizer, & low plasma level

Container Plasticizer Affects Long Term Storage

Assays (BTHC –PL 2209 Container Studies)	Storage Day 1 Control†	Storage Day 1 Test†	Storage Day 5 Control	Storage Day 5 Test
β-thromboglobulin release (%)	11.0 ± 7.0	14.8 ± 5.1	18.8 ± 7.9	24.6 ± 5.6
Percentage of osmotic recovery	51 ± 5	45 ± 9	57 ± 13	52 ± 12
Morphology score§	603 ± 39	572 ± 55	516 ± 61	527 ± 38
Lactate (mmol/L)	3.1 ± 0.5	3.9 ± 0.7	11.4 ± 2.3	14.8 ± 2.5
Glucose (mg/dL)	380 ± 25	376 ± 15	304 ± 38	286 ± 24

- Container permeability $K(O_2)$ α Plastic, Surface, & thickness
- Oxygen Consumption $C(O_2)$ α Plasticizer: BTHC > TOTM
- BTHC ↑ Lactate production rate with effect on PTR's

	Laboratory A†		Laboratory B‡		Laboratory C‡	
	PL-732§	PL-2209‖	PL-1240§	PL-2209	PL-732	PL-2209
Multiple-hit recovery (%)	38 ± 13	34 ± 17	36 ± 10	37 ± 11	51 ± 9	47 ± 10

Snyder. Transf 32: 736:1992

PAS-C, E, F & Experimental PAS In-vivo studies

- New FDA standard of simultaneous test/fresh using BEST SOP

In-Vivo - 5 day	PTR	Test/Control	Survival	Test/Control
PAS - C	46 %	81%	5.7 days	72%
PAS - F	54 %	87%	6.4 days	78%
PAS - E	37 %	54%	4.8 days	54%

- Slichter in-vivo PAS - SDP stored @ 80/20 PAS: Plasma ratio
  - Evaluated plasticizer, elutriation, & storage duration
  - BTHC Vs TOTM, surge Vs LRS, & 5 to 18 day storage

Integrated Effect of Separation, PAS, & BTHC

In-Vivo Value - 5 day	PAC-C	PAS-F	PAS-E
Recovery	46 %	54 %	37 %
Survival	5.7 days	6.4 days	4.8 days

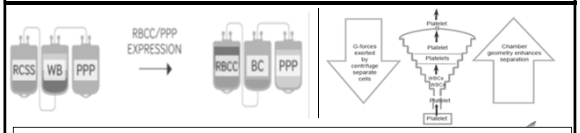
- Slichter evaluated Haemonetics MCS+ Vs Trima & Spectra:
  - Platelets were stored in CLX (PVC bags with TEHTM) and;
  - In CPP (BTHC) & CLX (TEHTM) with PAS:Plasma @ 80:20
  - Storage duration between 5 and 18 days at 22^{0}C
- Holme study used CLX, a PAS:Plasma @ 93:7, & PRP-PC

Study – PAS- (N)	Stored	Test	Survival	Container	Device
Slichter – F - (10)	9 Days	44%	5.0 days	PVC-TEHTM	Trima
Slichter - F - (8)	9 Days	29%	3.4 days	PVC-BTHC	Trima
*Holme – G - (6)	10 Days	34%	4.8 days	PVC-TEHTM	PRP-PC

- Long stored PAS - PC had acceptable recoveries
- BTHC was associated with ↓recoveries > 7 days

* Required HCO3 + 5% CO2 atmosphere. Slichter. Blood 123: 271: 2014 Holme. Vox Sang 59:12:1990

Leukodepletion → Smaller & More Activated Cells

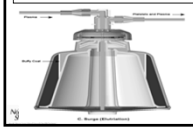


Apoptotic marker evaluation of 4BC-PC & Trima SDP (N=20):

Variable	Day 1	Day 5	Day 7
MPV (fL)			
AP-PCs	7.6 (±0.6)‡	7.9 (±0.6)†‡	8.1 (±0.7)†‡
BC-PCs	8.4 (±0.3)	8.5 (±0.3)	8.5 (±0.4)†

Albanyan. Transfusion 49: 108:2009

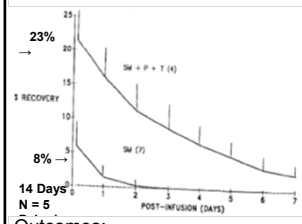
- SDP platelets smaller < BCPC (‡) & storage↑size → day 5(†)



Slichter (TEHTM)	Stored	Test	Survival
Surge elutriation	9 Days	55%	6.6 days
Platelet flotation	9 Days	44%	5.0 days

Relationships: PAS & ↓ Surface Area & Inhibitors

- 31 studies of RDP @ 22^{0}C in 60 mL of BSM (PAS-5) in PL 732
  - PC stored in normal Surface /Volume (7cm^2/L)or ~ ½ SV ratio (4cm^2/L)
  - Storage in PAS alone Vs PAS + 300nM/L PGE_1 & 1.9nm/L Theophylline



