BACKGROUND: Buffy-coat processing allows for the use of platelet additive solutions (PASs). PASs reduce plasma-associated transfusion reactions and conserve plasma for transfusion or fractionation. Platelet (PLT) storage in plasma was compared to storage in three commercially available PASs compared to assess their influence on in vitro laboratory variables.

STUDY DESIGN AND METHODS: Platelet concentrates (PCs) were prepared from leukoreduced pools of four buffy coats (BCPs) suspended in autologous plasma or one of PASs (Composol, Fresenius-Kabi; T-Sol, Baxter Corp.; or SSP+, MacoPharma). On Days 1, 2, 3, 5, and 7 of storage, samples were tested for PLT concentration, mean PLT volume (MPV), CD62P, morphology, pO2, pCO2, glucose, lactate and total protein concentration, pH, extent of shape change (ESC), and hypotonic shock response (HSR). Data were analyzed by analysis of variance (ANOVA) with repeated measures and t tests.

RESULTS: PLT recoveries from BCPs were higher (p < 0.05) with plasma than any PAS. Storage medium and duration did not affect PLT concentration or MPV over time. CD62P expression and morphology were significantly different among PCs pooled with different media. ANOVA showed (p < 0.05) differences among the rates of change of pCO2, pH, glucose consumption, lactate production, and ESC; PASs such as Composol and SSP+ offered excellent maintenance of pH and low rates of glucose consumption. PAS performed poorly in ESC and HSR compared to plasma. Correlation studies reveal far more significant correlations between variables of PLTs in PAS than in plasma.

CONCLUSION: Newer PASs, for example, SSP+ and Composol, can maintain PLT integrity and moderate metabolism similarly to plasma but offer consistently lower PLT recoveries and limited osmotic balance.

ABBREVIATIONS: BCP(s) = buffy-coat pool(s); ESC = extent of shape change; HSR = hypotonic shock response; MPV = mean platelet volume; PAS(s) = platelet additive solution(s); PC(s) = platelet concentrate(s).

In Canada, platelet concentrates (PC) derived from the buffy-coat production process are stored in donor plasma although the buffy-coat system would allow the use of platelet additive solutions (PASs) for the storage of platelets (PLTs), because commercially available PASs claim to maintain PLT quality in vitro similar to storage in plasma. Potentially, the use of PAS can conserve plasma resources and thereby decrease plasma-related transfusion reactions and disease transmission. As well, the use of PAS can contribute to the standardization of the blood component production process. Of the first generation of PAS was T-Sol (Baxter Corp., Alliston, Ontario, Canada), a sodium citrate-acetate-chloride formulation on which all subsequent PAS formulations have been based. More recently developed PASs contain phosphate to increase the buffering capacity, gluconate as a chelator, and potassium and magnesium to suppress metabolism (Table 1).
Historically, PASs were designed for the short-term maintenance of pH, PLT integrity, and PLT energy requirements. Recent multicenter studies demonstrated the effects of potassium and magnesium on PLT metabolic control as well as underlining the importance of glucose to PLT survival. Phosphates, a buffering component of the newer PASs, are also known to regulate glycolysis. Because the new-generation containers, for example, polyolefin or polyvinyl chloride, are gas-permeable, they allow the free exchange of gas molecules among PLTs, the medium, and the external environment. The PC must be stored with agitation so that glucose metabolism, which leads to CO₂ production, will neither a build-up of CO₂ nor a pH increase beyond the regulated 7.4 with concomitant poor PLT viability. Similarly, oxygen inside the PC storage container equilibrates with the external atmosphere and oxygen is consumed via the oxidative cycles of PLT metabolism during storage. A lack of oxygen promotes anaerobic respiration involving glycolysis, hence the rates of gas changes in a PC offer valuable insights into the rate of PLT metabolism.

Unfortunately, regardless of the storage medium, stored PLTs deteriorate, a phenomenon known as the “PLT storage lesion.” This can be characterized by several major changes such as PLT apoptosis and/or fragmentation, which occurs under ambient conditions or under thermostress at 37°C. Also, PLTs in storage tend to release their granular contents, which leads to increased P-selectin (CD62P) surface expression. As well, morphologic changes have been documented during storage.

