Storage of platelet concentrates from pooled buffy coats made of fresh and overnight-stored whole blood processed on the novel Atreus 2C+ system: in vitro study

Per Sandgren, Martine Callaert, Agneta Shanwell, and Hans Gulliksson

BACKGROUND: The Atreus 2C system (Gambro BCT) automatically separates whole blood (WB) into buffy coat (BC), red blood cells (RBC), and plasma and transfers the components into separate containers. After processing with the Atreus, 4 to 6 BC units can be pooled and processed into leukoreduced platelets (PLTs) by use of the automated OrbiSac BC system (Gambro BCT). The aim of our in vitro study was to investigate the effects of holding either WB or BC overnight before preparation of PLTs by use of the Atreus 2C+ system for BC preparation. A standard routine procedure involving conventional blood containers for the preparation of BC combined with the OrbiSac process (top-and-top system; Terumo) was used as a reference.

STUDY DESIGN AND METHODS: WB was either processed within 8 hours after collection ("fresh blood") or stored overnight before processing. WB units were separated into BC, RBC, and plasma units and transferred into individual containers. Either the BC or the WB units rested overnight at 22 ± 2°C. Six ABO-identical BCs, obtained from either fresh or overnight-stored WB, were pooled and processed with the OrbiSac BC system to obtain leukoreduced PLTs. In total, 20 Atreus and 10 reference (leukoreduced PLTs) samples were analyzed for various in vitro variables during the 7-day storage period.

RESULTS: No significant difference in glucose consumption, lactate production, mean PLT volume, LDH activity, bicarbonate, ATP, RANTES, and the expression of CD62p and CD42b between groups was detected. pH was maintained at greater than 7.0 (Day 7). Swirling remained at the highest levels (score, 2) for all units throughout storage.

CONCLUSION: PLTs derived from BCs, obtained from either fresh or overnight-stored WB processed on the novel automated Atreus 2C+ system, were equivalent to control PLTs with regard to PLT in vitro characteristics during 7 days of storage. Stable recovery of PLTs and satisfactory PLT content according to current standards were also found.

Several techniques for the preparation of red blood cells (RBCs), buffy coat (BC), and plasma from whole blood (WB) are used to satisfy different requirements. These techniques, however, require many manual steps affecting staff time and production flow in the blood center. A novel automated machine, the Atreus 2C+ system (Gambro BCT, Zaventem, Belgium), eliminates several of those manual steps by combining them into one integrated process, which provides several potential benefits.

Over the past 15 years, methods for preparing platelets (PLTs) from WB donations based on pooled BCs have been developed. These techniques have been progressively replaced at several blood centers by use of an automated technique (OrbiSac system, Gambro BCT). A synergy between the Atreus and the OrbiSac allows for automated production of PLTs from WB.

ABBREVIATIONS: Atreus F = platelets, derived from BCs, obtained from fresh WB processed on the Atreus system; Atreus S = platelets, derived from BCs, obtained from overnight stored WB processed with the Atreus system; BC = buffy coat; ESC = extent of shape change; HSR = hypotonic shock response; IgG1 = immunoglobulin G1; MPV = mean platelet volume; PAS = platelet additive solution; RANTES = regulated upon activation of normal T-cells expressed and secreted; TaT-F = platelets, derived from BCs, obtained from fresh WB processed with conventional blood containers, the top-and-top system; WB = whole blood.

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In routine practice, either WB units or BCs are kept overnight at room temperature preceding PLT preparation. The effect of holding BCs or WB overnight before preparation of PLTs, on in vitro PLT quality, has previously been described by several authors. Nevertheless, little is known about these effects with the novel Atreus 2C+ system in combination with the OrbiSac system for preparation of PLTs from pooled BCs.

PLTs have been shown to be variably affected by preparation and subsequent storage, which may be associated with different processing systems. For this reason, further development of such technique that will meet the increasing demands for quality, safety, standardization, and efficiency in preparation of blood components for transfusion is needed.

The aim of our in vitro study was to compare the effects of holding either BCs or WB overnight before preparation of PLTs stored in additive solution (T-Sol, Fenwal, La Châtre, France), in combination with use of a novel automated equipment, the Atreus 2C+ system for BC preparation (Gambro BCT). The results were compared with those of our standard routine procedure for BC preparation, a conventional quadruple top-and-top system (Terumo, Tokyo, Japan) in combination the OrbiSac to produce PLT units. The quality of the RBCs and plasma prepared from the Atreus system are evaluated in different studies.

