Exploratory Studies Of Extended Storage Of Apheresis Platelets In A Platelet Additive Solution (PAS)

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Running Head: Extended apheresis platelet storage in PAS

Key Words: Platelets, Platelet Apheresis, Platelet Storage, Platelet Radiolabeling, Plasmalyte

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KEY POINTS

Extended apheresis platelet storage is dependent on the collection method, storage in a storage solution, and storage bag composition.

The lifespan of the platelet is not intrinsic to the cell, and platelet viability is better maintained in vitro than in vivo.
ABSTRACT

To evaluate the post-storage viability of apheresis platelets stored for up to 18 days in 80% PAS/20% plasma, 117 healthy subjects donated platelets using the Haemonetics MCS+, COBE Spectra (Spectra), or Trima Accel (Trima) systems. Control platelets from the same subjects were compared to their stored test PAS platelets by radiolabeling their stored and control platelets with either $^{51}$Chromium or $^{111}$Indium. Trima platelets met FDA post-storage platelet viability criteria for only 7 days versus almost 13 days for Haemonetics platelets; i.e., platelet recoveries after these storage times averaged $44 \pm 3\%$ versus $49 \pm 3\%$ and survivals $5.4 \pm 0.3$ days versus $4.6 \pm 0.3$ days, respectively. The differences in storage duration are likely related to both the collection system as well as the storage bag. The Spectra and Trima platelets were hyperconcentrated during collection and PAS was added, while the Haemonetics platelets were elutriated with PAS which may have resulted in less collection injury. When Spectra and Trima platelets were stored in Haemonetics’ bags, post-storage viability was significantly improved. Platelet viability is better maintained in vitro than in vivo allowing substantial increases in platelet storage times. However, implementation will require resolution of potential bacterial overgrowth during storage.
INTRODUCTION

Platelet additive solutions (PAS) have been used to store platelets since the 1980’s.\textsuperscript{1,2} PAS storage of pooled buffy coat prepared platelet concentrates have long been used in Europe.\textsuperscript{3} The advantages of using a PAS for platelet storage are many including more plasma to meet patient needs or to fractionate into plasma-based products, reduce red cell hemolysis from ABO incompatible plasma, and reduce other adverse effects related to plasma transfusion.\textsuperscript{4,5}

To license platelets based on post-storage radiolabeled autologous platelet viability measurements in normal subjects, the FDA requires that the lower post-storage 95\% confidence limits (LCL) for platelet recoveries are $\geq 66\%$ and survivals are $\geq 58\%$ of the same subject’s radiolabeled fresh recoveries and survivals, respectively.\textsuperscript{6}

METHODS

Study Population

Healthy subjects who met allogeneic blood donor requirements were recruited between September 2000 and November 2011, and each signed a study consent in accordance with the Declaration of Helsinki. The study protocol and consents were approved by the University of Washington Institutional Review Board. Between 3 and 10 normal subjects participated in each study, with fewer subjects enrolled if the initial data suggested that FDA acceptance criteria would not be met.
Experimental Design

Three different apheresis systems – the COBE Spectra (Spectra) and Trima Accel (Trima) (Terumo BCT, Inc., Lakewood, CO) (these systems may be collectively referred to as Terumo BCT systems), and the Haemonetics MCS+ (Haemonetics Corporation, Braintree, MA) were used. For the Haemonetics collections, the in-line white cell leukoreduction filter was removed to give the highest cell counts to stress the system while the Terumo BCT platelets were in-process leukoreduced. Haemonetics collects whole blood into a spinning centrifuge bowl and then either plasma or PAS is pumped into the bottom of the bowel to push the supernatant platelets into a collection bag for storage.7 The Spectra has a dual-stage processing channel with the red and white cells removed in the first stage, and the platelets are concentrated in the second stage followed by transfer to a storage bag.7 The Trima has a single-stage channel where the cells separate into layers according to specific gravity, and each component leaves by its own outlet into a storage bag.7,8 All collections were within the manufacturer’s bag parameters for volume and total platelets per bag for 5- to 7-day plasma storage. However, these guidelines may not be applicable to the Plasmalyte (Baxter, Deerfield, IL) extended storage studies reported here. Both the Terumo BCT and Haemonetics bags have approximately the same surface area. The apheresis platelets were separated equally into two storage bags. One bag served as the control platelets that were stored for 1, 5, or 7 days in plasma or Plasmalyte while platelets in the other bag (test platelets) were stored for 5 to 18 days in Plasmalyte, subsequently referred to as a platelet additive solution (PAS). For some studies, the control platelets were “fresh” platelets prepared...
from a 43 ml whole blood sample drawn on the day the subject’s stored platelets were transfused.

**Platelet Radiolabeling**

At the end of storage, a 43 ml aliquot from both the control and the test platelets were alternately labeled with \(^{51}\)Cr or \(^{111}\)In using established techniques so that, at the end of an experiment, equal numbers of each platelet type had been labeled with both isotopes, and the platelets were transfused sequentially into their donor.\(^9,10\) Blood samples were drawn from the subject before, at 2 hours, and on days 1, 2, 3, 5, and 7 after transfusion to test for radioactivity using a Packard Model 5530 gamma counter (Downer’s Grove, IL). The radiolabeled data were not corrected for potential label elution nor for possible red cell bound isotope,\(^9\) except for the paired fresh and test platelet studies where a day 10 sample was obtained.\(^10\) Platelet recoveries and survivals were calculated using the COST program.\(^11\)

**PAS Platelets**

During the Terumo BCT studies, it was possible to change the usual collection procedure to hyperconcentrate the platelets with re-suspension in PAS.\(^12,13\) For the Haemonetics studies, the platelets were elutriated with PAS instead of plasma during collection by sterile docking a Haemonetics bag (Effluent #692) containing PAS to the collection set. The operator would unclamp and, as appropriate, clamp the tubing to the #692 bag to permit surging with PAS. A baseline sample of the subject’s plasma and
from their PAS stored platelet bag were assayed for albumin to determine the concentration of PAS versus residual plasma.