Although there are many methods to evaluate PLT quality in vitro, there is no “gold standard” that is the ultimate descriptor of PLT quality. Consequently, studies comparing buffy-coat PLTs with plasma or PAS are limited to contrasts among plasma and one or two additive solutions (ASs), between two ASs, or simply just one PAS alone and present comparisons with only selected evaluation methods. In this study, the most robust methods in the recent literature are used, and a comprehensive comparison of PCs stored in plasma and three PASs is provided.

### MATERIALS AND METHODS

#### Blood collection, centrifugation, and component separation

Ethical approval for the study was granted by Canadian Blood Services’ Ethical Review Board, and whole blood (WB) was collected from 80 consenting volunteer donors with the current Canadian Blood Services Research and Development Standard Operating Procedures (SOPs) into a quadruple blood collection system top and bottom bags (4-Optipure RC CPD+ SAGM and CGR8448b, respectively, Baxter Corp.). These WB units were stored on 1,4-butane-diol plates (CompoCool, Fresenius-Kabi, Redmond, WA) and cooled to and maintained at 20 to 23°C for 16 to 20 hours, as previously described. Centrifugation to produce buffy coats was performed in a centrifuge (Sorvall RC3BP, Thermo Scientific; radius 277 mm, 22°C) at 3800 g for 11 minutes in the collection system top and bottom bags procedures (SOPs) into a quadruple blood.

#### Preparation of PCs from buffy coats

Four ABO-matched buffy coats with a mean pooled volume of 200 ± 2 mL of 50 percent hematocrit were sterile-connected in a “train” configuration with a sterile connecting device (CompoDock, Fresenius-Kabi). A PLT PC pooling and/or storage bag system (OptiPure PL2410, Baxter Corp.) was attached to the bottom of the four buffy-coat trains. Five replicate pools received one of a 300-mL unit of plasma from one of the donors in the train or a 300-mL bag of PAS: SSP+ (MacoPharma, Tourcoing, France), Composol-PS (Fresenius-Kabi), or T-Sol (Baxter Corp.). The buffy-coat pools (BCPs) were sampled for PLT concentration and then weighed for volume determination. BCPs in plasma were centrifuged (Sorvall RC3BP, Thermo Scientific) at an optimized speed of 1860 × g for 5 minutes (slow start 9, slow stop 3). The buffy-coat/PAS mixtures were centrifuged at 310 × g for 11 minutes in the same centrifuge (slow start 10, slow stop 5). The resulting PLT-rich plasma was filter leukoreduced and tested for bacterial growth on Days 1 and 7 with 8-mL samples in aerobic bottles for a commercially available bacterial detection system (BacT/ALERT 3D bioMérieux, Inc., Durham, NC). The PCs were weighed first, sampled for PLT concentration, and then stored with agitation (60 strokes/min) at 22 to 24°C for 7 days. Samples, 13 mL, were collected aseptically on Days 1, 2, 3, 5, and 7.

#### Table 1. Composition of synthetic PASs

<table>
<thead>
<tr>
<th>Component</th>
<th>T-Sol</th>
<th>Composol</th>
<th>SSP+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na₃ citrate (mmol/L)</td>
<td>10.0</td>
<td>10.9</td>
<td>10.8</td>
</tr>
<tr>
<td>Na-acetate (mmol/L)</td>
<td>30.0</td>
<td>27.0</td>
<td>32.5</td>
</tr>
<tr>
<td>NaCl (mmol/L)</td>
<td>115</td>
<td>90.0</td>
<td>69.0</td>
</tr>
<tr>
<td>Na-gluconate (mmol/L)</td>
<td>23.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NaH₂PO₄ (mmol/L)</td>
<td></td>
<td></td>
<td>6.7</td>
</tr>
<tr>
<td>Na₂HPO₄ (mmol/L)</td>
<td></td>
<td></td>
<td>21.5</td>
</tr>
<tr>
<td>MgCl₂ •6 H₂O (mmol/L)</td>
<td>1.50</td>
<td>1.50</td>
<td></td>
</tr>
<tr>
<td>Na-acetate (mmol/L)</td>
<td></td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Viscosity (37°C), cP</td>
<td>0.7243 ± 0.0005</td>
<td>0.7249 ± 0.0013</td>
<td>0.7353 ± 0.0005</td>
</tr>
</tbody>
</table>
Evaluation of in vitro PLT quality

**PLT concentration and mean PLT volume**

PLTs were sampled into ethylenediaminetetraacetate tubes and tested immediately on a hematology analyzer (Advia 120, Siemens, Tarrytown, NY) for concentration and mean PLT volume (MPV).