**MATERIALS AND METHODS**

**Blood collection and preparation of blood components including BCs**

This study that is outlined in Fig. 1 evaluated in vitro quality during a 7-day storage period of BC-derived PLTs, prepared from either fresh (Atreus F) or overnight-stored WB (Atreus S) and processed with the Atreus system. The Atreus equipment includes an automatic centrifuge, a hydraulic expressor, valve/sealers, and a bar code reader integrated in the same equipment. The Atreus set for the preparation is similar to a conventional blood bag system (containing both citrate phosphate dextrose [CPD] and saline-adenine-glucose-mannitol), except that it also includes an additional separation bag ("round bag"). After the Atreus processing, RBC units are transferred through a leukoreduction filter to the appropriate bag for storage. By the Atreus centrifugal separation process, leukoreduced plasma is produced in the system with no further need of filtration.

The results from the Atreus groups were compared with pools obtained from BCs prepared from fresh WB (control units) and BCs stored overnight. Conventional quadruple top-and-top blood bag systems with inline RBC filters for white blood cell (WBC) removal were used for blood collection and preparation of blood components including BC (TaT-F, Terumo). The top-and-top system (Imuflex-CRC, Terumo) was made of polyvinylchloride (PVC) with di(2-ethylhexyl)phthalate as plasticizer, containing 63 mL of standard CPD. The WB bags were kept at room temperature (20-24°C) by cooling plates (Sebra, Tucson, AZ) and centrifuged at 2700 × g for 10 minutes at 22°C within 8 hours of collection. Centrifugation was immediately followed by separation into RBCs, plasma, and BCs with automated equipment, an automatic component extractor (T-ACE, Terumo). BCs were kept overnight without agitation at room temperature (20-24°C).

A total of 450 mL of WB was collected from healthy blood donors who met standard donation criteria and gave their written informed consent in accordance with our institution’s guidelines. WB was either processed within 8 hours after collection (Atreus F and TaT-F) or stored overnight (14-24 hr, Atreus S) before processing. Both the BC and the WB units rested overnight without agitation at 22 ± 2°C. BC units in all three groups were pooled and leukoreduced by the OrbiSac BC system to prepare PLTs.

**Preparation and storage of PLTs**

A well-established automated technique, the OrbiSac system (Gambro BCT), monitored by a system of pressure sensors and photocells, was used in the next step to prepare PLTs from six pooled ABO-identical BCs, obtained either from the Atreus system or the top-and-top system.
The synergy between the Atreus and the OrbiSac systems is outlined in Fig. 2. The PLTs were stored in 70 percent PLT additive solution (PAS; T-Sol, Fenwal) and 30 percent plasma on a flat-bed agitator (60 cycles/min, model LPR-3, Melco, Glendale, CA) in a temperature-controlled cabinet at 22 ± 0.1°C. The samples were drawn aseptically on Days 1, 3, 5, and 7. All sampling was done by sterile connection (TSCD-II, Terumo) of sampling bags to the respective containers (OrbiSac standard BC set, storage bag, Gambro BCT). The storage bag is made of PVC plastic with a citrate-based plasticizer and intended to contain up to 5 × 10¹¹ PLTs in 400 mL of plasma and PAS.

Analysis of metabolic and cellular variables
Metabolic and cellular in vitro variables were evaluated in a PLT storage study, including measurements of PLT counts (10⁹/L and 10⁹/unit) and mean PLT volume (MPV) by using a cell counter (Cellguard CA620, Boule Medical, Stockholm, Sweden). The volume (mL) was calculated by weighing the contents of the storage bag, in grams, on a scale (Mettler PB 3000, Mettler-Toledo, Albstadt, Switzerland) and the result, in grams, was divided by 1.01 (1.01 g/mL is the density of the storage medium composed of approx. 70% PAS and 30% plasma). By use of routine blood gas measurements (ABL 705, Radiometer, Copenhagen, Denmark), we also measured the pH, pO₂ (kPa at 37°C), pCO₂ (kPa at 37°C), glucose (mmol/L), and lactate (mmol/L). Bicarbonate (mmol/L) was calculated based on other measured variables.