**In Vitro Platelet Measurements**

Platelet counts of collected products were performed on the day following collection and after storage using an ABX Hematology Analyzer (ABX Diagnostics, Irvine, CA). After storage, *in vitro* measurements of glucose concentration, pH at 37°C, pCO₂, pO₂, extent of shape change (ESC), and hypotonic shock response (HSR),¹⁴ Annexin V binding, morphology score,¹⁵ and mean platelet volume (MPV) were performed. Residual donor plasma was added to the PAS stored platelets to adjust the platelet count to 300,000/μl before performing the ESC and HSR measurements.¹⁶

**Statistical Methods**

Summary statistics [n, mean, standard deviation (s.d.) or standard error (s.e.)] are presented for *in vivo* and *in vitro* measures of platelet quality grouped by apheresis machine and storage interval of the test platelets. *In vivo* measures of platelet viability, recovery and survival, from paired (test and control) studies were compared using a paired t-test. When paired with a fresh or 1-day stored control platelets, test platelets were evaluated to determine if they met FDA guidelines for post-storage platelet viability. The *p* values were not corrected for multiple comparisons.
RESULTS

Effect of PAS Concentration on Post-Storage Platelet Viability

Ten Haemonetics collections were stored for 7 days with PAS concentrations between 50% to 82% to determine the optimum PAS concentration. With lower PAS concentrations between 50% to 67% (n=5), recoveries averaged 65 ± 18% and survivals 5.7 ± 0.5 days, and, with higher concentrations of 77% to 82%, recoveries averaged 63 ± 11% and survivals 6.2 ± 0.7 (n=5) with no trends based on PAS concentrations. A targeted 80% PAS concentration was used for all studies.

Haemonetics Platelets

In Vivo Data

There were no significant differences between each subject’s paired plasma and PAS stored platelets at 5 and 7 days of storage except for 7-day PAS recoveries which were 52± 3% versus 44 ± 5% for plasma stored platelets (p<0.01) (Table 1). With the 9- to 18-day PAS storage studies, the guidelines for using paired 1-day plasma stored or “fresh” platelets as controls were established. There were no significant differences in post-storage results for 9-day PAS stored compared to 1-day plasma stored platelets. For PAS platelets stored for ≥13 days, there were significant decreases in both platelet recoveries and survivals compared to 1-day stored or fresh platelets. PAS stored platelet recoveries and survivals declined progressively over storage times of 5 to 18 days (Figures 1A and 1B). The equations for the regression lines are:

\[
\text{Recovery} = 62.8 - 1.2x\text{Days Stored}, \quad r^2=0.19, \quad p=0.002
\]

\[
\text{Survival} = 7.9 - 0.3x\text{Days Stored}, \quad r^2=0.42, \quad p<0.001
\]
For some of the 15-day storage studies, the Haemonetics bags had changed from CLX [polyvinyl-chloride (PVC) with tri-(2-ethylhexyl) trimillitate (TOTM plasticizer)] to CPP (PVC with tributyl citrate plasticizer). When platelets were stored for 15 days in the CLX versus the CPP bags, viability was significantly decreased; i.e., platelet recoveries were 57 ± 5% versus 24 ± 7% (p<0.001) and survivals were 3.4 ± 0.5 days versus 2.2 ± 0.4 days (p=0.004), respectively.

**In Vitro Data**

Storage intervals of 14 days or more showed unacceptable decreases in platelet counts compared to day 1 of 10% to 20% (Table 2). Glucose concentrations were very low even with only 9 days of storage. Morphology scores were relatively stable, while Annexin V binding increased and ESC values decreased over storage time. HSR values also decreased but not until ≥14 days of storage. Post-storage pH’s were stable, and none were less than 7.0.

**Spectra Platelets**

**In Vivo Data**

These studies were done before the FDA guidelines for platelet storage were established, and a variety of controls were used. PAS versus plasma-stored platelets at 7 days gave platelet recoveries of 37 ± 8% versus 52 ± 4%, respectively (p=0.05), while platelet survivals were not significantly different (Table 1). Eight and 9-day PAS stored platelets were compared to 5-day plasma stored platelets, and both the PAS recoveries and survivals were significantly less for all comparisons (p<0.05). There were progressive decreases in both platelet recoveries and survivals over storage time (Figures
1A and 1B). Studies were not extended beyond 9 days as platelet recoveries averaged only 24 ± 6% and survivals 3.2 ± 1.1 days.

Because the results of the Spectra platelets in PAS were so inferior to the Haemonetics platelets after only 9 days (Table 1), Spectra platelets from 6 subjects were stored for 13 days, half in a Terumo BCT storage bag [PVC with n-Butyryl tri-n-hexyl citrate (BTHC) as the plasticizer], and half in a Haemonetics CLX bag. This experiment was done to determine if the decreased viability of the Spectra platelets in PAS was due to the collection method or the storage bag. Recoveries and survivals of the platelets stored in the Terumo BCT and Haemonetics bags were 40 ± 5% and 41 ± 7% (p=0.89) and survivals were 2.2 ± 0.5 and 4.9 ± 0.8 days (p=0.06), respectively. These data suggest that at least some of the differences in results between the two systems may be related to the storage bag.

**In Vitro Data**

As with the Haemonetics data, by 9 days of storage, there is almost no residual glucose (Table 2). Morphology scores were relatively stable between 5 and 13 days of storage, Annexin V binding increased, and ESC and HSR decreased over storage time.