**CD62P analysis by flow cytometry**

PCs diluted to a concentration of $200 \times 10^9$ to $300 \times 10^9$ PLTs per L were stained for 30 minutes with anti-CD62-PE/CD42-FITC (Beckman Coulter, Inc., Mississauga, Ontario, Canada) in HEPES-buffered saline (15 mmol/L HEPES, 150 mmol/L NaCl, pH 7.40). Controls were incubated with thrombin and 25 μM gly-pro-arg-pro or with IgG-PE/IgG-FITC instead of CD62/CD42 antibodies (Beckman Coulter, Inc.). The samples were fixed in 0.2 percent formal saline and then analyzed on a flow cytometer (Coulter EPICS XL, Beckman Coulter, Hialeah, FL).

**PLT morphology**

For morphology scoring, PC samples were fixed with 4 percent paraformaldehyde and stored at room temperature for up to 1 week before analysis. Duplicate samples were scored on a light microscope (Nikon, Tokyo, Japan), 100× oil immersion lens, phase contrast 3; PLTs were scored as discoid (4 points), spiny (2 points), or spherical/balloon (1 point).

**Total protein assay**

Undiluted PLT samples were centrifuged in a bench-top centrifuge (CS-6R, Beckman Coulter, Inc.) for 30 minutes ($2300 \times g$), and the supernatant (~2.50 mL) was stored frozen at −20°C until assayed for total protein content with a commercially available protein assay (Biorad, Pierce, Inc., Nepean, Ontario, Canada).

**Evaluation of in vitro PLT metabolism**

On Days 1, 2, 3, 5, and 7, pooled PCs were sampled aseptically and analyzed for pH, pO2, pCO2, glucose concentration, and lactate concentration within 15 minutes of sampling on a self-calibrated blood gas analyzer (GEM Premier 3000, GMI, Inc., Ramsey, MN).

**PLT integrity**

Extent of shape change (ESC) in response to ADP and hypotonic shock response (HSR) were evaluated with a platelet aggregometer (Chrono-Log SPA-2000, Labmedics, Manchester, UK) according to the manufacturer’s instructions.

**Statistical analysis**

The results are presented with 95 percent confidence intervals. To pinpoint the day on which changes become significant for PCs stored with a particular medium (plasma, SSP+, Composol, or T-Sol), their respective Day 1 results were compared with those from subsequent days with t tests. A two-factor analysis of variance (ANOVA) with replication (with MS Excel 2003, Microsoft Corp., Redmond, WA) was performed to deduce significant interactions between storage medium and storage length and a p value of less than 0.05 was considered significant. Correlations were sought among PLT variables and are presented with critical values at 0.05 and 0.01.

**RESULTS**

**PLT component description**

When autologous plasma was used, PLT recovery from the BCP was significantly higher ($p < 0.05$) than PLT recovery with PAS (Fig. 1), and there were no differences among the PAS either individually or grouped. The PLT concentration of PCs stored in plasma or PAS was relatively constant over the time of storage (not shown), and ANOVA with replication did not reveal significant interactions between storage medium and storage length. PCs stored in plasma showed a significant increase of MPV between Day 1 and Day 2 ($p < 0.05$, not shown), but this increase did not persist to subsequent days as the PLTs returned to their initial volumes and the relative changes of MPV for PCs in plasma or PAS were not significant. The WBC concentrations were 1 to 2 logs below Canadian Blood Services’ and the Council of Europe’s quality control (QC) guidelines.

The soluble total protein concentration was used to estimate the residual plasma in PCs stored with T-Sol at 29 ± 2 percent, Composol at 27 ± 2 percent, and SSP+ at 30 ± 6 percent. Extended sample (8 mL) incubation by BacT/ALERT for 5 days did not detect the presence of...
aerobic bacterial contamination in any of the PCs sampled at any stage of the experiments.