The assessment of swirling was done by inspection and grading according to Bertolini and Murphy.¹² The WBC count on Day 1 was determined with a Nageotte chamber and a microscope (standard, Zeiss, Chester, VA).¹³ Hypotonic shock response (HSR) reactivity and extent of shape change (ESC) measurements were performed with a dedicated microprocessor-based instrument (SPA 2000, Chronolog, Havertown, PA) with the modifications of these tests described by VandenBroeke and coworkers.¹⁴ The total adenosine triphosphate (ATP) concentration (µmol/10¹¹) was determined with a luminometer (Orion microplate luminometer, Berthold Detection Systems GmbH, Pforzheim, Germany) on the basis of principles described by Lundin.¹⁵ Lactate dehydrogenase (LDH) activity, a marker for disintegration of PLTs, was measured with a spectrophotometric method (Sigma Aldrich kit 063K6003; Spectrophotometer DMS 100, Varian Techtron, Springvale, Australia).¹⁶

Cytokine analysis
Samples from the PLTs were collected in prechilled citrate theophylline adenosine dipyridamole tubes. The samples were centrifuged at 2500 × g per relative centrifugal force (Model 5810R, Eppendorf, Westbury, NY) for 30 minutes at +10°C and the supernatants were stored at −70°C pending analysis. RANTES plasma concentrations were determined with commercial enzyme-linked immunosorbent assay kits in accordance with the manufacturer’s recommendations (Quantikine R&D System, Abingdon, UK). The standards for RANTES were those provided by the manufacturer. Ranges for measurements in the assay were 31.2 to 2000 pg per mL. Dilution and reassay of specimens were done as required. All samples and standards were assayed in duplicate. All results are given in picograms per 10⁶ PLTs.

Monoclonal antibody labeling
PLT concentrate samples, fixed by adding an equal volume of 1 percent paraformaldehyde–phosphate-buffered saline (PBS; pH 7.2–7.4) at 22°C for 10 minutes, were then stained for 20 minutes at the same temperature in the dark by incubating with 20 µL of fluorochrome-labeled monoclonal antibodies (MoAb) per 1 × 10⁸ PLTs. The following MoAbs were used for single-color...
staining: fluorescein isothiocyanate (FITC)-conjugated (immunoglobulin G1 [IgG1] isotype) CD41 (clone P2), CD42b (clone SZ2), and CD61 (clone SZ21) and phycoerythrin (PE)-conjugated (IgG1 isotype) CD63 (clone CLB-gran12) and CD62p (p-selectin/GMP-140/PADGEM; clone CLB Thromb/6), all purchased from Immunotech (Beckman Coulter, Marseilles, France). Control specimens were processed as above, but incubated with a FITC- or PE-conjugated MoAb (IgG1 isotype) with irrelevant specificity, purchased from Immunotech (Beckman Coulter, Marseilles, France). After incubation with fluorochrome-conjugated antibodies, the samples were washed twice by adding 2.0 mL of filtered PBS-ethylenediaminetetraacetic acid with 0.1 percent Na-azide and centrifuged at 2760 g (Eppendorf 5810R) for 10 minutes at +18°C.

Flow cytometry analysis
A total of 100,000 PLT events were acquired on a flow cytometer (FACSCalibur, Becton Dickinson, Franklin Lakes, NJ) equipped with 15 mW argon ion lasers and its accompanying software (CellQuest Pro, Becton Dickinson). Daily controls of optics and fluorescence intensity were performed with standardized beads (Calibrite, Becton Dickinson). The flow cytometer settings were optimized for the acquisition of PLTs by logarithmic signal amplification in all four detectors (forward and side scatter channels and fluorescence channels FL1 and FL2). For analysis, the gate was set around intact PLT population as defined by forward and side scatter characteristics. The percentage of positive PLTs of total PLT expressing activation markers (CD62p and CD63) and surface membrane glycoproteins (CD41, CD42b, and CD61) above that of background (negative control) as well as the mean fluorescence intensity (MFI) was recorded.

Bacterial cultures
Bacterial cultures were performed on Day 7 with the routine methods of the bacteriologic laboratory at Karolinska University Hospital Huddinge, including aerobic cultures performed on agar plates, with 48 hours to final report.

