Automated preparation of whole blood–derived platelets suspended in two different platelet additive solutions and stored for 7 days

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BACKGROUND: The Atreus system (Terumo BCT) automates the preparation of blood components from whole blood donations. Intermediate platelet (PLT) products can be pooled manually or with the OrbiSac (Terumo BCT) and suspended in different PLT additive solutions (PASs) to obtain PLT concentrates (PCs). The aim of our study was to compare the in vitro PLT quality of PCs obtained with either the Atreus 2C+ and the OrbiSac or the Atreus 3C and suspended in PAS-II or PAS-IIIM during storage for up to 7 days.

STUDY DESIGN AND METHODS: We prepared eight PCs from buffy coats obtained with Atreus 2C+, pooled with the OrbiSac, and suspended in PAS-II and eight PCs from interim PLT units obtained with the Atreus 3C and suspended either in PAS-II or in PAS-IIIM. We measured volume, PLT content, and mean PLT component and performed metabolic assays (pH, glucose, lactate, pO₂, and pCO₂) and flow cytometry analyses (GPIb, GPIIbIIIa, GPIV, CD62P, CD63, von Willebrand factor [vWF], fibrinogen, Factor V, and annexin V).

RESULTS: PCs prepared with the Atreus 3C showed lower volume and higher PLT concentration when compared with PCs prepared with the Atreus 2C+ and the OrbiSac (p < 0.05). Glucose consumption rate and the expression of CD62P, CD63, and vWF were lower in PCs suspended in PAS-IIIM when compared with PCs suspended in PAS-II (p < 0.05).

CONCLUSION: PCs prepared with the Atreus 3C and suspended in PAS-IIIM preserve satisfactorily the in vitro PLT quality during 7-day storage. PLT activation during a 7-day storage period was lower when the storage solution was PAS-IIIM in comparison with PAS-II.

ABBREVIATIONS: BC(s) = buffy coat(s); IPU(s) = interim platelet unit(s); MPC(s) = mean PLT component(s); PAS(s) = platelet additive solution(s); PC(s) = platelet concentrate(s); PerCP = peridinin chlorophyll protein; PYI = platelet yield indicator; WB = whole blood.

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TRANSFUSION **,** **
of residual white blood cells (WBCs). Second, the 2C+ protocol was designed to obtain 1 unit of RBCs, one buffy coat (BC), and 1 unit of plasma. The obtained BCs can be pooled manually or automatically with either the OrbiSac (Terumo BCT) or the TACSI (Terumo BCT) to obtain a pooled platelet (PLT) concentrate (PC). Finally, the most recent 3C protocol was developed to obtain 1 unit of RBCs, 1 unit of plasma, 1 interim PLT unit (IPU), and 1 unit of residual WBCs. The Atreus 3C system also includes a PLT yield indicator (PYI). Without being a cell counter, the PYI provides an estimate of the PLT content in the IPU, displayed on the Atreus screen and recorded in the Atreus system manager. This indicator may be a helpful tool to increase and/or standardize PLT content in the pools either by removal of low-yield IPUs from inclusion in the pooling process or by offering the possibility to combine low- and high-yield IPUs in a more consistent manner (reducing variability in the final pool) or by both. The IPUs can be manually pooled with other IPUs to form a transfusable PLT dose, without the need for a second centrifugation step.

