

# In vitro viability effects on apheresis and buffy-coat derived platelets administered through infusion pumps

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**Background:** Different infusion pump systems as well as gravity infusion have been widely used in neonatal transfusion. However, the limited number of published studies describing the use of infusion pumps on platelets illustrates the necessity for more robust data.

**Methods:** To evaluate the potential in vitro effects on the cellular, metabolic, functional and phenotypic properties of platelets, we set up a four-arm paired study simultaneously comparing the use of different infusion pumps (Alaris<sup>®</sup> CC/GP) with unexposed platelets. The platelet units (n=8) were either produced by the apheresis technique and suspended in 100% plasma or derived from buffy coats to yield platelet units stored in approximately 30% plasma and 70% SSP+. Fresh and 5-day old platelets were tested.

**Results:** Regardless of the production system or storage time used, no significant differences were observed in glucose and lactate concentration, pH, adenosine triphosphate levels, response to extent of shape change, hypotonic shock response reactivity, and CD62P expression. Similarly, no differences were observed in expression of the conformational epitope on glycoprotein IIb/IIIa, determined using procaspase-activating compound 1, or in the expression of CD42b and platelet-endothelial cell adhesion molecule-1 in a comparison between platelets administered through infusion pumps versus unexposed platelets.

**Conclusion:** Using Alaris CC/GP infusion pumps had no influence on the cellular, functional, and phenotypic in vitro properties of platelets. This fact seems not to be affected by different production systems or storage time.

**Keywords:** platelets, neonatal platelet transfusion

## Introduction

Different platelet infusion pump systems<sup>1,2</sup> as well as gravity infusion have been widely used in neonatal transfusion practice.<sup>3</sup> Earlier data have shown that these infusion systems do not significantly injure or activate platelets and may also be of clinical benefit to pediatric or adult patients with a history of overload reaction, when precise control of the rate and volume of platelet transfusion is desired.<sup>4</sup> We note, however, that the favorable effects on platelets have only been evaluated using earlier versions of electromechanical infusion pumps<sup>1,2</sup> and not using automated pressure-based devices available for this purpose.

One of the key properties of platelets is their capacity to respond rapidly to different types of rheological conditions, become activated and secrete factors that promote blood clotting and tissue regeneration.<sup>5</sup> Therefore, platelets can be easily triggered when they encounter an artificial environment. Automated or manual pressure-based infusion pumps represent no exception to this notion. The extent to which these

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potential manipulations compromise platelet function in vivo is not fully known, but it is nevertheless important to characterize aberrant situations in which platelets capability may be affected. One such potential situation may be that the pressure forces within the infusion pumps, including passage through the pump hoses, further increase factors contributing to existing morphologic, biochemical, or functional derangements caused by processing and storage<sup>6–9</sup> of the platelet units. Subsequently, such a potential situation may be further associated with an increased risk of decreased post-transfusion survival.<sup>7,8,10</sup>

To evaluate the potential in vitro effects on the cellular, metabolic, functional, and phenotypic properties of platelets, we set up a four-arm paired study comparing the use of different infusion pumps with unexposed platelets. Considering the potential differences in quality of fresh and stored platelets<sup>11,12</sup> or platelets produced with different methods,<sup>13</sup> we also investigated the influence of such variables in synergy with the potential effects of using infusion pumps.

## Materials and methods

### Preparation and storage of apheresis platelet units

Apheresis platelets were collected from healthy blood donors who met standard donation criteria and gave their informed consent according to institutional guidelines. The donors were screened for leukocyte antibodies. The apheresis platelets studied were leukocyte-depleted and suspended in 100% plasma. A Trima Accel<sup>®</sup> automated blood collection system with a single needle (TerumoBCT, Lakewood, CO, USA) was used for the apheresis procedure.<sup>14</sup> A tubing set with a leukocyte reduction system was used to obtain a product containing  $<1 \times 10^6$  leukocytes.