**Trima Platelets**

**In Vivo Data**

To further explore the effects of the collection method, we evaluated the Trima system whose collection method differs from the Spectra system.\(^7,8\) As the survival of Spectra platelets differed from Haemonetics by 7 days (Table 3), we evaluated Trima platelets stored for 7, 9, and 13 days compared to “fresh” platelets. There were no
differences between the Spectra and Trima systems at any storage time (Table 1 and Figures 1A and 1B). Based on the improved results when Spectra platelets were stored in Haemonetics bags, Trima platelets were stored in Haemonetics bags for 9 days with significant improvements compared to Terumo BCT bags; i.e., recoveries averaged 44 ± 4% versus 29 ± 7%, \( p=0.05 \), and survivals averaged 5.0 ± 0.2 days versus 3.4 ± 0.6 days, \( p=0.01 \), respectively (Table 1). However, compared to 9-day Haemonetics platelets, Trima survivals in Haemonetics bags were still less (\( p=0.01 \)) (Table 3).

**In Vitro Data**

Because the Trima platelets were stored for only 7 and 9 days, neither trends in the data nor differences from the Spectra platelets could be determined (Table 2). However, the results appeared better when the platelets were stored for 9 days in Haemonetics versus Terumo bags.

**Viability Comparisons Between PAS-Store Haemonetics, Spectra, and Trima Platelets**

At 7 days, the recoveries of the Haemonetics, Spectra, and Trima stored platelets were not significantly different, while survivals were better for Haemonetics compared to Spectra platelets (\( p=0.05 \)) but not for Trima platelets (\( p=0.74 \)) (Table 3). At 9 days, Haemonetics recoveries and survivals were significantly better than both the Spectra and Trima platelets (\( p=0.007 \) and 0.03, respectively), and survivals (\( p=0.05 \) and 0.006, respectively). At 13 days, Haemonetics recoveries did not differ from Spectra, but survivals were significantly better (\( p=0.002 \)).
When Trima and Spectra platelets were stored in Haemonetics bags for 9 or 13 days, respectively, platelet recoveries were not different than similarly stored Haemonetics platelets. However, platelet survivals were significantly less for Trima stored platelets at 9 days ($p=0.01$) but not for 13-day stored Spectra platelets.

**Maximum Storage Duration Of Platelets That Meet FDA Post-Storage Viability Guidelines**

For Haemonetics platelets stored for 9, 13, and 14 days, comparisons were made to 1 day platelets and for 15-, 17- and 18-day storage to fresh platelets. For the Spectra data, no subjects had control platelets stored for either 1 day or fresh. For the Trima 7- and 9-day stored data, comparisons were made to “fresh” platelets. The data show that platelet recoveries met the FDA’s LCL of $\geq 66\%$ and $\geq 58\%$ of control criteria for 7 days for Trima-stored platelets (Figures 2A and B). For Trima platelets stored for 9 days in Haemonetics CLX bags, the LCL for recoveries were 67% and for survivals were 54%. At 13 days of storage, the LCL for Haemonetics platelets were 65% for recoveries and 55% for survivals.

**Effects Of Storage Volume, Platelet Concentration, Total Platelets, And Post-Storage pCO2, pO2, And Glucose On Post-Storage pH**

There were 12 Spectra or Trima platelets stored for 7 to 13 days in Terumo BCT bags that had post-storage pH’s of $\leq 6.4$. Ten of these units were transfused and recoveries averaged $18 \pm 16\%$ and survivals $1.8 \pm 0.7$ days (see legend to Table 1 for more details). In contrast, the lowest post-storage pH for Haemonetics platelets during
≤18 days of storage was 7.0. There was no apparent relationship between storage volume, platelet concentration, total platelet count or post-storage pCO₂ or pO₂ values, and post-storage pH (Figure 3A, 3B, 3C, 3D, and 3E, respectively). All of the Terumo BCT platelets with low pH’s had very low to absent residual glucose levels (Figure 3F). However, several other Terumo BCT and Haemonetics collections had similar low glucose levels with no effect on pH.

Comparisons Of Post-Storage Platelet Recoveries And Survivals To Post-Storage In Vitro Results

For both stored platelet recoveries and survivals, there are significant correlations with total platelet count of the product, storage volume, morphology score, glucose, Annexin V binding, ESC, HSR, pCO₂ and pO₂ (all p<0.05) (Figures 4A and 4B).

DISCUSSION

There is known to be a fair amount of heterogeneity in platelet recoveries and survivals among normal subjects. A recent study has further documented this heterogeneity, and, importantly, has demonstrated the reproducibility of fresh recoveries and survivals in the same subject. Murphy suggested that each subject serve as his/her own control by comparing their “fresh” platelet recoveries and survivals to their post-storage data, and the FDA has adopted this strategy for assessing post-storage platelet quality.

We have previously evaluated plasma-stored Haemonetics or Spectra collected platelets for 5 to 8 days, and platelet recoveries and survivals were not significantly
different between the two systems. With the plasma studies as background, we determined how long platelets could be stored in a PAS. Plasmalyte was selected as it was FDA licensed for intravenous use, it had previously been used for platelet storage, and no FDA-licensed PAS solutions were currently available. Unfortunately, many of our studies were completed before the FDA’s post-storage viability criteria were formulated, and so a variety of control platelets were used. Because there was little, if any, difference between 1-day stored and fresh platelet viabilities (Table 1), if either of these platelets were used as controls, FDA post-storage viability criteria were evaluated for the test PAS platelets.

As there were no differences in 7-day Haemonetics-stored platelet recoveries or survivals with PAS concentrations between 50% to 82%, we used a target PAS concentration of 80%. The Haemonetics platelets were close to meeting the FDA’s LCL for platelet recoveries and survivals after storage for 13 days (Figures 2A and 2B). In sharp contrast to the plasma platelet storage studies where the Haemonetics and Spectra systems gave the same results, the Spectra PAS platelets had similar recoveries compared to Haemonetics but survivals were significantly less \( (p=0.05) \) after storage for only 7 days, and by 9 days, all the results were significantly less for the Spectra platelets (Table 3).