During recent decades, there has been great interest in developing and using PLT additive solution (PAS) for the storage of PLTs, because PAS can improve storage conditions of PLTs. At present, such PASs are in use for transfusion purposes in several countries, mostly in Europe. PAS is generally used as a substitute for plasma to 1) reduce the amount of plasma transfused with PLTs and to recover plasma for other purposes, 2) avoid transfusion of large volumes of plasma with possible adverse reactions and circulatory overload, 3) make some photochemical treatments possible for the inactivation of pathogens in PLTs, and 4) improve storage conditions. The use of PAS offers the possibility of including substances in the storage solution that have specific effects on PLTs and that are not present in plasma or in the anticoagulant. PAS developed in 1980s are of historical interest; newer PASs, such as PAS-II (contains NaCl, citrate, and acetate) and PAS-III (contains the previous components and also phosphate), are widely used for preparation and storage of PLTs, mostly in Europe. The more recent PAS-IIIM, which contains the previous components and also potassium, magnesium, 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 BCs obtained from the Atreus 2C+ system were held undisturbed for a minimum of 4 hours and a maximum of 8 hours at 22 ± 2°C until they were processed in the afternoon of the day after the blood donation (Day +1) with the OrbiSac system. We pooled five ABO-identical BCs and 300 mL of PAS-II (SSP, MacoPharma, Lille, France) with a sterile connecting device (TSCD, Terumo BCT) in an OrbiSac standard BC set. This set consisted of a number of lines to dock the BCs and PAS, a round bag that served as the pooling and separation bag, a filter (LRP6, Pall, East Hills, NY), and a PLT storage bag. The bag system was placed into the OrbiSac device following the manufacturer’s instructions. The system first performed a pooling procedure and it continued on automatically by centrifugation, inline filtration, transfer of the PLT-rich supernatant into the storage bag, and sealing.

The IPUs obtained from the Atreus 3C system were left to rest at least 1 hour, followed by a minimum of 2

![Fig. 1. Study design.](image)
Sampling and determinations
We calculated the volume of the PCs according to the net weight and density (1.014 g/mL). We sampled PCs on Days 1, 5, and 7. We determined the PLT content of the PCs and the mean PLT component (MPC) in tubes containing EDTA (1.6 mg EDTA/mL; Sarstedt & Co., Nümbrecht, Germany) with a hematology analyzer (Advia 2120 hematology system, Siemens Healthcare Diagnostics, Deerfield, IL) and expressed the results as PLTs x 10^6/L and g/dL, respectively. We performed pH, pO₂, and pCO₂ measurements using a blood gas analyzer (Rapidlab 860, Bayer, Deerfield, IL). We determined levels of glucose and lactate, expressed as mg/dL, using an analyzer (ADVIA 2400, Siemens Healthcare Diagnostics). We determined glucose concentration in PLT samples collected in tubes anticoagulated with lithium heparin (Vacuette Premium, Greiner Bio One, Frickenhausen, Germany) and we determined lactate concentration in PLT samples collected in BD Vacutainer tubes (BD368920 plastic fluoride/oxalate tube, Becton Dickinson, San Jose, CA). We analyzed PLTs by dual-flow cytometry using combinations of antibodies or markers conjugated with fluorescein isothiocyanate (FITC), phycoerythrin, or peridinin chlorophyll protein (PerCP). Monoclonal antibodies (MoAbs) used were commercially available (Immuno-tech, Marseille, France; except where expressly indicated). Presence of major glycoprotein GPIbIIa was detected with an anti-CD41a-PerCP (Clone P2) from BD Biosciences (San Jose, CA), GPIb was detected with an anti CD42b-FITC (Clone SZ2), and GPIV with an anti-CD36-FITC (clone FA6.152). Activation markers were detected using antibodies against P-selectin with anti-CD62P-FITC (Clone CLBThromb/6) and the 53-kDa lysosomal membrane protein with anti-CD63-FITC (Clone CLBGran/12). The binding of adhesive proteins, fibrinogen, and von Willebrand factor (vWF) was detected with the corresponding antibodies (Dako A/S, Glostrup, Denmark, for fibrinogen and AbD Serotec, Kidlington, UK, for vWF). Detection of procoagulant activity was measured using coagulation Factor (F)V with a MoAb that recognizes the light chain of both FV and FVa (Clone V237 from American Diagnostica, Stamford, CT), and the exposure of anionic phospholipids on the outer leaflet of the PLT membrane was quantified using annexin V (BD Biosciences). Nonspecific membrane immunofluorescence was determined by using an immunoglobulin G1 (Clone 679.1Mc7) as a negative control.

Immunolabeling of PLTs with MoAbs was performed using dual-color analysis as previously described. Briefly, after collection 2.5-μL aliquots of PCs were added to polypropylene tubes preloaded with 50 μL of phosphate-buffered saline (PBS). Samples were first incubated with saturating concentrations of anti-CD41a-PerCP in the dark, without stirring, for 15 minutes at room temperature, followed by the addition of labeled conjugated MoAbs and an additional incubation for 15 minutes. Samples were then diluted with 1 mL of PBS and analyzed immediately with a flow cytometer (FACScan, Becton Dickinson, Mountain View, CA) as previously described.