The apheresis was performed in a closed system with continuous flow. Whole blood with anticoagulant (ACD-A) was pumped into a separation channel that spins in a centrifuge. The erythrocytes, which are heaviest, end up furthest out, then leukocytes, platelets and the plasma. Platelet-rich plasma was taken to a step-shaped funnel, ie, the leukocyte reduction system chamber, where platelets and leukocytes are separated. The leukocyte-depleted platelet-rich plasma then proceeded through the collection tube to the platelet collection bag (TerumoBCT). The whole procedure took 50–100 minutes. The total extracorporeal volume produced was approximately 196 mL. All units are then stored on a flat-bed agitator in a temperature-controlled cabinet at  $22^\circ\text{C} \pm 2^\circ\text{C}$  (Model PC900i, Helmer, Noblesville, IN, USA). Eight apheresis platelet units

were tested on day 2 (fresh) and day 5 (old), respectively. All tested apheresis platelet units were created to fall within clinical requirements.

### Preparation and storage of buffy coat platelet units

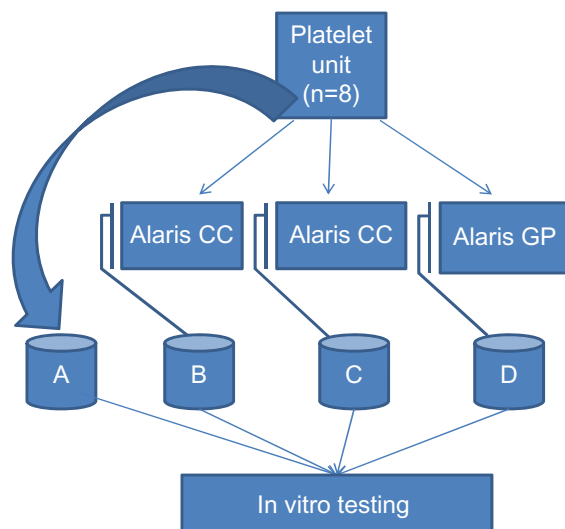
Buffy coat platelets were collected according to criteria similar to those for apheresis donation. In total, 450 mL of whole blood was drawn into either a CPD/SAG-M quadruple-bag blood container system (Fenwal, La Châtre, France) or an NPT 6280LE blood bag system (MacoPharma, Mouvaux, France). After storage at room temperature for 2–6 hours, all whole blood units were centrifuged (2,700 g) for 10 minutes at  $22^\circ\text{C}$ . Automatic equipment was used for preparation of the blood components (Optipress, Fenwal, or Macopress, Smart, MacoPharma), including buffy coat.

All buffy coat platelet units were prepared using the OrbiSac system (TerumoBCT)<sup>15</sup> to yield platelet units stored in approximately 30% plasma and 70% SSP+ (Macopharma). The buffy coat platelets were stored in platelet storage bags (OrbiSac Standard buffy coat set, storage bag, TerumoBCT Inc.) made of polyvinyl chloride plastic with a citrate-based plasticizer. To avoid disintegration and adverse negative effects on the platelets,<sup>16</sup> air and foam was excluded from the units immediately after preparation. All units were then stored in the same way as for the apheresis units described earlier (Helmer). Eight buffy coat platelet units were tested on day 2 (fresh) and day 5 (old). All tested buffy coat platelet units were created to fall within clinical requirements.

### Infusion pumps and study design

Alaris<sup>®</sup> GP volumetric and Alaris CC syringe pumps were used simultaneously from a single apheresis unit or a single buffy coat platelet unit in accordance with the recommendations of the manufacturer (Cardinal Health Inc., Dublin, OH, USA). All reference platelets remained unexposed to the infusion pumps. All samples for in vitro testing were collected in 50 mL Cellstar tubes (Greiner Bio-one, Monroe, NC, USA). The four-arm paired study is outlined in Figure 1 and was designed as follows:

- A, reference (no run)
- B, smooth aspiration in the syringe, ie, 50 mL at 90 sec with a Braun Omnifix<sup>®</sup> 50 mL syringe through a BD Connecta<sup>™</sup> three-way stopcock connected to a Mediplast<sup>®</sup> transfusion unit; the infusion was administered through a BD Connecta extension tube with BD Neoflon<sup>™</sup> 24 G (0.7×19 mm) attached to the tube, using the Alaris CC syringe pump



**Figure 1** Schematic overview of the study design.

**Note:** (B–D) was performed simultaneously from the attached platelet unit with the following pump settings: a total volume of 15 mL platelet suspension was administered through the different infusion pumps during 30 minutes of total run time while A remains untreated.

- C, heavy aspiration in the syringe as above but with an aspiration time of less than 10 seconds
- D, infusion was administered using the Alaris GP volumetric pump with the Alaris GP volumetric pump transfusion set, with BD Neoflon 24 G (0.7×19 mm) connected to the set.

## Analysis of cellular, metabolic, in vitro functional, and phenotypic markers

Immediately after administered through the different infusion pumps or unexposed to infusion pumps, in vitro cellular parameters including measurements of platelet counts ( $10^9/L$ ) and mean platelet volume using CA 620 Cellguard (Boule Medical, Stockholm, Sweden) was performed on platelets from the collecting tubes (Greiner Bio-one) (see Figure 1). In the same way, the extracellular metabolic environment was studied using routine blood gas equipment (ABL 800, Radiometer, Copenhagen, Denmark) including pH ( $37^\circ C$ ),  $pCO_2$ ,  $pO_2$  (kPa at  $37^\circ C$ ), glucose (mmol/L), and lactate (mmol/L). Bicarbonate (mmol/L) was calculated based on the other measured variables. The pH of all samples was measured at  $37^\circ C$ . Therefore, Rosenthal's factor of 0.0147 unit/ $1^\circ C$  was used to correct pH to the temperature of sampling ( $22^\circ C$ ). This factor gives an approximation of the change in pH of the sample per degree Celsius when it is warmed anaerobically from the collecting temperature of  $22^\circ C$  to  $37^\circ C$ .

According to Bertolini and Murphy,<sup>17</sup> the assessment of swirling was scored as 0, 1, and 2. The white blood cell count on day 1 was determined using a Nageotte chamber

and a standard microscope (Zeiss, Chester, VA, USA).<sup>18</sup> Hypotonic shock response reactivity as well as the extent of shape change measurements were performed using a dedicated microprocessor-based instrument (SPA 2000, Chronolog, Havertown, PA, USA) with modifications of these tests as described by VandenBroeke et al.<sup>19</sup> The total adenosine triphosphate concentration ( $\mu mol/10^{11}$  platelets) was determined using a luminometer (Orion Microplate, Berthold Detection Systems GmbH, Pforzheim, Germany) on the basis of the principles described by Lundin.<sup>20</sup>

Extracellular lactate dehydrogenase activity (% of total), a marker for cell disintegration, was measured using a spectrophotometric method (kit 063K6003, Sigma-Aldrich, St Louis, MO, USA; 6500 spectrophotometer, Jenway, Stone, UK).<sup>21</sup> Expression of procaspase-activating compound 1 (a marker of cellular responsiveness towards adenosine diphosphate), CD62P (a marker of activation), CD42b (a marker of adhesive capability), and platelet endothelial cell adhesion molecule 1 (PECAM-1) was measured by flow cytometry (FC500, Beckman Coulter, Villepinte, France). MLP acquisition and analysis software packages (Beckman-Coulter) were used for data acquisition and analysis, respectively. All methods including staining were performed as described in recent publications.<sup>16,22,23</sup>

## Statistical analysis

Mean values and standard deviations are given unless otherwise indicated. A one-way analysis of variance including post hoc test Bonferroni's adjustment was performed. The results of Bonferroni's test are presented in Tables 1–4 and considered to be statistically significant at  $P < 0.05$ . The analyses were carried out using Statistica version 9 software (StatSoft, Inc. 1984–2007 (SPSS, Chicago, IL, USA).