The discrepant results could be due to the different methods used to process the platelets for PAS storage; i.e., hyperconcentration of the platelets with Spectra and re-suspension in PAS \textit{versus} platelet elutriation with PAS for the Haemonetics platelets. The hyperconcentration may have resulted in platelet damage, and so we evaluated the Trima system which uses a different collection system and hyperconcentration
Unfortunately, there were no differences in post-storage platelet viability regardless of the Terumo BCT system that was used (Tables 1 and 3 and Figures 1A and 1B).

The next question was whether the storage bag made a difference. The Haemonetics CLX bag is composed of PVC plastic with TOTM plasticizer. Haemonetics stopped manufacturing the CLX bags and, for 8 of the 11 15-day storage studies, a CPP bag composed of PVC plastic with a tributyl citrate plasticizer was used. Average recoveries for CLX versus CPP stored platelets were 56 ± 3% versus 24 ± 2% (p ≤ 0.001) and survivals 3.7 ± 0.4 days versus 2.2 ± 0.2 days (p = 0.004), respectively. Both the Terumo BCT systems use the same PVC bag with N-butyryl-tri-n-hexyl citrate (BTHC) plasticizer; i.e., the same plasticizer as in the Haemonetics CPP bags. These data may suggest the plasticizer might have a substantial effect on post-storage platelet viability when platelets are stored in PAS but not in plasma. Certainly, red cell storage studies showed the DEHP plasticizer helps maintain red cell viability during storage. In vitro platelet assays suggest that the storage bag and even the method of bag sterilization may affect platelet quality during storage. When Terumo BCT platelets were stored in Haemonetics CLX bags, there were clear improvements in post-storage platelet viability, but the results may still not be as good as Haemonetics platelets stored in Haemonetics CLX bags (Table 3).

Additional evidence that the storage bag could be a problem was the frequency with which pH values fell below acceptable levels during storage with the Terumo BCT but not with Haemonetics platelets (Tables 1 and 2). Twelve Terumo BCT collections had pH values of <6.4, and, of the 10 injected, platelet recoveries averaged 18 ± 16% and
survivals 1.8 ± 0.7 days (Table 1). In contrast, even though the Haemonetics platelets
had higher cell counts because they were not leukoreduced compared to the in-process
leukoreduced Terumo BCT platelets, all pH’s were above 7.0 for ≤18 days of storage.
However, even when Haemonetics platelets were stored in CPP bags for 15 days, the
lowest post-storage pH was 7.5, suggesting that the collection method plus the storage
bag may be producing the low pH. There was no relationship between platelet volume,
platelet concentration, total platelet count, or post-storage pCO₂ or pO₂, and post-storage
pH regardless of the storage conditions (Figures 3A, B, C, D, and E). All of the Terumo
BCT collections that had low pH’s had low to absent residual glucose at the end of
storage, while the residual glucose concentration did not appear to effect pH values for
Haemonetics collected platelets and for some of the Terumo BCT collections (Figure 3F).
These data suggest that the Haemonetics bags allow the platelets to metabolize the
acetate in the PAS to maintain pH better than platelets collected and stored in Terumo
BCT bags. Studies by Murphy, et al.34 demonstrated that platelets metabolize 2 mM of
acetate per day of storage. As PAS contains 27 mM of acetate, this suggests maintenance
of platelet viability for at least 13 days of storage as our studies demonstrated. Some
studies have suggested that enough plasma must be present during platelet storage to
maintain glucose levels,35,36 while others have indicated that residual glucose is not
required.37,38 Our studies have indicated that, depending on the storage conditions
(mainly the storage bag), acetate can substitute for glucose to maintain platelet viability.

There were significant correlations between most of the in vitro assays and both
platelet recoveries and survivals at the end of storage (all p<0.05) (Figures 4A and B).
Importantly, the Haemonetics CLX bags tended to have higher post-storage pCO₂ values
and lower pO₂ values compared to platelets stored in the Haemonetics CPP or the Terumo BCT bags. These combined high pCO₂ and low pO₂ results correlated with both better platelet recoveries and survivals suggesting the platelets were actively utilizing O₂ and releasing CO₂ to maintain viability. The interactions between platelet metabolic parameters and post-storage platelet viability requires further explanation.

Several prior studies have evaluated the effects of various PAS compared to plasma using radiolabeled autologous platelet recovery and survival measurements with variable results.²⁶,²⁹-⁴¹ de Wildt-Eggen, et al. have provided an excellent review of both in vitro and in vivo results of platelets stored in plasma or PAS.⁴¹ Only two prior studies have evaluated platelets stored in PAS beyond 7 days. At storage times of either 10 or 14 days, radiolabeled paired autologous PRP platelet concentrates were stored in a PAS or plasma, and PAS results were better than plasma.⁴² In the second study,²¹ 11 stable thrombocytopenic patients were given 4 to 12 day PAS stored pooled buffy-coat platelets, and the patients had good increments but shortened survivals.