	SM (7)	SM + P + T (4)
pH	6.6 ± 0.3*	7.0 ± 0.2
O_2 consumption (nmol/10^{12} platelets)	0.6 ± 0.4*	1.0 ± 0.3
ATP (μmol/10^{11} platelets)	1.2 ± 1.1*	3.5 ± 0.6
Lactate (nmol/10^8 platelets)	37.7 ± 16.6*	18.7 ± 4.0
HSR (percent response)	15 ± 16*	44 ± 8

Outcomes:
- Platelet ATP levels α O_2 consumption rate & with GP 1b levels
- PTR's correlated with residual GP 1b (r=-0.91) & HSR (r =0.84)

Cell inhibition ↓metabolic loss & ↓ surface contact ≈ ↓ activation

Holme. J Lab Clin Med 119:144:1992

PAS-PC Washing: BRS-A Low Plasma Studies

- SDP diluted in 250 mL BRS-A & centrifuged @ 2650g x 10 min
- Manually separated pellet resuspended (> 30 min) in 200 mL
- Platelet wash recovery= 90 ±1 % with 2 ± 0.7% residual

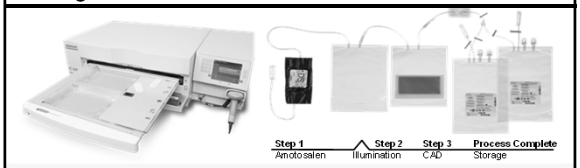
Oikawa. Transfusion 53:655:2013

Characteristic	Day 1	3	5	7
pH at 37°C				
Control	7.15 ± 0.05	7.09 ± 0.03	7.00 ± 0.05	6.84 ± 0.10
Test	7.24 ± 0.04†	7.18 ± 0.09†	7.31 ± 0.12†	7.50 ± 0.04†
Glucose (mmol/L)				
Control	22.21 ± 1.95	20.88 ± 1.97	19.23 ± 2.20	17.37 ± 1.51
Test	5.00 ± 0.72†	1.95 ± 0.86†	0.12 ± 0.14†	0.03 ± 0.01†
HSR (%)				
Control	69.7 ± 6.4	63.2 ± 3.7	58.6 ± 5.7	49.2 ± 4.4
Test	70.4 ± 3.5	75.5 ± 6.7†	64.5 ± 6.8	66.9 ± 4.1†

NaCl	95.2
KCl	3.8
$MgCl_2$	0.9
$NaHCO_3$	26.6
Glucose	5.8
Trisodium citrate	4.2
Citric acid	1.8
$CaCl_2$	1.4

- Study utilized sterile fill mixture
- Designed for wash + brief storage
- Glucose was absent after day 3
- In-vitro studies ≈ plasma controls
- HCO_3 & Glucose needed for ↑quality

Pathogen Reduction: PAS/Plasma Viral Inhibition

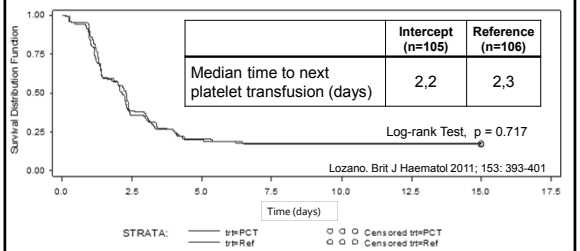


Amotosalen & UVA effective in ↓ bacterial contamination

Enveloped Viruses Reduction in Plasma & PAS	PC 35% Plasma	PC/PL 100% Plasma
Human immunodeficiency virus type (cell)	>6.1	>6.7
Hepatitis C virus (HCV)	>4.5	>4.5
BVDV (model for HCV)	>6.0	≥6.0 (≥5.4)
Hepatitis B virus (HBV)	>5.5	>4.5
Human T-cell lymphotropic virus type 1	4.7	≥4.5
West Nile virus (WNV)	>6.0	≥6.8
Chikungunya virus Company data	>6.4	≥7.6

Intercept®/PAS-C: 7 day storage clinical outcome

Time to Next Transfusion and post Tx increments similar with < 7 day PC



	Intercept (n=105)	Reference (n=106)
Median time to next platelet transfusion (days)	2,2	2,3

Log-rank Test, p = 0.717

Lozano. Brit J Haematol 2011; 153: 393-401

1 Hour Increments	Intercept® n =101	PAS-B, SSP, n = 98	p-value
Count increment (±SD)	19.4 ± 13.44	21.6 ± 14.56	0.27
Corrected count increment	8,163 ± 5,370	9,383 ± 5,905	0.007*