Statistical analyses
The mean values and standard deviations (SDs; n = 100) are usually given. Repeated measurement analysis of variance was performed on data as well as post hoc tests multiple comparisons in an attempt to identify differences between specific groups. Three different groups were studied over time (days). “Days” was the repeated factor and “group” was a between factor. The p value for the interaction term between days and groups is reported. The analyses were carried out with computer software (Statistica, Version 14.0, SPSS, Chicago, IL).

RESULTS
In this study, PLTs were studied, which were derived either from BCs obtained from fresh WB (Atreus F, n = 10) or overnight-stored WB (Atreus S, n = 10) and prepared with the Atreus 2C+ system. PLTs (n = 10), obtained from BCs prepared from fresh WB with conventional blood bags (TaT-F, control) were studied in parallel and used as a reference.

Cellular assays
The PLT counts and contents on Day 1 are given in Table 1. During the following storage period, the PLT counts and contents were significantly higher in the Atreus S group than in the Atreus F and TaT-F groups (data not shown). No significant difference in PLT counts and contents between the Atreus F and TaT-F groups was detected throughout storage (data not shown). No significant difference in MPV and LDH activity between groups was detected (Table 2).

Metabolic assays
No significant differences in glucose consumption, lactate production, and bicarbonate and ATP levels between groups were detected (Table 2). pH was slightly higher in the Atreus F group. No significant difference in pH levels between the other two groups was detected (Table 3). Carbon dioxide (pCO2) was higher in the Atreus S group, and no significant difference in pCO2 between the other two groups was detected (Table 3). The oxygen tension (pO2) differed significantly in the Atreus S group from the other two groups (Table 3).

HSR, ESC, and swirling
HSR was slightly higher in the Atreus S group. No significant difference between the Atreus F and TaT-F groups

<table>
<thead>
<tr>
<th>WB processing system</th>
<th>Volume (mL)</th>
<th>PLTs (x10^3/L)</th>
<th>WBCs (x10^3/units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atreus F</td>
<td>357 ± 27</td>
<td>896 ± 136</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>Atreus S</td>
<td>365 ± 10</td>
<td>988 ± 122</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>TaT-F</td>
<td>381 ± 14</td>
<td>854 ± 62</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

* Results are expressed as mean ± SD (n = 10).
was detected. ESC was significantly higher in the TaT-F than in the other two groups. No significant difference with regard to ESC between the Atreus F and Atreus S groups was detected (Table 3). Swirling remained at the highest levels (score, 2) for all units at all times.

**Cytokine analysis**

The concentrations of RANTES (Table 3) increased during storage in all units. No significant difference in the concentration of released RANTES between groups was detected.

**Flow cytometry analysis**

The percentage of PLTs expressing the activation markers CD62p as well as the MFI (data not shown) increased during storage in all units. No significant difference in the percentage of PLTs expressing the activation marker CD62p between groups was detected (Fig. 3). CD63 increased moderately during storage in all units and was lower (MFI [Fig. 3] in the Atreus F group compared with the Atreus F group, p = 0.032; and TaT-F group, p < 0.01). No significant difference between the Atreus F and TaT-F groups was detected.

The surface expression (MFI) of CD41 (Fig. 3) and CD61 (Fig. 3) as well as the percentage of PLTs (data not shown) remained almost unchanged during storage in all units. A significant difference (MFI) between all groups (p < 0.01; CD41) and a significant difference (MFI) between the Atreus S and TaT-F groups (CD61) was detected (p = 0.026). The percentage of PLTs expressing CD42b (Fig. 3) as well as MFI (data not shown) decreased during storage in all units. No significant difference in the percentage of CD42b between groups was detected. All bacterial cultures were negative.

**DISCUSSION**

In this study, the in vitro quality of PLTs derived from BCs, obtained from either fresh or overnight-stored WB processed on the Atreus 2C+ system (Gambro, BCT), was evaluated and compared with data from our current routine procedure, based on the preparation of BCs with a conventional top-and-top blood container system. We found significant differences in a variety of cellular, metabolic, and flow cytometry variables between the various PLT groups. For most variables, however, the degree of change during storage showed little difference between the groups and appeared to be insignificant with regard to current in vitro standards and previously published data.