## Results

The platelet counts, cellular, metabolic and in vitro functional and phenotypic parameters are listed and given in Tables 1–4. Initially, we hypothesized that potential differences in the quality of fresh and stored platelets or platelets produced with the different methods might act in synergy with the potential effects of using infusion pumps to further accelerate the aberrant lesion effects on platelets. We note, however, that regardless of production system or storage time, no significant difference in platelet count between the groups was observed by simultaneously using the different infusion pumps. Subsequently, no differences were detected in extracellular lactate dehydrogenase activity in percent of total which remained stable at low levels in both apheresis as

**Table 1** Cellular, metabolic, functional and phenotypic in vitro effects in fresh apheresis platelets administered through Alaris GP and Alaris CC infusion pumps\*

	In vitro marker		
	Platelets 10 <sup>9</sup> /L	MPV (fL)	LDH (%) of total
A Reference (no run)	1,393±189	8.6±1.2	10.5±4.8
B Alaris CC (smooth)	1,404±174	8.6±1.2	6.6±4.9
C Alaris CC (heavy)	1,395±190	8.5±1.1	9.0±5.4
D Alaris GP	1,394±198	8.6±1.2	8.6±6.3
	Glucose (mmol/L)	Lactate (mmol/L)	pH (22°C)
A Reference (no run)	16.7±7.8	6.6±2.8	7.509±0.084
B Alaris CC (smooth)	17.0±8.0	6.7±2.6	7.579±0.090
C Alaris CC (heavy)	17.0±8.0	6.7±2.6	7.597±0.082
D Alaris GP	17.1±7.9	6.8±2.9	7.609±0.082
	pCO <sub>2</sub> (kPa at 37°C)	Bicarbonate	ATP (μmol/10 <sup>11</sup> platelets)
A Reference (no run)	3.88±0.61	13.5±2.3	7.9±0.6
B Alaris CC (smooth)	3.22±0.40 <sup>†</sup>	13.4±2.4	8.4±1.0
C Alaris CC (heavy)	3.06±0.32 <sup>†</sup>	13.3±2.4	8.4±0.4
D Alaris GP	2.97±0.37 <sup>†</sup>	13.3±2.4	9.0±0.8
	HSR (%)	ESC (%)	CD62P (%)
A Reference (no run)	61.7±8.0	25.5±5.6	11.28±3.26
B Alaris CC (smooth)	54.4±4.4	24.9±7.2	11.97±3.93
C Alaris CC (heavy)	56.9±7.1	25.5±4.2	12.81±3.58
D Alaris GP	60.7±8.7	28.4±6.4	11.44±3.43
	PAC-1 (%)	CD42b (%)	PECAM-1 (%)
A Reference (no run)	53.39±6.58	99.50±0.15	99.59±0.35
B Alaris CC (smooth)	50.28±6.78	99.55±0.12	99.64±0.38
C Alaris CC (heavy)	44.97±4.94	99.55±0.15	99.53±0.56
D Alaris GP	48.37±5.47	99.50±0.19	99.63±0.35
	PECAM-1 (MFI)	pO <sub>2</sub> (kPa at 37°C)	
A Reference (no run)	14.8±4.9	16.4±2.7	
B Alaris CC (smooth)	14.9±4.8	20.2±2.1 <sup>†</sup>	
C Alaris CC (heavy)	14.6±4.5	21.1±1.7 <sup>†</sup>	
D Alaris GP	14.7±4.2	20.1±3.3 <sup>†</sup>	

**Notes:** \*Values are reported as the mean ± standard deviation. <sup>†</sup>P<0.05 versus A. **Abbreviations:** ATP, adenosine triphosphate; ESC, extent of shape change; HSR, hypotonic shock response reactivity; LDH, lactate dehydrogenase; MFI, mean fluorescence intensity; MPV, mean platelet volume; PAC-1, procaspase-activating compound 1; PECAM-1, platelet endothelial cell adhesion molecule 1.

well as buffy coat platelet units. Thus, mean platelet volume remained unaffected when using infusion pumps.