There were several weaknesses of our studies. The studies were done sequentially and not randomized, the apheresis collections were done outside manufacturers guidelines, and Plasmalyte was used for elutriation of platelets on the Haemonetics system and it is also not a licensed storage solution.⁶

Our data are the best results yet reported in the literature for extended stored platelets and demonstrate that platelet viability is better maintained in vitro than in vivo. Specifically, fresh radiolabeled autologous platelet survivals in the 38 normal subjects in our studies averaged 8.2 ± 0.2 days, while platelets could be stored in vitro for 13 days with a residual in vivo lifespan of 4.6 ± 0.3 for a combined in vitrolin vivo lifespan of
almost 18 days fully 9 days longer than \textit{in vivo} (Table 1). This \textit{in vivo versus in vitro} difference may be related to an ongoing “work related” platelet utilization to maintain vascular integrity. Further studies are needed to confirm our results and to determine how to reduce any collection injury, identify the best PAS, the optimal PAS concentration, and the storage bag. These parameters may all interact in ways we do not yet understand. We also recognize that either pathogen-reduction or a sensitive and specific point of release bacterial assay will be needed before the FDA will license extended stored platelets.
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AUTHORSHIP CONTRIBUTIONS

S.J. Slichter: Designed research, analyzed and interpreted data, wrote the manuscript.
J. Corson: Recruited normal subjects.
M. K. Jones: Performed research, analyzed and interpreted data.
T. Christoffel: Performed research, analyzed and interpreted data.
E. Pellham: Performed research, analyzed and interpreted data.
S. L. Bailey: Performed research, analyzed and interpreted data.
D. Bolgiano: Analyzed and interpreted data, performed statistical analysis, designed figures and tables.

DISCLOSURE OF CONFLICTS OF INTEREST / DISCLAIMERS

The authors certify that neither they nor their institution have an affiliation or financial involvement in any organization or entity with a direct financial interest in the subject matter or materials discussed in this manuscript. S.J. Slichter, J. Corson, M.K. Jones, T. Christoffel, and D. Bolgiano participated in a prior commercial study supported by the Haemonetics Corporation. The studies reported in this article were supported only by a grant from the NIH, and only supplies were received from any company. No company was involved in the design or execution of the studies.

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T. Christoffel: None
E. Pellham: None
S. L. Bailey: None
D. Bolgiano: None
REFERENCES


(dual-stage filler) and the Trima Accel (single-stage filler) in the same donors. 


TABLE 1
IN VIVO PAIRED RECOVERIES AND SURVIVALS OF AUTOLOGOUS RADIOLABELED HAEMONETICS, SPECTRA, AND TRIMA PLATELETS

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<th>N</th>
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<th>STORAGE TIME (DAYS)</th>
<th>PLATELET RECOVERIES (%)</th>
<th>PLATELET SURVIVALS (DAYS)</th>
<th>STORAGE TIME (DAYS)</th>
<th>PLATELET RECOVERIES (%)</th>
<th>% OF CONTROL</th>
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<table>
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<th>PLATELET RECOVERIES (%)</th>
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<th>% OF CONTROL</th>
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<td>PAS</td>
<td>1</td>
<td>61 ± 6</td>
<td>7.3 ± 1.5</td>
<td>7</td>
<td>49 ± 6**</td>
<td>77 ± 4</td>
<td>5.0 ± 0.3</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>5</td>
<td>PAS</td>
<td>5</td>
<td>67 ± 4</td>
<td>6.6 ± 0.5</td>
<td>7</td>
<td>60 ± 2</td>
<td>91 ± 4</td>
<td>5.3 ± 0.5*</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>7</td>
<td>Plasma</td>
<td>7</td>
<td>52 ± 4</td>
<td>4.8 ± 0.3</td>
<td>7*</td>
<td>37 ± 8*</td>
<td>68 ± 13</td>
<td>3.2 ± 0.5</td>
<td>71 ± 16</td>
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<tr>
<td>5</td>
<td>Plasma</td>
<td>5</td>
<td>55 ± 6</td>
<td>6.7 ± 0.9</td>
<td>8*</td>
<td>45 ± 8*</td>
<td>80 ± 8</td>
<td>4.0 ± 0.8*</td>
<td>60 ± 12</td>
</tr>
<tr>
<td>5</td>
<td>Plasma</td>
<td>5</td>
<td>50 ± 5</td>
<td>6.0 ± 0.1</td>
<td>9*</td>
<td>24 ± 6*</td>
<td>49 ± 13</td>
<td>3.2 ± 1.1*</td>
<td>52 ± 18</td>
</tr>
<tr>
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<td>PAS</td>
<td>13</td>
<td>40 ± 5</td>
<td>2.2 ± 0.5</td>
<td>13****</td>
<td>41 ± 7</td>
<td>110 ± 20</td>
<td>4.9 ± 0.8</td>
<td>320 ± 109</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>N</th>
<th>STORAGE SOLUTION</th>
<th>STORAGE TIME (DAYS)</th>
<th>PLATELET RECOVERIES (%)</th>
<th>PLATELET SURVIVALS (DAYS)</th>
<th>STORAGE TIME (DAYS)</th>
<th>PLATELET RECOVERIES (%)</th>
<th>% OF CONTROL</th>
<th>PLATELET SURVIVALS (DAYS)</th>
<th>% OF CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>None</td>
<td>“Fresh”</td>
<td>58 ± 5</td>
<td>8.3 ± 0.2</td>
<td>7</td>
<td>44 ± 3**</td>
<td>77 ± 4</td>
<td>5.4 ± 0.3</td>
<td>66 ± 3</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>“Fresh”</td>
<td>54 ± 4</td>
<td>7.7 ± 0.4</td>
<td>9*</td>
<td>29 ± 7**</td>
<td>54 ± 12</td>
<td>3.4 ± 0.6**</td>
<td>46 ± 8</td>
</tr>
<tr>
<td>10</td>
<td>None</td>
<td>“Fresh”</td>
<td>57 ± 4</td>
<td>8.5 ± 0.2</td>
<td>9****</td>
<td>44 ± 4**</td>
<td>78 ± 5</td>
<td>5.0 ± 0.2**</td>
<td>59 ± 2</td>
</tr>
<tr>
<td>2</td>
<td>None</td>
<td>“Fresh”</td>
<td>64.35</td>
<td>8.4, 7.5</td>
<td>13f</td>
<td>3.17</td>
<td>5.49</td>
<td>0.6, 2.3</td>
<td>7.13</td>
</tr>
</tbody>
</table>
Fresh platelets were prepared from a blood sample drawn from the donor on the day the stored platelets were injected. Percent of control results were determined by dividing the test results by the control x 100.