The novel Atreus 2C+ system, which in synergy with the OrbiSac system allows for automated production of pooled PLT units has several distinct advantages such as: 1) elimination of several manual steps by combining them into one integrated process and by that reducing staff labor time. 2) PLTs can be derived from BCs, obtained from either fresh or overnight-stored WB, whatever preferred, potentially increasing efficacy in the production flow of PLTs. 3) The possibility of processing PLTs from fresh WB potentially will give positive side effects with regard to higher plasma and RBC quality.17,18 Negative effects associated with blood collection, preparation, and storage of PLTs are generally referred to as the PLT storage lesion.19-21 These changes affect PLT ultrastructure, function, metabolism, and membrane properties. Some of the morphologic and biochemical changes that characterize this storage lesion are reminiscent of cell death by apoptosis. Nevertheless, recent work

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**TABLE 2. In vitro effects of PLTs, obtained from different WB processing systems and stored in T-Sol for 7 days**

<table>
<thead>
<tr>
<th>In vitro measure</th>
<th>Day</th>
<th>Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPV (fL)</td>
<td>Atreus F</td>
<td>8.9 ± 0.6</td>
<td>5.3 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>Atreus S</td>
<td>8.7 ± 0.4</td>
<td>5.5 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>TaT-F</td>
<td>8.6 ± 0.5</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td>LDH (%)</td>
<td>Atreus F</td>
<td>5.3 ± 1.2</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Atreus S</td>
<td>5.5 ± 1.7</td>
<td>5.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>TaT-F</td>
<td>5.0 ± 1.1</td>
<td>5.7 ± 1.1</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>Atreus F</td>
<td>6.4 ± 0.3</td>
<td>5.1 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>Atreus S</td>
<td>6.7 ± 0.4</td>
<td>5.6 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>TaT-F</td>
<td>6.4 ± 0.3</td>
<td>5.5 ± 0.1</td>
</tr>
<tr>
<td>Bicarbonate (mmol/L)</td>
<td>Atreus F</td>
<td>8.0 ± 0.2</td>
<td>8.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>Atreus S</td>
<td>8.3 ± 0.3</td>
<td>8.3 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>TaT-F</td>
<td>7.9 ± 0.5</td>
<td>7.7 ± 0.8</td>
</tr>
<tr>
<td>ATP (μmol/10^11)</td>
<td>Atreus F</td>
<td>8.36 ± 0.48</td>
<td>7.87 ± 0.51</td>
</tr>
<tr>
<td></td>
<td>Atreus S</td>
<td>7.97 ± 0.79</td>
<td>8.65 ± 0.63</td>
</tr>
<tr>
<td></td>
<td>TaT-F</td>
<td>8.26 ± 0.40</td>
<td>7.56 ± 0.39</td>
</tr>
</tbody>
</table>

* Data are reported as mean ± SD (n = 10). The p value represents the significance of the differences from baseline (Day 1) between groups (heterogeneity) during 7 days.
suggests that proteins involved in cell apoptosis are unlikely to account for the PLT storage lesion, because caspase activation is a late event during PLT storage.22

Although a variety of methods have been applied to evaluate PLT quality during storage,23 there appears to be no one in vitro test that clearly predicts the in vivo recovery, survival, and function of transfused PLTs. A battery of in vitro tests reflecting the different aspects of PLT function, however, is so far the best tool and is widely used in an attempt to identify the PLT storage lesion by detecting changes that occur during processing and storage of PLTs.

In this study, PLTs, derived from BCs, obtained from the Atreus system showed a satisfactory PLT content (Table 1) according to current European standards (>240 × 10^6/ unit). In contrast to a previous investigation,6 our results are in accord with those of others24 who have shown that PLT yield significantly improved when the WB units were stored overnight before processing. Indicators of PLT metabolism associated with preparation with the Atreus system were similar to those reported for PLTs from the routine procedure, except for occasional small differences in pH, PCO2, and PO2 values between the groups. Because no evident differences in PLT metabolism are observed, we suggest that these differences may be associated with impacts in the chemical equilibrium due to sampling, measurements, and differences in PLT yield.

HSR and ESC values decreased during storage in all units. There was a small statistically significant decrease in HSR in PLTs obtained from fresh WB compared with those from stored WB, which may be explained by a larger amount of T-Sol being used, owing to a lower PLT content in fresh WB, to achieve the dilution required for the HSR test.14 We found that the differences in the ESC values between the reference system and the Atreus system were small, except on Day 7. All HSR and ESC scores on Day 725 were below the level in a plasma storage environment, for which poor in vivo viability earlier has been predicted to occur in PLTs stored for 7 days. Although we modified our ESC and HSR tests based on the technical implications described by VandenBroeke and colleagues,14 we still experienced decreased responses to HSR and in particular ESC when PLTs were stored in PAS. This phenomenon is likely to be associated with the technique of measurement rather than effects associated with PLT quality and PLT storage environment.