Differences, albeit slight, were found in extracellular pO<sub>2</sub> and pCO<sub>2</sub> levels (*P*<0.05), while glucose and lactate concentration, pH, and bicarbonate and adenosine triphosphate levels remained unaffected by the use of the infusion pumps. Subsequently, hypotonic shock response reactivity, the response to extent of shape change, and CD62P expression showed similar responses and expression levels without any significant differences between the units. No differences

**Table 2** Cellular, metabolic, functional, and phenotypic in vitro effects in fresh buffy coat-derived platelets administered through Alaris GP and Alaris CC infusion pumps\*

	In vitro marker		
	Platelets 10 <sup>9</sup> /L	MPV (fL)	LDH (%) of total
A Reference (no run)	927±86	9.0±0.8	4.7±1.2
B Alaris CC (smooth)	923±98	8.8±1.2	4.7±1.3
C Alaris CC (heavy)	926±90	8.8±0.8	4.6±1.9
D Alaris GP	926±98	8.8±0.8	3.5±1.1
	Glucose (mmol/L)	Lactate (mmol/L)	pH (22°C)
A Reference (no run)	4.4±0.7	7.7±1.2	7.226±0.071
B Alaris CC (smooth)	4.4±0.7	7.6±1.0	7.265±0.068
C Alaris CC (heavy)	4.3±0.7	7.8±1.0	7.218±0.039
D Alaris GP	4.4±0.8	7.8±1.0	7.230±0.048
	pCO <sub>2</sub> (kPa at 37°C)	Bicarbonate	ATP (μmol/10 <sup>11</sup> platelets)
A Reference (no run)	3.11±0.61	5.1±0.8	7.9±0.9
B Alaris CC (smooth)	2.47±0.51 <sup>†</sup>	4.8±0.4	8.1±1.0
C Alaris CC (heavy)	2.69±0.33 <sup>†</sup>	5.1±0.6	7.9±1.0
D Alaris GP	2.78±0.54 <sup>†</sup>	5.0±0.5	7.6±0.8
	HSR (%)	ESC (%)	CD62P (%)
A Reference (no run)	55.0±9.0	22.1±4.1	16.68±2.24
B Alaris CC (smooth)	55.2±5.7	21.7±3.3	16.41±3.24
C Alaris CC (heavy)	55.6±5.2	22.9±2.8	18.72±2.96
D Alaris GP	56.2±9.1	23.2±4.3	16.37±2.71
	PAC-1 (%)	CD42b (%)	PECAM-1 (%)
A Reference (no run)	46.98±5.75	97.62±2.34	99.48±0.12
B Alaris CC (smooth)	44.78±7.43	97.56±2.33	99.53±0.12
C Alaris CC (heavy)	43.68±7.63	97.19±1.75	99.42±0.14
D Alaris GP	44.79±6.40	97.97±1.72	99.51±0.17
	PECAM-1 (MFI)	pO <sub>2</sub> (kPa at 37°C)	
A Reference (no run)	14.2±0.9	11.9±4.2	
B Alaris CC (smooth)	14.9±1.6	13.9±2.9 <sup>†</sup>	
C Alaris CC (heavy)	14.4±0.8	13.8±4.6 <sup>†</sup>	
D Alaris GP	15.2±1.5	14.4±5.0 <sup>†</sup>	

**Notes:** \*Values are reported as the mean ± standard deviation. <sup>†</sup>P<0.05 versus A. **Abbreviations:** ATP, adenosine triphosphate; ESC, extent of shape change; HSR, hypotonic shock response reactivity; LDH, lactate dehydrogenase; MFI, mean fluorescence intensity; MPV, mean platelet volume; PAC-1, procaspase-activating compound 1; PECAM-1, platelet endothelial cell adhesion molecule 1.

were observed in expression of the conformational epitope on glycoprotein (Gp)IIb/IIIa determined by using procaspase-activating compound 1, as well as in the expression of PECAM-1 and CD42b between groups. Additionally, swirling remained at the highest level (=2) in all tested apheresis and buffy coat platelet units.