Data are given as the average ±1 S.E.

* p<0.05.  p values for PAS platelet recoveries and survivals are based on the differences between the PAS data and the control platelet data for each study.

** p<0.01.  p values for PAS platelet recoveries and survivals are based on the differences between the PAS data and the control platelet data for each study.

*** These platelets were stored in Haemonetics CPP bags versus all other Haemonetics collection studies which were stored in Haemonetics CLX bags.

**** These Terumo BCT platelets were stored in Haemonetics CLX rather than Terumo BCT bags.

Four units had post-storage pH’s of ≤6.0 with platelet recoveries of 57%, 18%, 17%, and 9% and associated survivals of 1.7, 2.3, 2.2, and 2.9 days, respectively.

One unit had post-storage pH of 6.3 and platelet recovery of 24% and survival of 2.6 days.

Three units had post-storage pH’s of 6.4, 6.3, and 5.7 with associated platelet recoveries of 8%, 13%, and 28% and survivals of 1.6, 1.7, and 1.1 days, respectively.

One collection not injected as pH <6.0 for both bags at the end of storage.

One unit not injected as post-storage pH <6.0.  Two other units both had post-storage pHs of 6.1 and recoveries of 2%, 3% and survivals of 1.7 and 0.5 days, respectively.

One unit with post-storage pH of 6.4 had a platelet recovery of 3% and platelet survival of 0.6 days.
**TABLE 2**

**IN VITRO ASSAYS OF HAEMONETICS, SPECTRA, AND TRIMA APERHESES PLATELETS STORED IN PAS**

<table>
<thead>
<tr>
<th>Storage Time (Days)</th>
<th>Donor’s Platelet Count (μl)</th>
<th>Unit Volume (mls)</th>
<th>Total Platelet Count (x 10¹¹)</th>
<th>PAS (%)</th>
<th>Glucose (mgm/dl)***</th>
<th>MPV</th>
<th>Morphology Score</th>
<th>Annexin V Binding (%)</th>
<th>ESC (%)</th>
<th>HSR (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>240,000 ± 29,000</td>
<td>189 ± 23</td>
<td>ND</td>
<td>1.76 ± 0.46</td>
<td>78 ± 7</td>
<td>ND</td>
<td>ND</td>
<td>229 ± 14</td>
<td>16 ± 2</td>
<td>26 ± 5</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>232,000 ± 51,000</td>
<td>215 ± 23</td>
<td>ND</td>
<td>2.02 ± 0.43</td>
<td>69 ± 12</td>
<td>ND</td>
<td>ND</td>
<td>250 ± 26</td>
<td>17 ± 7</td>
<td>17 ± 3</td>
</tr>
<tr>
<td>4</td>
<td>9</td>
<td>288,000 ± 59,000</td>
<td>217 ± 53</td>
<td>ND</td>
<td>2.65 ± 0.70</td>
<td>79 ± 3</td>
<td>77 ± 10</td>
<td>5.5 ± 6.1</td>
<td>325 ± 35</td>
<td>23 ± 5</td>
<td>18 ± 3</td>
</tr>
<tr>
<td>10</td>
<td>13</td>
<td>234,000 ± 31,000</td>
<td>258 ± 28</td>
<td>ND</td>
<td>2.59 ± 0.58</td>
<td>81 ± 1</td>
<td>74 ± 7</td>
<td>7.0 ± 15</td>
<td>265 ± 48</td>
<td>26 ± 14</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>10</td>
<td>14</td>
<td>215,000 ± 45,000</td>
<td>263 ± 47</td>
<td>ND</td>
<td>2.22 ± 0.53</td>
<td>82 ± 1</td>
<td>74 ± 1</td>
<td>ND</td>
<td>252 ± 52</td>
<td>34 ± 0</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>245,000 ± 43,000</td>
<td>200 ± 48</td>
<td>ND</td>
<td>2.33 ± 0.92</td>
<td>80 ± 2</td>
<td>76 ± 2</td>
<td>0.3 ± 0.6</td>
<td>220 ± 25</td>
<td>33 ± 5</td>
<td>9 ± 5</td>
</tr>
<tr>
<td>8</td>
<td>15*</td>
<td>254,000 ± 73,000</td>
<td>230 ± 56</td>
<td>ND</td>
<td>2.26 ± 0.63</td>
<td>76 ± 4</td>
<td>81 ± 9</td>
<td>1.1 ± 0.8</td>
<td>245 ± 46</td>
<td>40 ± 5</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>269,000 ± 67,000</td>
<td>218 ± 20</td>
<td>ND</td>
<td>2.88 ± 0.65</td>
<td>82 ± 3</td>
<td>ND</td>
<td>ND</td>
<td>197 ± 10</td>
<td>35 ± 10</td>
<td>10 ± 6</td>
</tr>
<tr>
<td>3</td>
<td>18</td>
<td>242,000 ± 56,000</td>
<td>252 ± 78</td>
<td>ND</td>
<td>2.95 ± 0.35</td>
<td>78 ± 4</td>
<td>72 ± 0.3</td>
<td>0.3 ± 0.6</td>
<td>225 ± 56</td>
<td>32 ± 0</td>
<td>9 ± 6</td>
</tr>
</tbody>
</table>