Our data showed no significant difference in the concentration of released RANTES and the expression of the activation marker CD62p between the groups. In all units, we found that CD62p, a constituent of α-granules in resting PLTs that can be detected on the surface of activated PLTs after α-granule secretion,26 has a close relation to modifications in PLT morphology as judged by a reduction in ESC values, followed by an increase in the release of RANTES. The effect of passive transfusion of RANTES into recipients of PLTs is not clear. Recent work indicates that no correlation was seen between the concentration of RANTES and nonhemolytic transfusion reactions.27 In contrast, higher concentrations (668 ± 223 ng/mL) of RANTES were associated with allergic reactions in another study.28 In our study, the concentrations of RANTES given in picograms per 10^6 PLTs on Day 7 were below the value noted in the latter study.

Increased expression of CD63 is suggested to reflect increased dense granule release,29 a condition associated with PLT activation. In our study, the CD63 antigen was expressed along a continuum with no distinct positive or negative cell. Therefore, the percentage of PLTs expressing CD63 are of no interest. In view of the fact that MFI values reflect the density of the antigen, our MFI values reflect

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**TABLE 3. In vitro effects of PLTs, obtained from different WB processing systems and stored in T-Sol for 7 days**

<table>
<thead>
<tr>
<th>In vitro measure</th>
<th>Day 1</th>
<th>Day 3</th>
<th>Day 5</th>
<th>Day 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (37°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atreus F</td>
<td>6.97 ± 0.039</td>
<td>7.189 ± 0.032</td>
<td>7.176 ± 0.026</td>
<td>7.148 ± 0.027†</td>
</tr>
<tr>
<td>Atreus S</td>
<td>6.935 ± 0.042</td>
<td>7.133 ± 0.048</td>
<td>7.132 ± 0.034</td>
<td>7.067 ± 0.054‡</td>
</tr>
<tr>
<td>TaT-F</td>
<td>6.956 ± 0.040</td>
<td>7.128 ± 0.058</td>
<td>7.154 ± 0.031</td>
<td>7.136 ± 0.032‡</td>
</tr>
<tr>
<td>pCO2 (37°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atreus F</td>
<td>4.89 ± 0.37</td>
<td>3.06 ± 0.20</td>
<td>3.01 ± 0.25</td>
<td>2.85 ± 0.23‡</td>
</tr>
<tr>
<td>Atreus S</td>
<td>5.50 ± 0.53</td>
<td>3.44 ± 0.33</td>
<td>3.16 ± 0.36</td>
<td>3.05 ± 0.44‡</td>
</tr>
<tr>
<td>TaT-F</td>
<td>4.97 ± 0.32</td>
<td>3.23 ± 0.22</td>
<td>3.11 ± 0.19</td>
<td>2.9 ± 0.2‡</td>
</tr>
<tr>
<td>pO2 (37°C)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atreus F</td>
<td>19.2 ± 1.0</td>
<td>19.7 ± 1.1</td>
<td>19.2 ± 1.1</td>
<td>19.5 ± 0.9†</td>
</tr>
<tr>
<td>Atreus S</td>
<td>19.1 ± 1.2</td>
<td>18.6 ± 1.2</td>
<td>19.5 ± 1.1</td>
<td>18.9 ± 1.2†</td>
</tr>
<tr>
<td>TaT-F</td>
<td>20.3 ± 0.6</td>
<td>19.0 ± 0.8</td>
<td>19.6 ± 0.6</td>
<td>21.2 ± 0.6‡</td>
</tr>
<tr>
<td>HSR (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atreus F</td>
<td>55.5 ± 4.9</td>
<td>41.1 ± 3.8</td>
<td>34.4 ± 3.0</td>
<td>33.1 ± 3.2‡</td>
</tr>
<tr>
<td>Atreus S</td>
<td>56.4 ± 3.4</td>
<td>46.6 ± 3.9</td>
<td>42.4 ± 2.7</td>
<td>39.7 ± 4.0‡</td>
</tr>
<tr>
<td>TaT-F</td>
<td>54.5 ± 4.6</td>
<td>46.0 ± 3.2</td>
<td>35.4 ± 2.5</td>
<td>34.9 ± 5.2‡</td>
</tr>
<tr>
<td>ESC (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atreus F</td>
<td>19.0 ± 1.0</td>
<td>11.4 ± 0.7</td>
<td>9.3 ± 0.9</td>
<td>4.9 ± 0.4‡</td>
</tr>
<tr>
<td>Atreus S</td>
<td>19.7 ± 1.0</td>
<td>13.3 ± 0.9</td>
<td>9.9 ± 0.8</td>
<td>6.5 ± 0.7§</td>
</tr>
<tr>
<td>TaT-F</td>
<td>19.1 ± 0.7</td>
<td>14.8 ± 1.0</td>
<td>11.5 ± 0.7</td>
<td>9.4 ± 1.6‡</td>
</tr>
<tr>
<td>RANTES (pg/10^6 PLTs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atreus F</td>
<td>65.2 ± 31.2</td>
<td>244.8 ± 47.6</td>
<td>348.4 ± 57.6</td>
<td>427.6 ± 46.5§</td>
</tr>
<tr>
<td>Atreus S</td>
<td>32.2 ± 31.9</td>
<td>250.0 ± 31.0</td>
<td>347.0 ± 44.0</td>
<td>416.8 ± 36.1§</td>
</tr>
<tr>
<td>TaT-F</td>
<td>65.1 ± 26.4</td>
<td>261.4 ± 38.2</td>
<td>358.5 ± 47.6</td>
<td>429.4 ± 64.9§</td>
</tr>
</tbody>
</table>