- Unpublished 7 day isotope data supports this clinical study

PAS-E & RiboFlavin (Mirasol®) Effect on Storage

- 15 In-vitro studies in SSP+ & Mirasol with Trima double SDP's
- M units 3.89 ± .3 x 10^{11}/ unit & Control units 3.95 ± .3 x 10^{11}/unit
  - Annexin V was ↑ from day # 1 together with ↑depolarization

Variable	Storage day 0*	1	5	7
pH value (at 22°C)				
C	7.31 ± 0.01	7.31 ± 0.03	7.40 ± 0.04	7.43 ± 0.05
M	7.31 ± 0.01	7.27 ± 0.04†	7.17 ± 0.09†	7.09 ± 0.09†
Glucose content (mmol/L)				
C	6.4 ± 0.5	5.8 ± 0.5	4.3 ± 0.5	3.5 ± 0.6
M	6.4 ± 0.5	5.6 ± 0.5†	2.5 ± 1.0†	0.6 ± 0.8†
Lactate content (mmol/L)				
C	1.3 ± 0.1	2.0 ± 0.4	5.2 ± 0.6	6.7 ± 0.9
M	1.3 ± 0.1	2.5 ± 0.4†	8.6 ± 1.7†	11.3 ± 1.7†
P-selectin (CD62P) expression (%)				
C	12.7 ± 7.1	17.6 ± 7.7	13.3 ± 5.2	18.5 ± 6.0
M	12.7 ± 7.1	31.2 ± 11.6†	43.9 ± 14.1†	57.0 ± 10.8†

- Metabolic studies: Glucose ↓ rate 0.3 Vs 0.15 mM/hr/10^{12} cells
  Lactate Rate ↑ 0.5 Vs 0.28 mM/hr/10^{12} cells

Mirasol Rx ↑metabolic stimulation @ acceptable limit

† = Sig Diff between M & C units

Picker. Transfusion 52:510:2012

Evolution of PAS & Next Steps

Early generation PAS studies showed:

- BTHC Plasticizer ↑metabolism: Δ ↑ nutrient requirement
- Elutriation can affect cell population & metabolic rates
- Pathogen inactivation support 7 day storage but↑energy need

Technology advances identified:

- Bacterial contamination was limiter to storage longevity
- Platelet storage lesion mostly metabolic:
  - O_2 levels, glucose, & acetate are required for 7 days
  - PO_4, K^+, Mg^{++} are important
  - ↓ stimulation separation & 5th gen PAS may offset PI effect

Fifth Generation/Current Studies include:

- Interactive effects of elutriation, plasticizer, & formulation
- Effects of lower residual plasma

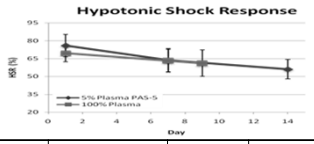
4th/5th Gen Platelet Additive Solutions

(mM/L)	BRS - A	M- Sol	PAS - F	PAS -5/G
Na/Cl	95	77	98	110
$KCl/CaCl_2$	3.8/1.4	3/1	5	5/1
$MgCl_2$	0.9	1.6	3/1.5	3
Na_3Cit/Citric Acid	1.8/4.2	14	-	10 - 7.5/2.5
NaH_2PO_4/Na_2HPO_4	-	-	1	**4**
Na-acetate	-	21	27	30/15
Na-gluconate/Glucose	**-/5.8**	-/**15**	23/-	**17/30**
BiCarbonate (HCO_3)	**26.6**	**44**		**10-20/18**

Additive Solution Generations			
• PAS C (PAS-3)	2009	PO_4 enhanced acetate solution
• PAS F	2013	Addition of K^+ and MG^{++}
• PAS V/G/M-Sol	2010-?	Glucose nutrient + HCO_3 buffer

5th Generation PAS-V and PAS-G In Vivo Studies

PAS-5: 5% plasma
- Invitro 20mM HCO_3
- Invivo 10mM HCO_3
  - 95% PAS-5



*In-Vivo - 5 day**	PTR	Test/Control	Survival	Test/Control
PAS – 5 (N =6)	54%	74%	150 hrs	75%

PAS-G @ Day 7
- Glucose only
- Solution + pellet
  - 80% PAS-G
- HCO_3 18mM

^{111}In In- Vivo Viability Day 7*	Plasma	PAS-G
Recovery (%)	56.3	58.0
Survival (hrs)	141.0	159.4

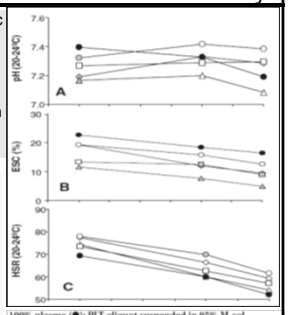
Radwanski, Wagner. Transfusion 51 Supp: 2011 * Company communication

Ca++ with/without Citrate in M-Sol: In-vitro Study

- M-Sol + Ca^{++} 1mM or NaCit or + CitAc
  - 9.4 mM Citrate/4.8mM Citric Acid
  - 15mM Glucose & 44mM HCO_3^-
- Formulation → local available product
- Developed for low (**5%**) plasma option
- 10 Studies in PL 732 & 2209 plastics