## Discussion

This four-armed paired study describes the in vitro viability effects on fresh and stored apheresis and buffy coat platelet

**Table 3** Cellular, metabolic, functional, and phenotypic in vitro effects in stored apheresis platelets administered through Alaris GP and Alaris CC infusion pumps\*

	In vitro marker		
	Platelets 10 <sup>9</sup> /L	MPV (fl)	LDH (%) of total
A Reference (no run)	1,327±201	8.4±0.7	6.1±1.0
B Alaris CC (smooth)	1,337±196	8.4±0.6	6.5±0.5
C Alaris CC (heavy)	1,355±185	8.4±0.6	7.2±1.6
D Alaris GP	1,327±166	8.3±0.6	6.8±1.8
	Glucose (mmol/L)	Lactate (mmol/L)	pH (22°C)
A Reference (no run)	11.2±1.9	12.0±2.6	7.328±0.142
B Alaris CC (smooth)	11.3±1.7	11.9±2.5	7.345±0.153
C Alaris CC (heavy)	11.5±1.7	11.9±2.6	7.354±0.155
D Alaris GP	11.6±1.5	12.2±2.8	7.384±0.156
	pCO <sub>2</sub> (kPa at 37°C)	Bicarbonate	ATP (μmol/10 <sup>11</sup> platelets)
A Reference (no run)	3.38±0.43	7.9±2.5	7.0±0.7
B Alaris CC (smooth)	2.93±0.11 <sup>†</sup>	8.0±2.3	6.9±1.0
C Alaris CC (heavy)	3.13±0.1	7.9±2.2	6.6±1.3
D Alaris GP	2.92±0.17 <sup>†</sup>	7.8±2.3	7.0±1.6
	HSR (%)	ESC (%)	CD62P (%)
A Reference (no run)	63.2±2.6	24.0±3.2	22.08±4.15
B Alaris CC (smooth)	54.6±3.5	24.2±3.1	24.03±4.51
C Alaris CC (heavy)	60.4±8.2	22.1±4.4	22.68±2.87
D Alaris GP	52.6±7.7	24.4±1.6	23.26±6.47
	PAC-1 (%)	CD42b (%)	PECAM-1 (%)
A Reference (no run)	27.43±2.07	99.42±0.14	99.73±0.19
B Alaris CC (smooth)	27.01±3.12	99.40±0.20	99.81±0.04
C Alaris CC (heavy)	26.06±2.31	99.30±0.27	99.80±0.04
D Alaris GP	25.27±1.65	99.37±0.07	99.77±0.09
	PECAM-1 (MFI)	pO <sub>2</sub> (kPa at 37°C)	
A Reference (no run)	15.0±3.3	15.7±0.2	
B Alaris CC (smooth)	15.3±3.0	17.9±0.4 <sup>†</sup>	
C Alaris CC (heavy)	14.6±3.2	18.0±1.2 <sup>†</sup>	
D Alaris GP	15.1±3.3	18.8±0.8 <sup>†</sup>	

**Notes:** \*Values are reported as the mean ± standard deviation. <sup>†</sup>P<0.05 versus A. **Abbreviations:** ATP, adenosine triphosphate; ESC, extent of shape change; HSR, hypotonic shock response reactivity; LDH, lactate dehydrogenase; MFI, mean fluorescence intensity; MPV, mean platelet volume; PAC-1, procaspase-activating compound 1; PECAM-1, platelet endothelial cell adhesion molecule 1.

units administered through different infusion pumps as described previously.