* Data are reported as mean ± SD (n = 10). The p value represents the significance of the differences from baseline (Day 1) between groups (heterogeneity) during 7 days.  †p < 0.01 vs. the other two groups.  ‡p < 0.05 vs. the other two groups.  § p Value nonsignificant.
low degree of expression with barely any differences between the different WB processing systems.

In agreement with previous data, there was no change during storage in the expression of CD41 (GPIIb), usually linked with CD61 (GPIIIa) to form the GPIIb/IIIa complex, respectively. Because we observed greater than 98 percent CD41/CD61 in all our experiments, we suggest that MFI reflecting density of the antigen on PLT surface to be more informative. The GPIIb/IIIa complex seems to be involved in PLT aggregation, serving as a receptor for soluble fibrinogen, fibronectin, thrombospondin, von Willebrand factor, and vitronectin. This complex has great impact in the regulation of PLT aggregation and adhesion throughout hemostasis. Because the expression levels of CD41/CD61 remain almost unchanged during storage, it seems most unlikely that the differences in density of the antigen on the surface between groups will be of any clinical relevance.

CD42b (GP1ba) is a subunit of the GPIb-IX-V complex and is the receptor for vWF and a high-affinity receptor for thrombin. A strong correlation between the percentage of PLTs able to bind antibodies that recognize the N-terminal of CD42b and in vivo recovery has been reported. In our study, the percentage of PLTs expressing CD42b were all higher (>91% on Day 7) than those associated with poor in vivo recovery in the mentioned study. We found no statistical difference in the percentage of PLTs expressing CD42b between the groups and our findings are in accordance with those of others who have shown that storage of PLTs reduces the expression of GP1ba on the surface of the PLTs. This reduction is assumed to decrease the adhesive capacity. In contrast, previous data suggest that a 65 percent reduction in this expression of GPIb has no effect on PLT adhesion during flow conditions.

Previous data have shown that CD42b can be cleaved by the binding of thrombin. In contrast, it seems unlikely that generation of thrombin will occur during PLT storage. Consequently, because apoptosis is unlikely to account for changes related with PLT storage lesion,
seems unclear which mechanism is responsible for the decrease in CD42b expression during PLT storage.

In summary, the results of this study suggest that PLTs, derived from pooled BCs, obtained either from fresh or from overnight-stored WB that has been processed with the Atreus system, are equivalent to PLTs derived from standard routine procedure with regard to PLT in vitro characteristics during 7 days of storage. Satisfactory PLT recovery and content were found according to current standards. Overnight storage of WB improved PLT recovery and content. Because the automated Atreus system allows for holding either WB or BC overnight before the preparation of blood components including PLTs, the new system certainly will have the potential to facilitate and meet demands for increased efficacy and standardization.

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