**SPECTRA PLATELETS**

<table>
<thead>
<tr>
<th>Storage Time (Days)</th>
<th>Donor’s Platelet Count (μl)</th>
<th>Unit Volume (mls)</th>
<th>Total Platelet Count (x 10¹¹)</th>
<th>PAS (%)</th>
<th>Glucose (mgm/dl)***</th>
<th>MPV</th>
<th>Morphology Score</th>
<th>Annexin V Binding (%)</th>
<th>ESC (%)</th>
<th>HSR (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>≤1</td>
<td>194,000 ± 1,000</td>
<td>275 ± 24</td>
<td>2.36 ± 0.2</td>
<td>ND</td>
<td>80 ± 1</td>
<td>ND</td>
<td>ND</td>
<td>368 ± 6</td>
<td>7 ± 2</td>
<td>16 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>5</td>
<td>260,000 ± 68,000</td>
<td>250 ± 22</td>
<td>ND</td>
<td>2.73 ± 0.46</td>
<td>78 ± 2</td>
<td>ND</td>
<td>ND</td>
<td>298 ± 50</td>
<td>10 ± 5</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>15</td>
<td>7</td>
<td>242,000 ± 58,000</td>
<td>276 ± 46</td>
<td>ND</td>
<td>2.38 ± 0.12</td>
<td>80 ± 3</td>
<td>ND</td>
<td>ND</td>
<td>240 ± 107</td>
<td>29 ± 25</td>
<td>11 ± 4</td>
</tr>
<tr>
<td>8</td>
<td>8</td>
<td>252,000 ± 128,000</td>
<td>268 ± 27</td>
<td>2.17 ± 0.10</td>
<td>2.21 ± 0.88</td>
<td>80 ± 1</td>
<td>ND</td>
<td>ND</td>
<td>296 ± 58</td>
<td>28 ± 6</td>
<td>9 ± 3</td>
</tr>
<tr>
<td>5</td>
<td>9</td>
<td>225,000 ± 43,000</td>
<td>261 ± 67</td>
<td>ND</td>
<td>1.80 ± 1.1</td>
<td>80 ± 1</td>
<td>ND</td>
<td>3 ± 7</td>
<td>233 ± 46</td>
<td>61 ± 0</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>13</td>
<td>228,000 ± 8,000</td>
<td>268 ± 11</td>
<td>2.16 ± 0.10</td>
<td>1.81 ± 0.1</td>
<td>84 ± 1</td>
<td>ND</td>
<td>ND</td>
<td>240 ± 14</td>
<td>52 ± 4</td>
<td>8 ± 2</td>
</tr>
<tr>
<td>6</td>
<td>13**</td>
<td>228,000 ± 8,000</td>
<td>288 ± 13</td>
<td>2.14 ± 0.12</td>
<td>2.09 ± 0.1</td>
<td>84 ± 1</td>
<td>ND</td>
<td>ND</td>
<td>251 ± 17</td>
<td>44 ± 3</td>
<td>7 ± 1</td>
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**TRIMA PLATELETS**

<table>
<thead>
<tr>
<th>Storage Time (Days)</th>
<th>Donor’s Platelet Count (μl)</th>
<th>Unit Volume (mls)</th>
<th>Total Platelet Count (x 10¹¹)</th>
<th>PAS (%)</th>
<th>Glucose (mgm/dl)***</th>
<th>MPV</th>
<th>Morphology Score</th>
<th>Annexin V Binding (%)</th>
<th>ESC (%)</th>
<th>HSR (%)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>7</td>
<td>261,000 ± 81,000</td>
<td>297 ± 11</td>
<td>2.37 ± 0.62</td>
<td>2.34 ± 0.60</td>
<td>83 ± 1</td>
<td>ND</td>
<td>ND</td>
<td>258 ± 38</td>
<td>14 ± 6</td>
<td>13 ± 3</td>
</tr>
<tr>
<td>8</td>
<td>9</td>
<td>300,000 ± 89,000</td>
<td>311 ± 31</td>
<td>2.16 ± 0.56</td>
<td>2.10 ± 0.61</td>
<td>83 ± 4</td>
<td>ND</td>
<td>ND</td>
<td>193 ± 186</td>
<td>26 ± 5</td>
<td>9 ± 5</td>
</tr>
<tr>
<td>10</td>
<td>9**</td>
<td>257,000 ± 40,000</td>
<td>298 ± 12</td>
<td>2.49 ± 0.62</td>
<td>2.38 ± 0.56</td>
<td>82 ± 2</td>
<td>ND</td>
<td>ND</td>
<td>302 ± 19</td>
<td>16 ± 6</td>
<td>12 ± 2</td>
</tr>
</tbody>
</table>

Data are given as the average ± S.D.
* These platelets were stored in Haemonetics CPP bags versus other studies which were stored in Haemonetics CLX bags.
** Platelets stored in Haemonetics CLX bags.
*** Glucose levels can be accurately measured to 0 mgm/dl.

ND = Not done.
<table>
<thead>
<tr>
<th>Collection Machine</th>
<th>N</th>
<th>Storage Bag</th>
<th>Storage Time (Days)</th>
<th>Platelet Recoveries (%)</th>
<th>P value (H versus S or T)</th>
<th>Platelet Survivals (Days)</th>
<th>P value (H versus S or T)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemonetics (H)</td>
<td>10</td>
<td>H</td>
<td>7</td>
<td>52 ± 3</td>
<td>---</td>
<td>6.0 ± 0.3</td>
<td>---</td>
</tr>
<tr>
<td>Spectra (S)</td>
<td>15</td>
<td>T</td>
<td>7</td>
<td>49 ± 5</td>
<td>0.71</td>
<td>4.7 ± 0.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Trima (T)</td>
<td>9</td>
<td>T</td>
<td>7</td>
<td>44 ± 3</td>
<td>0.06</td>
<td>5.4 ± 0.3</td>
<td>0.74</td>
</tr>
<tr>
<td>Haemonetics</td>
<td>4</td>
<td>H</td>
<td>9</td>
<td>55 ± 5</td>
<td>---</td>
<td>6.6 ± 0.6</td>
<td>---</td>
</tr>
<tr>
<td>Spectra</td>
<td>5</td>
<td>T</td>
<td>9</td>
<td>24 ± 6</td>
<td>0.007</td>
<td>3.2 ± 1.1</td>
<td>0.05</td>
</tr>
<tr>
<td>Trima</td>
<td>8</td>
<td>T</td>
<td>9</td>
<td>29 ± 7</td>
<td>0.03</td>
<td>3.4 ± 0.6</td>
<td>0.006</td>
</tr>
<tr>
<td>Trima</td>
<td>10</td>
<td>H</td>
<td>9</td>
<td>44 ± 4</td>
<td>0.20</td>
<td>5.0 ± 0.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Haemonetics</td>
<td>10</td>
<td>H</td>
<td>13</td>
<td>49 ± 3</td>
<td>---</td>
<td>4.6 ± 0.3</td>
<td>---</td>
</tr>
<tr>
<td>Spectra*</td>
<td>6</td>
<td>T</td>
<td>13</td>
<td>40 ± 5</td>
<td>0.14</td>
<td>2.2 ± 0.5</td>
<td>0.002</td>
</tr>
<tr>
<td>Spectra*</td>
<td>6</td>
<td>H</td>
<td>13</td>
<td>41 ± 7</td>
<td>0.25</td>
<td>4.9 ± 0.8</td>
<td>0.77</td>
</tr>
</tbody>
</table>