With the exception of differences detected in the extracellular environment, all of the in vitro parameters selected on the basis of different aspects of platelet function were not significantly different between the four groups at the measured time points. The data presented demonstrate that platelets administered through infusion pumps maintain their in vitro characteristics to a great extent and do not tend to reinforce potential negative cellular

**Table 4** Cellular, metabolic, functional, and phenotypic in vitro effects in stored buffy coat-derived platelets administered through Alaris GP and Alaris CC infusion pumps\*

	In vitro marker		
	Platelets 10 <sup>9</sup> /L	MPV (fl)	LDH (%) of total
A Reference (no run)	863±151	8.9±0.3	5.5±2.4
B Alaris CC (smooth)	864±115	8.9±0.3	5.9±2.1
C Alaris CC (heavy)	858±156	8.9±0.4	3.8±1.3
D Alaris GP	843±154	8.8±0.4	5.2±2.0
	Glucose (mmol/L)	Lactate (mmol/L)	pH (22°C)
A Reference (no run)	3.4±0.5	9.5±0.8	7.319±0.022
B Alaris CC (smooth)	3.5±0.6	9.6±0.8	7.342±0.019
C Alaris CC (heavy)	3.5±0.6	9.6±0.8	7.351±0.025
D Alaris GP	3.5±0.5	9.6±0.9	7.356±0.027
	pCO <sub>2</sub> (kPa at 37°C)	Bicarbonate	ATP (μmol/10 <sup>11</sup> platelets)
A Reference (no run)	2.52±0.12	5.1±0.7	7.5±0.5
B Alaris CC (smooth)	2.16±0.35 <sup>†</sup>	5.0±0.7	7.5±0.8
C Alaris CC (heavy)	2.09±0.33 <sup>†</sup>	5.0±0.6	7.2±0.4
D Alaris GP	2.03±0.36 <sup>†</sup>	4.9±0.7	6.9±1.1
	HSR (%)	ESC (%)	CD62P (%)
A Reference (no run)	51.3±2.9	23.4±1.0	30.50±3.52
B Alaris CC (smooth)	46.5±7.3	21.1±1.6	30.66±3.53
C Alaris CC (heavy)	45.9±3.8	18.2±0.6	31.37±1.74
D Alaris GP	51.4±3.2	21.8±3.1	30.34±4.84
	PAC-1 (%)	CD42b (%)	PECAM-1 (%)
A Reference (no run)	32.37±0.92	95.25±6.72	99.30±0.37
B Alaris CC (smooth)	29.50±3.22	94.87±6.97	99.38±0.34
C Alaris CC (heavy)	30.16±3.65	95.14±7.42	99.45±0.24
D Alaris GP	30.48±3.29	96.38±4.60	99.38±0.36
	PECAM-1 (MFI)	pO <sub>2</sub> (kPa at 37°C)	
A Reference (no run)	17.7±2.7	11.8±3.0	
B Alaris CC (smooth)	18.3±1.7	16.5±2.1 <sup>†</sup>	
C Alaris CC (heavy)	17.7±2.7	17.8±2.0 <sup>†</sup>	
D Alaris GP	18.3±1.7	18.2±1.6 <sup>†</sup>	

**Notes:** \*Values are reported as the mean ± standard deviation. <sup>†</sup>P<0.05 versus A. **Abbreviations:** ATP, adenosine triphosphate; ESC, extent of shape change; HSR, hypotonic shock response reactivity; LDH, lactate dehydrogenase; MFI, mean fluorescence intensity; MPV, mean platelet volume; PAC-1, procaspase-activating compound 1; PECAM-1, platelet endothelial cell adhesion molecule 1.

changes, respectively. Hence, the capacity of the different Alaris infusion pump systems (GP/CC) to act as a potential source of morphologic, biochemical, or functional derangements seems to be insignificant. This fact seems not to be affected by the age of the platelets, different production systems used, or heavy aspiration in the syringe preceding use of Alaris CC.