- ↑ Day 7 Lactate with BTHC
- Citric Acid offered no advantage
  - Ca^{++} equivalent: pH, ESC, CD62
  - BTHC/M-Sol α ↓HCO_3, ↑CD62
    - Less in plasma

Δ in a Ca^{++} enhanced M-Sol:
(incl: Glucose, acetate, HCO_3,≠ PO_4)
- **HSR/% discoid cell > Plasma**

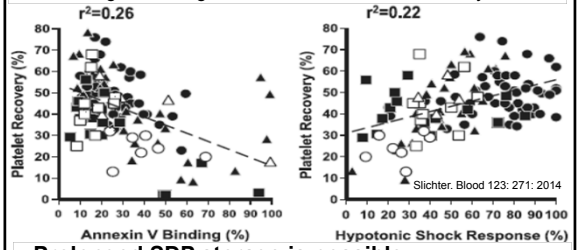


100% plasma (●); PLT aliquot suspended in 95% M-sol without citric acid, with 5% plasma (□); PLT aliquot suspended in 95% M-sol, with 5% plasma (○); PLT aliquot suspended in 95% M-sol, without calcium and citric acid, with 5% plasma (□); PLT aliquot suspended in 95% M-sol, without calcium, with 5% plasma (Δ).

Wagner Transf 50::1028:2010

Separation, Plastics, & Storage on PAS-F PC

Plasmalyte stored platelets in CLX, ELP, & CPP containers:
Range of storage duration between 5 and 18 days

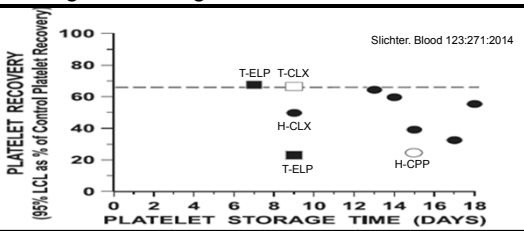


Slichter. Blood 123: 271: 2014

- **Prolonged SDP storage is possible**:
- Consistent in-vitro/vivo relationship across wide range
  - PTR ↓ by ~ 2.3%/day
  - PTR = 31% + 0.5*HSR – 0.25*Annexin V (α 48% variance)

Slichter. Transf 50: 2199: 2010

Storage Advantages of Elutriation & Plasticizer



Slichter. Blood 123:271:2014

9 Day @ 22^{0}C	TEHTM		BTHC	
Devices (N)	MCS+ (4)	Trima (10)	Trima (8)	Spectra (5)
Recoveries %	55%	44%	*29%	*24%
Survivals days	^{0}6.6	5.0	3.4	3.2

- N = 83: MCS+ & Trima in TEHTM Vs. BTHC…< 7 d comparable
- Graph ≈ relative PTR @ % of control ^{0} = Sig Diff to any T-ELP. * = Sig Diff to H-CLX

PAS-5 Low Plasma Can Support 7 Day Storage

10 mM HCO_3
N = 10

Sample	Trima: Total protein (mg/ml)	Trima: % Plasma removed	Amicus: Total protein (mg/ml)	Amicus: % Plasma removed
100% Plasma	61·6 ± 6·4	–	61·5 ± 8·0	–
65% PAS 5	19·0 ± 2·3	68·8 ± 5·9	19·3 ± 2·3	66·9 ± 5·5
95% PAS 5	1·8 ± 0·4	97·0 ± 0·5	1·9 ± 0·3	96·7 ± 0·8

- Amicus stored **5** and **7** days @ different plasma:PAS ratios
- 6 mL plasma, little difference Amicus:Trima, pH < Amicus
  - 7 day low plasma PAS-5 products ≈ acceptable in-vitro

Amicus SDP	100% Plasma		65% Plasma		95% Plasma	
pH	7.24	7.05	7.48	7.4	7.5	7.44
Platelet MPV fl	7.3	7.5	7.3	7.5	7.2	7.3
HSR - %	59	49	72	67	68	59
Annexin-V - %	21	24	17	18	16	16
Lactate mM (x↑)	16 (1.5)	18 (1.8)	*8(2.7)	*12 (3.9)	*6 (5.9)	*9 (8.8)