PLT activation as detected by CD62P expression was approximately 32 percent (Fig. 2A) as a baseline level on Day 1. An apparent decrease of CD62 expression was seen on Day 2 for PC in plasma, Composol, and SSP+, while the activation level of PLTs in T-Sol increased. These changes of activation levels, although graphically apparent, were not significantly different relative to their respective Day 1 values. CD62 expression became significantly higher ($p < 0.05$) than their respective Day 1 values for PCs stored in T-Sol on Day 2, SSP+ on Day 5, and Composol and plasma on Day 7. This is supported by ANOVA as significant interaction ($p < 0.05$) between storage medium and storage length.

Compared to their own Day 1 morphology scores, PCs stored in plasma and Composol revealed significant decreases by Day 3, while PCs in SSP+ showed significant decreases ($p < 0.05$) only by Day 5. PLTs stored in T-Sol presented with a relatively constant but extremely poor morphology score ($<250$) on all 7 days of storage (Fig. 2B). Significant interactions ($p < 0.05$) between storage medium and storage length in ANOVA analysis confirm these differences.

**PLT metabolism and function**

As expected, the partial pressures of oxygen (Fig. 3A) for PCs during storage increased while those of carbon dioxide (Fig. 3B) decreased. The $pO_2$ increases for all the storage media were similar (parallel lines) and differences among the PAS were nonsignificant. The significant interactions ($p < 0.05$) observed for $pCO_2$ confirm that the absolute changes of $CO_2$ levels were different, with PCs stored in plasma showing the steepest slope, indicating the fastest rate of $CO_2$ decrease, but differences among PAS were nonsignificant.

Glucose was consumed steadily during storage in all PCs (Fig. 4A), and the rate of lactate production was approximately twice that of glucose consumption (Fig. 4B). Note that the initial glucose concentration in plasma-stored PCs was approximately three times that of PCs stored in PAS. As well, the rate of glucose consumption for PCs in plasma was visibly higher than...
glucose consumption in PAS. The individual rates of glucose consumption and lactate production in the PAS were also significantly different (ANOVA, p < 0.05), with those in T-Sol showing the highest glucose consumption rate, followed by SSP+ and then by Composol, which suggests that the other components of the PAS affect glucose utilization. Glucose consumption rates were plasma, 0.8447 mmol per L per day; T-Sol, 0.6231 mmol per L per day; SSP+, 0.5326 mmol per L per day; and Composol, 0.4178 mmol per L per day. Lactate production rates were plasma, 1.2259 mmol per L per day; T-Sol, 1.1438 mmol per L per day; SSP+, 1.1327 mmol per L per day; and Composol, 0.8453 mmol per L per day.

The pH for all PCs increased between Day 1 and Day 5 before leveling off and/or decreasing (Fig. 5). Relative to their respective Day 1 values, PCs stored in PAS showed significantly higher (p < 0.05) pH on all subsequent days. The pH of plasma-stored PC increased significantly (p < 0.05) only on Days 3 and 5 and returned to their Day 1 values by Day 7. The patterns of changes of pH among PCs stored in the different media over 7 days were significantly different by ANOVA (p < 0.05).

The ESC of PCs stored in plasma were visibly higher than those stored in PAS (Fig. 6A). Decreases of ESC did not become significant (p < 0.05) from their respective Day 1 values until Day 7 for plasma, Day 5 for SSP+ and Composol, and Day 3 for T-Sol. The differences among the
curves were confirmed to be significant (p < 0.05) by ANOVA, which indicates that the relative trends in ESC were significantly different from each other in terms of both overall performance and rates of change over time.

The HSR of PCs stored in plasma was also higher compared to those stored in PAS (Fig. 6B). Relative to their respective Day 1 HSR scores, decreases of PCs stored with plasma over 7 days were not significant; decreases of PCs stored with Composol became significant (p < 0.05) from Day 5 onward and with SSP+ from Day 3 onward, but not on Days 1 and 2; and decreases in PCs stored with T-Sol became significant (p < 0.05) from Day 3 onward. ANOVA analysis, however, did not detect significant interactions between storage medium and storage length and therefore the relative decreases of HSR scores are not considered to be significant.