Bacterial cultures
We performed bacterial cultures on Day 7 with the routine methods of the bacteriologic laboratory at Hospital Clínica, including aerobic and aerobic cultures performed on BD BACTED Standard 10 (Becton Dickinson), with 5 days to final report.

Statistical analysis
Data are expressed as mean ± standard deviation (SD). One-way analysis of variance test for independent experiments was applied when multiple comparisons were required, followed by Bonferroni’s test if significance was detected. The level of statistical significance was established at a p value of less than 0.05. Statistical analysis was carried out with computer software (SPSS Software, release 18.0, IBM Corp., Armonk, NY).

RESULTS
We analyzed eight PCs prepared in each of the three arms of the study. All bacterial cultures were negative.

Cellular assays (Table 1)
The volume of PCs prepared with the Atreus 3C system was 21% lower when compared with the volume of PCs...
The expression of major glycoproteins (GPIb, GPIIb/IIIa, and GPIV) on PLT membrane showed no significant difference among the three arms of the study.

The expression of activation markers (CD62P and CD63) remained stable up to 7-day storage and it was lower in PCs prepared with the Atreus 3C and stored in PAS-IIIM when compared with the other two arms of the study, and the difference was significant at 5-day and at 7-day storage (p < 0.05).

In the case of adhesive proteins, the expression of vWF was lower in PCs prepared with the Atreus 3C and stored either in PAS-II or in PAS-IIIM when compared with PCs prepared with the Atreus 2C and the OrbiSac and stored in PAS-II, and the difference was significant at 5-day storage (p < 0.05), and the expression of fibrinogen showed no significant difference among the three arms of the study. There was a progressive increase in procoagulant markers (FV/Va and annexin V to PLTs) throughout the study. There was a progressive increase in procoagulant markers (FV/Va and annexin V to PLTs) throughout the study. There was a progressive increase in procoagulant markers (FV/Va and annexin V to PLTs) throughout the study. There was a progressive increase in procoagulant markers (FV/Va and annexin V to PLTs) throughout the study. There was a progressive increase in procoagulant markers (FV/Va and annexin V to PLTs) throughout the study. There was a progressive increase in procoagulant markers (FV/Va and annexin V to PLTs) throughout the study.
the storage in the three arms of the study, and the difference was not significant.

**DISCUSSION**

Our study showed that PCs prepared with the Atreus 3C and suspended in PAS-IIIM preserve the in vitro PLT quality during 7-day storage satisfactorily. Those PCs had also the advantage of a smaller total volume when compared with PCs prepared with the Atreus 2C+ and the OrbiSac. Moreover, direct and indirect signs of PLT activation markers were lower when the storage solution was PAS-IIIM in comparison with PAS-II.

To our knowledge, this is the first study to compare PLT in vitro quality variables of PCs obtained with two different configurations of the Atreus system and stored in two different PASs. There were previous validation studies performed in a controlled environment focused on analyzing PLT characteristics of PCs prepared from BCs obtained from Atreus 2C+ and either pooled manually and suspended in plasma or pooled with OrbiSac and suspended in PAS-II (T-Sol, Fenwal Europe sprl, Mont Saint Guibert, Belgium). Other authors focused on analyzing RBCs, plasma, and BCs obtained with the Atreus 2C+ or PCs prepared from IPUs obtained with the Atreus 3C and stored in PAS-II (SPP, MacoPharma). Finally, in a routine environment, other authors focused on operational values and in vitro quality of blood components obtained with the Atreus 3C.