Consequently, a combination of several factors, including different production systems, composition of the platelet storage medium, and biochemical storage effects, needs to

be considered to conclude that infusion pumps do not affect platelet function.

Extracellular blood gas measurements offer valuable insights into the equilibrium between the cytosolic and mitochondrial oxidative pathways of the platelets, as physiological challenges that disturb metabolic homeostasis cause increased cytosolic glycolysis<sup>23–25</sup> and promote platelet activation,<sup>6,23,26–30</sup> as well as aggregation and release reactions.<sup>22,23,26,31–34</sup> Importantly, the pH in all the infusion pump-administered fractions was maintained above Council of Europe recommendations (pH>6.4), along with oxygen consumption and the ability to generate an equivalent concentration of adenosine triphosphate,<sup>35,36</sup> indicating that oxidative phosphorylation was likely maintained in such platelets. The slight observed differences between the groups, including for oxygen and carbon dioxide, may be attributable to differences in the gas exchange capacity of the storage container versus the environmental conditions within the infusion pumps. Because the results for platelets administered through these infusion pumps seem to agree with the functional integrity of the reference platelets, strengthens the conclusion that cellular effects cannot be the cause of the extracellular differences observed.

Platelets have been shown to be variably activated as a consequence of collection, processing, storage, and different pathogen inactivation procedures,<sup>27,37–43</sup> although it has been more difficult to directly correlate in vitro platelet activation with the risk of decreased post transfusion recovery and survival. However, it makes sense to consider the consequences of platelet activation as potentially harmful effects<sup>22,31,33,44–48</sup> and try to minimize and characterize situations in which aberrant platelet activation may occur preceding transfusion.

One such potential situation that caught our attention was aspiration when using the Alaris CC in stressful situations was carried out with vigorous (heavy) aspiration in the syringe, which rationally justifies an additional study arm to examine possible cell influence of this practical procedure. In contrast, offers the volumetric pump no such opportunity to influence the cell content, which eliminates the need for a fifth study arm. In addition, there is great variability worldwide in neonatal platelet transfusion practices,<sup>49</sup> and guidelines differ widely between countries. Therefore, we tested pump settings only according to our local clinical guidelines.

With exception of slight storage effects that were detected for apheresis as well as buffy coat platelets, we observed no statistically verified increase in activation levels in the

units tested versus the reference units, which strengthens the conclusion that infusion pumps do not tend to reinforce platelet activation. However, it is important to emphasize that the results presented here are representative of the level of activation of platelets prepared by our specific methods of apheresis and buffy coat platelets, and may not be representative of other devices.

Additionally, using the tested infusion pumps did not affect the ability of the platelets to respond to agonists (extent of shape change and procaspase-activating compound 1), indicating that cellular responsiveness was maintained relative to the activation state. Further, all tested hypotonic shock response reactivity scores were well above the level for which poor in vivo viability has been predicted to occur in a plasma storage environment.<sup>50</sup>

CD42b (Gp1ba) is a subunit of the GpIb-IX-V complex and is the receptor for von Willebrand factor and a high-affinity receptor for thrombin.<sup>51</sup> A strong correlation between the percentage of platelets able to bind antibodies that recognize the N-terminal of CD42b and adhesive capacity under flow conditions has been reported.<sup>52</sup> In our study, the percentages of platelets expressing CD42b were all higher (>90%) than those associated with decreased adhesive capacity in the abovementioned study. Similarly, we found no difference in the percentage of platelets expressing PECAM-1 (CD31)<sup>53</sup> between the groups, and our findings are in accordance with recently presented data on PECAM-1,<sup>54</sup> indicating ultrastructural maintenance and no upregulation of this essential structure.

In summary, our data clearly show that all parameters reflecting different aspects of platelet function remained largely unaffected when administered through Alaris GP and Alaris CC infusion pumps. This observation seems not to be affected by use of different production systems or storage time. However, whether the in vivo efficacy of such platelets is affected will require clinical studies.

## Disclosure

The authors report no conflicts of interest in this work.

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