Correlation studies
For PCs stored in plasma and PAS, there were prevailing correlation trends between glucose and lactate, glucose and morphology, lactate and pCO2, lactate and morphology, pH and pCO2, pO2 and pCO2, and morphology and pH and pCO2, lactate and morphology (Table 2).

**DISCUSSION**
To blood product manufacturers, the quality of the final pooled PLT component is not only a function of meeting QC limits, but also of the PLTs’ perceived ability to provide in vivo hemostatic support. Because very few in vitro PLT variables measurements have any direct relationship to in vivo function, we sought correlations on an in vitro panel to find differences in the effects of storage in various PAS. Consequently, we measured a number of variables and assessed their aggregate results to make comparisons.

Viable PLT count is probably the most important information for clinicians; thus PLT recovery from BCP is essential to the manufacture of a good transfusion product. PLT recovery is dependent on the centrifugation settings and the viscosities of the storage medium used to pool theuffy coats. PASs have viscosities similar to that of water while the soluble proteins in plasma enhance its viscosity; thus pooling with plasma results in higher recoveries than pooling with PAS (Fig. 1). From a blood component manufacturing perspective, pooling with plasma results, in a purely numerical sense, in a superior transfusion product.

Reduction of PLT numbers during storage could be caused by activation resulting in microaggregation, fragmentation, or losses of PLT integrity. These may be caused by the storage medium itself or as a by-product of the PLTs’ metabolism and consequent pH changes. Previous studies have ruled out apoptosis as a major contributor to PLT loss in vitro, and only one article describes significant PLT losses in multiple electrolytes (PlasmaLyte, Baxter Corp.) compared to 5-day storage in plasma. PlasmaLyte is similar to Composol but without sodium citrate, which suggests that certain formulations can better maintain PLT integrity. It has also been suggested that not only the formulation but a minimum of 25 percent residual plasma and plasma factors in the PAS is required for integrity. This had been met intrinsically by the production process such that absolute decreases of PLT concentration were less than 1 percent per day.

Evidence of PLT stress in storage is measured by α-granule content release, which in turn leads to surface expression of CD62 P-selectin. PLT activation on Day 1 can run between 15 and 30 percent (Fig. 2A). These initial levels are attributed to the collection and production processes and are consistent with other studies on buffy-coat PLTs. During storage, surface CD62 expression

<p>| TABLE 2. Correlations among metabolic characteristics of PLTs stored in plasma and PAS* |
|-------------------------------------|----|----|----|----|----|----|----|----|</p>
<table>
<thead>
<tr>
<th>CD62</th>
<th>Glucose</th>
<th>Lactate</th>
<th>pH</th>
<th>pO2</th>
<th>pCO2</th>
<th>ESC</th>
<th>HSR</th>
<th>Morphology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>-0.87†</td>
<td>-0.39</td>
<td>0.46</td>
<td>0.062</td>
<td>0.275</td>
<td>-0.41</td>
<td>-0.12</td>
<td>-0.18*</td>
</tr>
<tr>
<td>Lactate</td>
<td>0.69‡</td>
<td>-0.72†</td>
<td>-0.96†</td>
<td>-0.11</td>
<td>-0.52†</td>
<td>0.78‡</td>
<td>0.76‡</td>
<td>0.61‡†</td>
</tr>
<tr>
<td>pH</td>
<td>-0.38</td>
<td>0.12</td>
<td>0.19</td>
<td>0.062</td>
<td>0.275</td>
<td>-0.41</td>
<td>-0.12</td>
<td>-0.18*</td>
</tr>
<tr>
<td>pO2</td>
<td>0.15</td>
<td>0.030</td>
<td>0.30</td>
<td>0.31†</td>
<td>0.71†</td>
<td>0.030</td>
<td>0.31†</td>
<td>-0.14</td>
</tr>
<tr>
<td>pCO2</td>
<td>-0.12</td>
<td>0.13</td>
<td>-0.60‡</td>
<td>-0.83†</td>
<td>-0.78†</td>
<td>0.64‡</td>
<td>0.55‡</td>
<td>0.55‡</td>
</tr>
<tr>
<td>HSR</td>
<td>-0.11</td>
<td>-0.087</td>
<td>-0.099</td>
<td>-0.22</td>
<td>-0.14</td>
<td>0.11</td>
<td>0.11</td>
<td>0.50</td>
</tr>
<tr>
<td>Morphology</td>
<td>-0.41</td>
<td>0.58‡</td>
<td>0.81†</td>
<td>-0.35</td>
<td>-0.49</td>
<td>0.76‡</td>
<td>0.30</td>
<td>-0.097</td>
</tr>
<tr>
<td>MPV</td>
<td>-0.62‡</td>
<td>-0.42</td>
<td>-0.15</td>
<td>-0.066</td>
<td>0.21</td>
<td>0.030</td>
<td>-0.56†</td>
<td></td>
</tr>
</tbody>
</table>