* = Sig diff Vs Day 1 & Low Vs 100% plasma. Morrison. Vox Sang 107:epub:2014

PAS benefits for PI-PC,↓ Plasma, & → Storage

- **Reduced reactions (& ↑Plasma Harvest) require low residual**
  - 5th gen PAS offers ↑buffering & plasma nutrient replacement
  - Low plasma will facilitate TRALI mitigation
- **Pathogen Reduction reduces viral & bacterial load:**
  - Allows long storage opportunity → improved metabolic suppor
  - PI stimulates platelets Δ platelet metabolic support desirable
- **Container Plasticizer, & Elutriation method supports:**
  - ↑ Oxygenation, ↓ glycolysis, &: selective cell harvest
  - Membrane & apopototic effector modification is possible
- **Patient Dosing & Reaction Reduction require PAS changes:**
  - Patient dosing is decreasing…increased focus on cell quality
- **PAS development represents a winning combination:**
  - ↑ Storage quality & ↓ antigen load → offset PI & BTHC effect

Next developments in Single Donor Platelets

- **PAS development:** (no negative sequelae in 20 years)
  - Low plasma ≈ ↓ reactions, ↓ TRALI risk, & ↓ABO matching
  - Tighter manufacturing ≈ (containers, content, & agitation)
- **Benefits likely to create a Standard of Practice**
  - Patient - reduces reactions & mitigates TRALI risk
  - Doctor – prophylactic drug Rx, & less patient intervention
  - Blood Center - frees up fractionation plasma & is profitable
  - Hospital – reduces ABO matching & may reduces cost
- **The Next Steps:** PAS uptake will be driven by hospital request
  - Pathogen Inactivation/Bacterial Testing offers 7 day dating
  - PAS development → offset the adverse effects of PI

Sanquin
Blood Supply

In vitro function of platelets in PAS

Pieter F. van der Meer
Sanquin Blood Bank, Unit Production
Sanquin Research, Clinical Transfusion Research

Disclosures

None related to the topics today

Objectives

- Discuss the platelet storage lesion, and how platelet additive solutions (PASs) can help prevent it
- Understand the roles – and interactions – of various ingredients of PAS
- Assess the role of glucose during platelet storage
- Evaluate recovery and survival of platelets stored in different PASs

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 limited buffering capacity of plasma; maintain pH>6.0

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Platelet additive solution

Additional benefits
- More plasma for transfusion/fractionation
- Standardized composition
- Ability to control storage environment
- Sterile, pathogen-free
- Less protein – fewer allergic reactions
- Lower AB0 titer
- (Reduction of antibody-mediated TRALI)

| 5

Nomenclature

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-II T-Sol SSP	PAS-3 PAS-III InterSol	Composol	PAS-IIIM SSP+	Plasma-Lyte A Isoplate	M-Sol
Citrate	X	X	X	X	X		X
Phosphate	X		X		X		X
Acetate		X	X	X	X	X	X
Magnesium				X	X	X	X
Potassium	X			X	X	X	X
Gluconate				X		X	
Glucose							X

Ashford, Vox Sang 2010;98:577, modified

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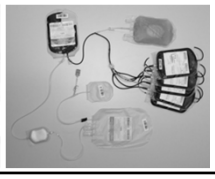
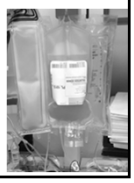
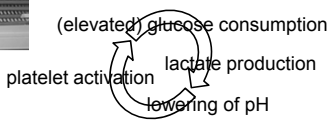
PAS and platelet processing

Integration in platelet preparation process
- PRP
- BC
- Apheresis

Platelet storage

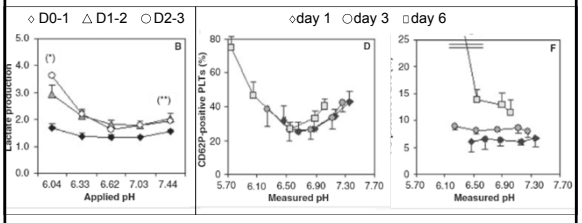
Platelet storage lesion (PSL)



Platelet storage lesion

- All things “bad” happening to a platelet
  - increased activation and metabolism
  - increased signals for removal
  - poorer response to stimuli
  - reduced adhesion
  - changed internal signaling
  - ...
  - less functionality
  - lower recovery, shorter survival
  - less able to stop or prevent bleeding

Platelet storage lesion



"We suggest that the pH decrease is a result of the PLT storage lesion and not the cause."

Dekkers, Transfusion 2006;46:1889

Platelet additive solutions

Composition of PASs used to 'tweak' platelet quality during storage

- What needs to be in the solution?
- Interactions?

Citrate

Table 4. The effect of lowering amounts of citrate on factor VIII recovery. Fibrinopeptide A (FpA) is measured to indicate activation.