Taking together all the previous studies, it seems that automation of PC preparation from WB bags helps in standardization and reduction of variability in the final pool. In this sense, Cid and colleagues published a study in 2007, where authors compared the results of pooled PCs...
prepared with the OrbiSac system with pooled PCs prepared manually. Authors showed that automation of the pooling process increased not only the PLT content but also the consistency of the final product; the coefficients of variation for the volume and the PLT content decreased from 30% to 20% and 17% to 11%, respectively. Interestingly, the present study, as reported previously, showed that the use of PYI in the Atreus 3C configuration helped to improve the PLT content of the final pooled PC when compared with pooled PCs prepared with the Atreus 2C and the OrbiSac, because the content of PLTs in the final pooled PC was the same pooling 4 IPUs obtained from the Atreus 3C than pooling 5 BCs obtained from the Atreus 2C+. Because the introduction of automation in blood component preparation is changing the classic way of preparing PLTs, and we are reporting here changes in PLT volume, PLT concentration, metabolic changes, and lower expression of vWF; it should be desirable to perform future studies to compare the clinical outcome of PLT transfusions prepared by these two automated methods, as others did in the past comparing PLTs prepared either manually or semiautomatically with OrbiSac.

Our study showed a slightly stronger decrease in glucose concentration and increase in lactate concentration in PCs prepared with the Atreus 3C when compared with PCs prepared with the Atreus 2C+ and the OrbiSac, and both variables were significant on Day 5 and Day 7 (p < 0.05). However, the glucose consumption rate was similar for both systems. This observation could be related to the fact that PCs prepared with the Atreus 3C resulted in a lower volume and higher PLT concentration when compared with PCs prepared with the Atreus 2C+ and the OrbiSac. Interestingly, when we compared the two groups of PLTs prepared with the Atreus 3C, the presence of PAS-IIIM resulted in a decrease of glucose consumption rate when glucose consumption was corrected by PLT concentration in the PCs. Thus, in all PCs prepared with both systems and both PASs, there was still plenty of glucose at the end of the storage.

Regarding PAS used in our study, PCs suspended in PAS-IIIM showed higher pH, lower expression of GPIb/IIa, lower expression of activation markers on PLT membrane (CD62P and CD63), and lower expression of vWF when compared with PCs suspended in PAS-II and prepared either with the Atreus 2C+ and OrbiSac or with the Atreus 3C (p < 0.05). We believe that these results can be explained in part because PAS-IIIM contains phosphate, potassium, and magnesium, in addition to NaCl, citrate, and acetate, present in PAS-II, and it is known that the combination of these three new components in PAS-IIIM is associated with complex effects and interdependence. For example, previous studies have shown that the presence of magnesium in PAS significantly inhibited exposure of P-selectin, decreased binding of fibrinogen to ADP-activated PLTs, and significantly decreased agonist-induced PLT aggregation. Additional studies showed that magnesium present in PAS reduced PLT activation and metabolic rate.

In our study, we also measured MPC as an indicator of PLT activation. MPC is a new PLT variable reported by modern ADVIA cell blood count analyzers that measure the mean refractive index of the PLTs. The previous hematology analyzer measures the intensity of light scattered by PLTs at two different angles (2-3° and 5-15°) and from the paired values computes the PLT volume and the PLT component concentration on a cell-by-cell basis. These values are then averaged to provide the MPC expressed in g/dL. MPC is linearly related to PLT density and is reduced when PLTs degranulate, thus indicating that PLTs have undergone activation. Several studies have recently demonstrated that MPC values are inversely correlated with PLT membrane activation and subsequent P-selectin expression. In fact, the MPC variable has been used as an indicator of PLT activation in different conditions and physiologic states as well as stored PCs. In the present study, we observed that MPC decreased during 7-day PLT storage indicating PLT activation in the three arms of the study. However, higher MPC indicating lower PLT activation was seen in PCs prepared with the Atreus 3C and suspended in PAS-IIIM when compared with the other two arms of the study. Therefore, using MPC as a variable of PLT activation, PCs prepared with the Atreus 3C and suspended in PAS-IIIM showed lower PLT activation when compared with the other two arms of the study.

In conclusion, our study showed that PCs prepared with the Atreus 3C and suspended in PAS-IIIM had similar or slightly improved in vitro quality during a 7-day storage period compared to PCs prepared with the Atreus 2C+ and the OrbiSac and suspended in PAS-II. Moreover, those PCs had the advantage of a smaller total volume. Finally, PLT activation during a 7-day storage period was lower when the storage solution was PAS-IIIM in comparison with PAS-II.

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**CONFLICT OF INTEREST**

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