* Correlations of PLT in vitro variables on Days 1, 5, and 7, n = 13. Shaded areas represent correlations for PCs stored in plasma and unshaded areas represent correlations for PCs stored in PAS.
† Significant correlation, α = 0.01.
‡ Significant correlation, α = 0.05.
increases. P-selectin, however, is also cleaved from the PLT surface. Thus, if the rate of activation is slower than the rate of cleavage, CD62 surface expression should decrease, leading to an apparent recovery from activation. We found such an apparent recovery in PCs stored in plasma, SSP+, and Composol but not in T-Sol. The apparent recovery is observed between Days 1 and 3 and is most pronounced in PCs stored in Composol. Similar data were obtained in other studies. The rate of storage-induced PLT activation may be related to the ability to control increasing calcium levels. The components responsible for sequestering free calcium ions are suspected to be phosphates in SSP+, gluconate in Composol, a multitude of entities including phosphates, and calcium-binding proteins in plasma. As a result, PCs in Composol experience greater activation recovery because gluconate is a potent chelator of calcium. The activation trends for SSP+ and Composol approximate plasma much more closely than does T-Sol, and this may be the result of efficient metabolic control via potassium and magnesium offered by the latest generation of PAS (Composol and SSP+).

An important measure of in vitro PLT quality is the morphology score. The absolute morphology scores of PLTs stored in SSP+ and plasma were quite similar, while Composol trailed in parallel (Fig. 2B). As higher morphology scores have been correlated to higher pH, because of the additional buffering capacity provided by sodium phosphates in SSP+, the superior ability to preserve PLT morphology by SSP+ is not entirely surprising. As well, the contribution of potassium and magnesium to PLT viability becomes evident when comparing the morphology scores of plasma, SSP+, and Composol to T-Sol that does not contain these ions. This is also consistent with significant correlations linking morphology to glucose and lactate in both plasma and PAS (Table 2).

Other factors, such as dissolved gases, also affect PLT metabolism and survival in storage. The partial pressures of dissolved gases such as pO2 and pCO2 are a function of equilibration with their respective solubility at the beginning of storage (pO2, approx. 160 mmHg in plasma and PAS; pCO2, 62 mmHg in plasma, 21 mmHg in PAS, assuming pH 7.2, T = 295K, [HCO3-] = 24.0 mmol/L in plasma, [HCO3-] = 8.0 mmol/L in PAS); the nature of the breathable container, gas consumption and/or production via oxidative metabolism, and bicarbonate decomposition via pH changes.

The change of blood gas levels reflects the gas-exchanging ability of the storage container. Maintaining high pO2 and low pCO2 is crucial during PLT storage because hypoxia may lead to increased glycolysis, lactate production, and subsequent pH decrease. O2 equilibration is faster than O2 consumption (Fig. 3A) because pO2 increases steadily during storage. On Day 1 all the PCs contain a pO2 of 75 to 80 mmHg; therefore, they all experience the same driving force toward equilibration with atmospheric pO2 at 180 to 200 mmHg.