Final plasma citrate (mM)	VIII:C at 22 h (IU/dl)	FpA (ng/ml)	Ca^{2+} (μM)	pH
20 (neat CPD)	68 ± 17	40	25	7.6
16	71 ± 13	30	36	7.7
12	80 ± 16	28	61	7.7
10	76 ± 20	25	77	7.7
8	86 ± 17	17	96	7.7
4	clot at 30′	13.350	276	7.8
Heparin	92 ± 22	23	955	7.9

Most PASs therefore contain at least 8 mM citrate

Smit Sibinga, Cryopreservation and low temperature biology in blood transfusion 1990:129

Citrate

Whole blood collected in 0.5CPD (10 mM citrate)

Day 7	PAS-2 **Citrate ±12 mM**	PlasmaLyte **±3 mM**
ß-TG, 10^3 IU/10^{11} platelets	98±9	153±70*
pH	6.92±0.02	7.19±0.09*
Lactate production#	1.0±0.1	0.7±0.7

#mmol/L/7 days; *p<0.05

Van Rhenen, transfusion 1995;35:50

Platelet metabolism

Platelets are extremely metabolically active cells:
- Oxygen consumption rate of 3 µmol/10^{10} cells/h
- Six times as fast as resting muscle; 30% as fast as mammalian brain

Guppy Vox Sang 1990;59:146

Platelet metabolism

Percentage contribution to:

	Total oxygen consumption, %	Total ATP turnover, %
Carbohydrates	64	60
Amino acids	11	10
Free fatty acids	5	5
Lactate production	0	6
Unknown	20	19
Total	100	100

Niu, Br J Haem 1997;97:908

Platelet metabolism

Glucose consumption
- into lactate 3.13±0.44 µmol/10^{11} platelets/h (net yield 2 ATP)
- full oxidation 0.05±0.01 µmol/10^{11} platelets/h (net yield 30 ATP)

PASs need to provide a fuel that can readily be used by platelets

Guppy Vox Sang 1990;59:146

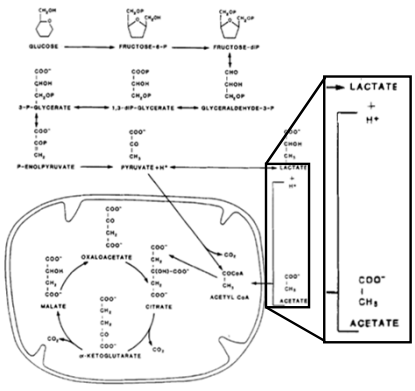
Acetate

Serendipity:
Acetate was present in infusion fluids that happened to be used in the early PAS studies

Lactate production
- No acetate: 2.4±0.5 µmol/day/10^{11} platelets
- 23 mM acetate: 1.3±0.3 µmol/day/10^{11} platelets

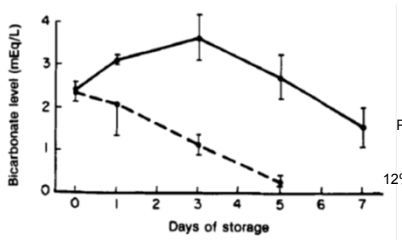
Platelets have a maximum need of 2 mmol/L/day of acetate

Shimizu Transfusion 1993;33:304; Murphy Blood 1995;86:3951



Murphy, Blood 1995;85:1929-35

Acetate



Shimizu Transfusion 1993;33:304

Acetate

	lactate production
Saline (reference)	0.07
Additive solution A acetate (10 mmol/l)	0.12
Additive solution B acetate (20 mmol/l)	0.10
Additive solution C acetate (30 mmol/l)	0.07

Mean production of lactate (mmol/day/10^{11} platelets) in PCs stored in CPD plasma (40%) diluted with saline or platelet additive solutions (PASs).

At 10 mM citrate in the PAS, the addition of 10 or 20 mM acetate still leads to higher lactate production; at 30 mM back to baseline values

Gulliksson Vox Sang 1995;68:152

Buffers

Acetate provides it's own buffer
However, with pathogen reduction (causing elevated lactate production), additional buffering capacity was needed: phosphate

Buffers

Phosphate
- Provides buffering capacity
- (Provides inorganic phosphate for ATP)
- Induces a 50% higher lactate production rate

Production of lactate, mmol/day/10^{11} platelets (days 1–7)	
Plasma	0.08±0.02
T-Sol	0.07±0.01
PAS-III	0.11±0.02[a]