Data are given as the average ±1 S.E.

Bags: H=Haemonetics CLX; T=Terumo BCT. Same bags were used to store both Spectra and Trima collected platelets.

*These were paired observations from the same donor’s collection stored in either C or T bags.
FIGURE LEGENDS

Figure 1  Recoveries and Survivals of Stored Apheresis Platelets.

Figure 1A  Recoveries of Haemonetics, Spectra, and Trima Platelets For 1 to 18 Days.

Figure 1B  Survivals of Haemonetics, Spectra, and Trima Platelets For 1 to 18 Days.

Data for Haemonetics stored platelets in CLX bags are given as closed circles (●), for Spectra stored platelets as closed triangles (▲), for Trima stored platelets as closed squares (■). All data are given for platelets stored in each system’s own bags. Data are given as average ±1 S.E.

Figure 2  Stored Platelet Recoveries and Survivals As A Percentage Of Control Platelets (Fresh or 1 Day Plasma Stored Platelets).

Figure 2A  Stored Platelet Recoveries Compared To Control Platelets.

Figure 2B  Stored Platelet Survivals Compared To Control Platelets.

Shown are the 2-sided, lower 95% confidence limits (LCL) for the mean difference between the stored platelets and the proportion of the fresh or 1 day plasma platelets (control platelets) specified by the FDA criteria. The LCLs are a function of both the means and the standard deviations of these differences. These limits have been transformed to a “percent of control scale.” The horizontal, dashed lines show the critical values specified by FDA’s post-storage platelet viability criteria; i.e., platelet recoveries should be ≥66% and survivals ≥58% of each subject’s paired control platelets.
Data for Haemonetics MCS+ platelets in CLX bags are shown as closed circles (●) and in CPP bags as open circles (○), and, for Trima platelets in Terumo BCT bags, closed squares (■) and in Haemonetics CLX bags as open squares (□).

Figure 3  Relationship Between Post-Storage pH And Storage Volume, Platelet Concentration, Total Platelet Count, and Post-Storage pCO2, pO2, And Glucose for Haemonetics, Spectra, or Trima Collected Platelets.

Figure 3A  Post-Storage pH Versus Storage Volume.
Figure 3B  Post-Storage pH Versus Platelet Concentration.
Figure 3C  Post-Storage pH Versus Total Platelet Count.
Figure 3D  Post-Storage pH Versus Post-Storage pCO2.
Figure 3E  Post-Storage pH Versus Post-Storage pO2.
Figure 3F  Post-Storage pH Versus Post-Storage Glucose Concentration.

Data for Haemonetics platelets in CLX bags are shown as closed circles (●) and in CPP bags as open circles (○), for Trima platelets in Terumo BCT bags as closed squares (■) and in Haemonetics CLX bags as open squares (□), and for Spectra platelets in Terumo BCT bags as closed triangles (▲) and in Haemonetics CLX bags as open triangles (△).

Figure 4  Relationship Between Post-Transfusion In Vivo Versus In vitro Data.
Figure 4A  Post-Storage Platelet Recoveries Versus In Vitro Measurements.
Figure 4B  Post-Storage Platelet Survivals Versus In Vitro Measurements.
Data for Haemonetics platelets in CLX bags are shown as closed circles (●) and in CPP bags as open circles (〇), for Trima platelets in Terumo BCT bags as closed squares (□), and in Haemonetics CLX bags as open squares (□), and for Spectra platelets in Terumo BCT bags as closed triangles (▲) and in Haemonetics CLX bags as open triangles (△). The regression lines for the data are shown as the hatched lines and the $r^2$ values are given on the figures.
Figure 1A

![Graph A](image)

Figure 1B

![Graph B](image)
Figure 4A

4A

- Platelet Recovery (%)
- Annexin V Binding (%)
- Extent of Shape Change (%)
- Hypotonic Shock Response (%)

- pCO₂ (mmHg)
- pO₂ (mmHg)
- Glucose (mg/dl)
- pH

Figure 4B

4B

- Platelet Survival (Days)
- Annexin V Binding (%)
- Extent of Shape Change (%)
- Hypotonic Shock Response (%)

- pCO₂ (mmHg)
- pO₂ (mmHg)
- Glucose (mg/dl)
- pH

Correlation coefficients (r²) are indicated for each graph.
Exploratory studies Of extended storage Of apheresis platelets in a platelet additive solution (PAS)

Sherrill J. Slichter, Jill Corson, Mary Kay Jones, Todd Christoffel, Esther Pellham, S. Lawrence Bailey and Doug Bolgiano