The pCO2 of stored PCs decreased (Fig. 3B) and reached equilibrium. Although all stored PCs show the pCO2 decrease, the trend of pCO2 behavior is drastically different for PCs stored in plasma compared to those in PAS due to different carbonate content, which affects the solubility of CO2. For plasma-stored PCs, venous collection resulted in high pCO2 on Day 1: roughly three times that of PCs in PAS (25 mmHg). HCO3- and CO32- produce CO2(aq) in the presence of H+ ions. In plasma and PAS, pH increases until Day 5, which has a negative impact on CO2(aq) solubility and pCO2 decreases. When apparent pH decreases past Day 5, carbonates are converted to CO2(aq) and a pCO2 increase is observed. While bicarbonate is the major buffer for plasma, however, PASs contain other buffering ions in high concentration so there is a more significant pCO2 increase in plasma.

The availability of an energy supply also has implications for long-term storage. None of the PASs used in this study contain glucose; thus, all of the initial glucose in PCs stored in PAS is assumed to have come from the roughly 30 percent residual plasma. Glucose is crucial for maintaining the viability of PLTs in vitro. As well, PCs stored in Composol and SSP+ showed higher rates of glycogen and triacylglycerol consumption relative to T-Sol.3 As well, PCs stored in Composol show lower rates of glycogen and triacylglycerol consumption relative to those in SSP+ and T-Sol. As well, PCs stored in Composol show lower rates of glycogen and triacylglycerol consumption relative to those in SSP+ and T-Sol. As well, PCs stored in Composol show lower rates of glycogen and triacylglycerol consumption relative to those in SSP+ and T-Sol.
storage medium results in increased PLT metabolism and eventually leads to pH decrease, pO₂ increase, and loss of viability.⁴,¹⁵,¹⁶

The primary contributor to the decrease of pH in PCs is glucose metabolism via glycolysis and the production of lactate.⁴ Since the PASs under evaluation contain only approximately 30 percent residual plasma, the plasma-derived glucose (approx. 6.0 mmol/L) can be consumed quickly, and the pH will remain stable thereafter. In contrast, PLTs stored in plasma will experience further pH decreases due to the higher initial glucose content of approximately 18.0 mmol per L. The pH of PCs stored in plasma can decrease as low as 6.0 while PCs in PAS can maintain a stable pH at 6.5 to 6.7 after glucose depletion.³⁴ PAS may have lower buffering capacity compared to plasma and thus experience significantly faster pH decreases.⁷⁵

In Fig. 5, the pH of PCs stored in plasma and PAS increased between Day 1 and Day 4, before declining. In other studies, the same phenomenon has been observed albeit to a lesser degree.¹⁵,¹⁶,²⁶ Preprocessing storage of WB in nonpermeable storage containers for up to 24 hours (the buffy-coat method) could lead to initial hypoxic conditions that promote glycolysis and consequent pH decreases caused by lactate produced by the blood cells and released into the plasma which is then used for storage. Over time, the bicarbonate buffer in the plasma decomposes into H₂O and CO₂ and along with other buffering components (such as phosphates and acetate) increases the pH of the PCs. Hence PCs stored in plasma, which had the highest initial bicarbonate content, experienced the greatest pH recovery. Apparent decreases began when the bicarbonate was either depleted or when the rate of glycolysis exceeded the rate of bicarbonate decomposition as well as the buffering capacity of the remaining buffering components. This is illustrated by the rapid pH decrease of T-Sol in which the main buffering component is acetate (Table 1) that removes hydrogen ions by conversion to a protonated form before subsequent oxidation.³⁶ It is reasonable to assume that the acetate’s buffering capacity alone is not sufficient for pH control for the number of PLTs in the storage container.

Although Composol does not contain additional buffers compared to T-Sol, its potassium and magnesium content suppress a rapid pH decrease indirectly, by influencing glycolysis.³⁴ Studies have shown that citrate and magnesium increase potassium efflux through the PLT membrane¹⁶ and decrease the ADP-activated binding of fibrinogen that takes place during PLT activation.³⁶ These influences may be indirectly responsible for reducing the rate of glycolysis through a set of as yet unknown mechanisms. The additional 25 mmol per L sodium phosphate in SSP⁺ should prolong pH maintenance until the depletion of glucose.