Probably balance out at current phosphate concentration

Gullikkson Vox Sang 2000;78:176

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Effect of K/Mg

Day 7	pH	CD62P
PAS-2	6.98±0.07	49±10
PAS-2 + Mg	7.10±0.07*	41±14

1.5 mM Mg, 4.5 mM K; *p<0.05;**p<0.01

De Wildt-Eggen Transfusion 2002;42:76-80

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Effect of K/Mg

Day 7	pH	CD62P
PAS-2	6.98±0.07	49±10
PAS-2 + Mg	7.10±0.07*	41±14
PAS-2	6.93±0.04	55±6
PAS-2 + K	7.19±0.03**	35±8*

1.5 mM Mg, 4.5 mM K; *p<0.05;**p<0.01

De Wildt-Eggen Transfusion 2002;42:76-80

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Effect of K/Mg

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

1.5 mM Mg, 4.5 mM K; *p<0.05;**p<0.01

De Wildt-Eggen Transfusion 2002;42:76-80

Effect of K/Mg

Potassium
- maintaining membrane potential

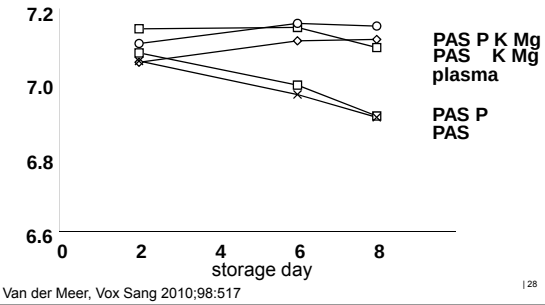
Magnesium
- activates potassium pumps
- decreases the PLT activation
- influences influx of calcium, thereby intracellular potassium concentration
- inhibits agonist-induced PLT aggregation, by changing membrane fluidity and/or by triggering of cAMP

Comparison of current PASs

- Pool and split buffy coats
- Add plasma or either of 4 PASs
- Centrifuge
- Storage in the same storage containers for 8 days
- *In vitro* analysis

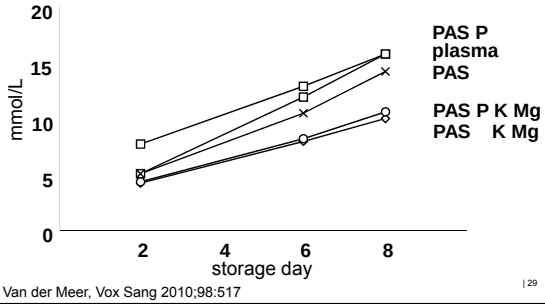
Van der Meer, Vox Sang 2010;98:517

$pH_{37°C}$



Van der Meer, Vox Sang 2010;98:517

Lactate



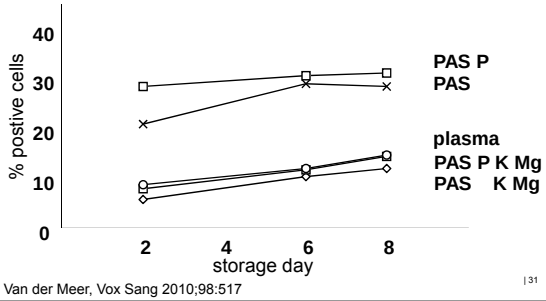
Van der Meer, Vox Sang 2010;98:517

Lactate production rate

plasma	0.13±0.04	
PAS	0.14±0.02	PAS-2
PAS P	0.17±0.03	PAS-3
PAS P K Mg	0.11±0.03	SSP+
PAS K Mg	0.10±0.02	Composol

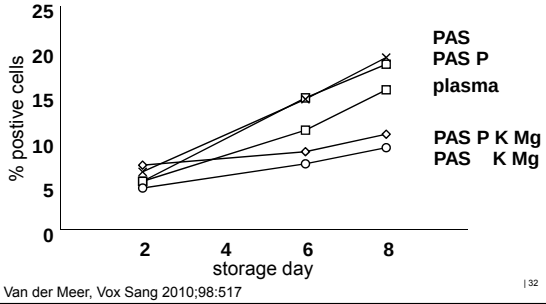
Van der Meer, Vox Sang 2010;98:517

CD62P expression



Van der Meer, Vox Sang 2010;98:517

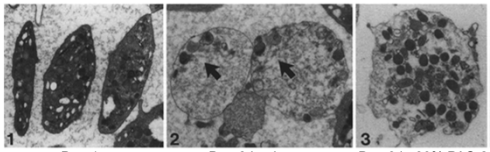
Annexin A5 binding



Van der Meer, Vox Sang 2010;98:517

Percent residual plasma

At least ±30% needed to preserve structural integrity



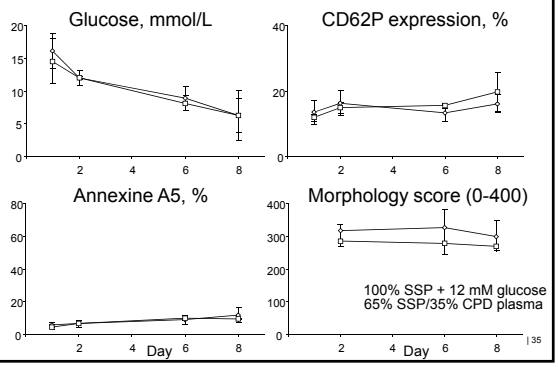
Day 1 Day 8 in plasma Day 8 in 80% PAS-2

Klinger, Vox Sang 1996;71:13

Percent residual plasma

- Pool and split 2 platelet concentrates
- Add 10% ACD, centrifuge, remove all supernatant
- Unit A: SSP+ and 12 mM glucose 16.1±2.7 mM
- Unit B: 35% plasma/65% SSP+ 14.6±3.3 mM
- Store for 8 days
- Various in vitro measures
- n=3

Percent residual plasma



Percent residual plasma

- No difference between 100% PAS with added glucose versus 65% PAS/35% CPD plasma
- Therefore, the plasma carry over was necessitated by the glucose requirement

Is glucose really needed?