The ESC ad HSR are a reflection of the PLTs’ morphology³⁷ and integrity and energy reserves, respectively. ESC, the optical detection of the response to ADP activation, decreased for PLTs stored in all the types of media and was especially evident in PLTs stored with PAS.³⁸ PCs stored in PAS generally have lower morphology scores than those stored in plasma; multicenter studies have found correlations between morphology and ESC to be as high as 0.93,³⁸ suggesting that PLTs, which are not morphologically discoid, may not experience the same degree of shape change in response to ADP.

Similar to ESC, HSR values also decrease steadily (Fig. 6). Although perhaps ambiguous at first, PLT HSR behavior correlates (0.58) strongly with ESC, especially during the latter days of storage. Highly stressed PLTs often exhibit high metabolism, including higher rates of glycolysis, oxidation, oxygen consumption,⁴ and low energy reserves.⁴⁰ In addition, PLT integrity is affected by osmotic balance, maintained by proteins both inside and outside the PLTs. Because PCs stored with PAS only contain approximately 30 percent residual plasma and the PASs themselves do not contain any components that support osmotic balance, PLTs stored in PAS experience a net water gain during storage which expands them and may compromise their membrane integrity and energy supplies as they attempt to pump out water. In turn, this could lead to increased metabolic requirements to replenish ATP reserves, with an increased rate of oxidation, increased rate of glycolysis, faster pH decreases, poor morphology, and finally poor performance in ESC and HSR.

**Correlations**

Although correlation studies can distinguish trends but cannot be used to identify causal relationships, the prevailing correlations for storage in both plasma and PAS can identify major relationships among the measured PLT storage variables (Table 2). Some of the correlations may not be as much due to PLT metabolism as to gas exchange, such as the strongly negative correlations between pCO₂ and pO₂, lactate and pCO₂, and pCO₂ and morphology, where opposing trends exist probably coincidentally.

PLT metabolism requires some direct correlations such as those between glucose and lactate, where glucose consumption directly results in lactate production, as well as between pH and pCO₂, where the pH correlates negatively with pCO₂ (as H⁺ ions drive the reaction of carbonates toward production of CO₂(aq)).

There are some indirect but intuitive correlations between glucose and morphology, as well as between lactate and morphology. Morphology is generally a measure of PLT viability; low glucose or no glucose is usually the result of extended storage, which indirectly results in loss of viability.⁴ As well, lactate has been nega-
tively correlated with in vivo recovery,\textsuperscript{41} which is also a measure of PLT viability.

The major differences between plasma- and PAS-stored PLTs are the number of strong correlations for PAS among glucose, lactate, pO\(_2\), pCO\(_2\), ESC, HSR, and morphology. A higher degree of apparent interdependence of these variables is shown for PAS-stored PCs compared to plasma-stored PCs. The main cause for glucose loss, aside from normal metabolism as observed for plasma, is due to the constant energy requirement by ion pumps to maintain osmotic balance. It is probable that this is responsible for the observed higher correlations between glucose and lactate. Because osmotic balance is lost over time, as suggested by the significant decreases of ESC, HSR, and morphology, PLTs in PAS are fighting a losing battle while consuming a valuable and yet dangerous resource: glucose.

In plasma, which is osmotically balanced with proteins, most of the strong correlations observed for PAS, including strong dependence on glucose and lactate, are less apparent. There are strong correlations among CD62 and glucose/lactate/MPV/morphology, however, as well as between pO\(_2\) and pH, which indicates that high metabolism is linked to degranulation, osmotic destabilization and/or swelling, and oxygen availability is strongly associated with pH maintenance in an osmotically balanced medium.

**CONCLUSION**

All storage media, whether synthetic or natural, were able to maintain pH levels above 7.0 over a 7-day storage period. PLTs stored in plasma, SSP\textsuperscript{+}, and Composol, in that order, however, appear to possess greater buffering capacity than T-Sol and may be more suited for extended storage up to Days 10 or 12. Although PASs were designed with only ionic balance in mind, it can be inferred from the ESC and HSR experiments that PLTs suffer loss of osmotic balance during storage in PAS, which do not have components to moderate PLT fluid uptake. Based on these comparisons, plasma appears to best support PLTs in storage. The newer PAS formulations, however, offer sufficiently good storage potential to merit financial consideration.

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