- Bothersome to add to PASs, as it caramelizes at neutral pH
- two-bag system, one with acidic glucose solution and one with a basic PAS
- acidic PAS and bicarbonate pills to correct pH

Is glucose really needed?

- Pool and split 3 platelet concentrates
- Add 10% ACD, centrifuge, remove all supernatant
- Add SSP+ and
  - no glucose
  - to 12 mM glucose
  - to 24 mM glucose
- Store for 8 days
- Various *in vitro* measures

Is glucose really needed?

Glucose added, mM	0	12	24
Day 1 (mean±SD)			
Glucose, mM	1.9±0.3	14.4±2.3	25.6±4.7
Day 8			
pH	7.05±0.02	6.93±0.16	6.85±0.04
Lactate prod*	0.05±0.00	0.11±0.01	0.10±0.01
CD62P, %	21±4	17±3	17±4
Annexin A5, %	20±5	11±6	13±9
Morphology	305±26	303±26	272±23
HSR, %	45±14	67±7	52±7
ATP**	3.8±1.0	4.4±1.9	5.0±1.7

*mmol/10^{11} platelets/day
**µmol/10^{11} platelets

Is glucose really needed?

- Based on these results, glucose is probably not needed at all during platelet storage
- Platelets can survive on acetate only?
- Further confirmation needed

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Recovery and survival

	n	Source	Solution	Storage, d	Recovery, %	Survival, d
Adams, 1986	4	PRP	Plasma	5	51±4	8.7±0.3
		PRP	PAS-F/citrate/glucose	5	**39±5**	**6.0±1.4**
Holme, 1987	10	PRP	Plasma	7	36±11	4.6±1.3
		PRP	"PAS"	7	**51±8**	**6.0±0.7**
Heaton, 1990	10	PRP	Plasma	5	43±10	7.3±1.2
		PRP	"CSM"	5	**40±9**	**6.4±1.2**
	10	PRP	Plasma	7	56±8	6.2±1.1
		PRP	"CSM"	7	**59±6**	**7.3±1.2**
Holme, 1990	5	PRP	Plasma	7	41±11	6.1±1.7
		PRP	"PAS"	7	**45±12**	**6.7±1.3**
	5	PRP	Plasma	10	23±9	3.1±1.8
		PRP	"PAS"	10	**34±7**	**4.8±1.9**
Holme, 1994	18	Apheresis	Plasma	5	53±9	6.5±0.8
		Apheresis	"PAS"	5	**50±8**	**6.8±0.9**
Turner, 1996	11	BC	Plasma	5	51±16	6.5±1.5
	11	BC	PAS-F	5	**53±16**	**5.9±1.3**
	11	BC	PAS-B	5	**30±14**	**5.1±1.3**

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Recovery and survival

	n	Source	Solution	Storage, d	Percentage of 'fresh'	
					Recovery, %	Survival, %
Slichter, 2010	12	PRP	Plasma	7	72±11	51±16
	12	PRP	PAS-F	7	**46±20**	**24±10**
Vassallo, 2010	33	Apheresis	PAS-C	5	**80.5**	**72.1**
Dumont, 2013	66	Apheresis	PAS-F	5	**86.7**	**78.0**
Slichter, 2014a	10	BC	Plasma	5	89±10	78±16
	10	BC	Plasma	6	80±9	67±10
	10	BC	Plasma	7	79±6	59±16
	6	BC	PAS-F	5	**89±11**	**72±13**
	10	BC	PAS-F	6	**80±10**	**70±14**
	5	BC	PAS-F	7	**79±5**	**55±10**

Requirements
Recovery ≥67%
Survival ≥58%

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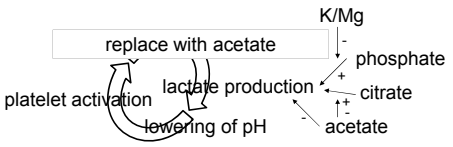
Recovery and survival

	n	Source	Solution	Storage, d	Recovery, %	Survival, d
Slichter, 2014b	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

PAS and storage lesion



Conclusions

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

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

In fact, acetate partially replaces glucose consumption, limiting lactate formation and the platelet storage lesion

Conclusions

Addition of phosphate, potassium, magnesium further optimizes platelet quality
There are *many* interactions between these ingredients

Glucose is probably not needed by platelets, and does not need to be included in the PAS, nor is plasma carry over necessary

Recovery and survival of platelets stored in PAS conform to FDA requirements

Summary

***In vitro* function of platelets in PAS**

Similar to that of platelets in plasma

Opportunities to have a longer storage time, and/or have better quality during current storage time