

# **Pathogen Inactivation and In Vitro Functions of Platelet Concentrates Collected in 100% Plasma Treated with INTERCEPT Blood System**

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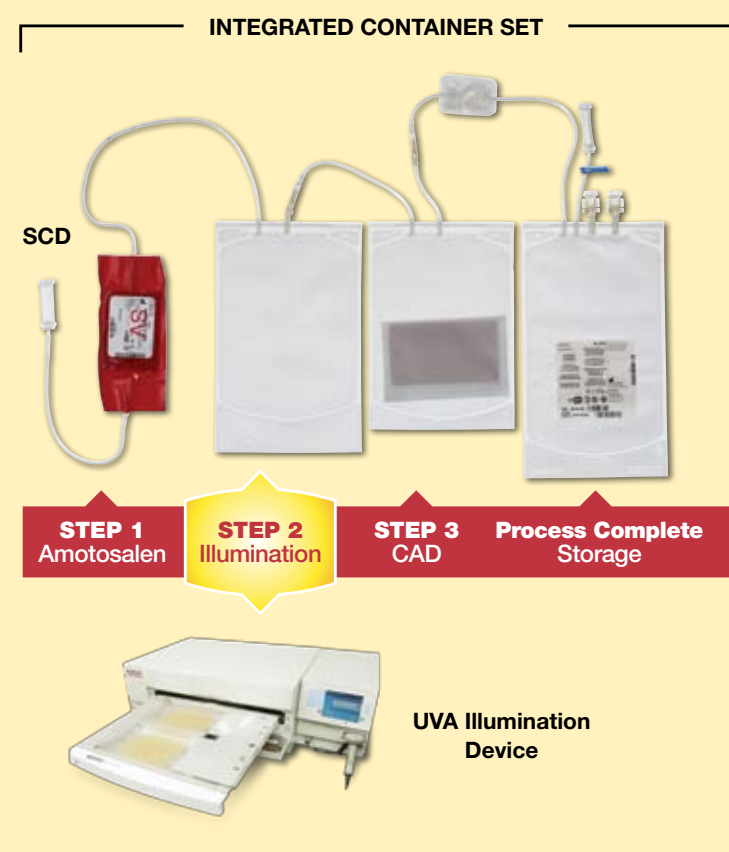
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## Background

The INTERCEPT Blood System™ was initially developed to inactivate pathogens and leukocytes in platelet components (PCs) suspended in a combination of approximately 35% plasma and 65% platelet additive solution (InterSol™). This system was CE Mark registered in 2002 (Figure 1). To date >350,000 INTERCEPT™ PCs have been transfused in routine clinical use. In many regions, platelet components prepared for transfusions are suspended in 100% plasma and there is no mechanism to incorporate the addition of platelet additive solution. In order to expand the treatment range, a series of studies were performed to evaluate the INTERCEPT treatment of platelet components suspended in 100% plasma with respect to pathogen inactivation and in vitro platelet functions (Brucher et al, Transfusion 2007;47:1896-1901, Wagner et al, Transfusion 2009;49:704-710).

**Figure 1: The INTERCEPT Blood System for Platelets**

Using a sterile connecting device (SCD), the platelet container is sterilely connected to the INTERCEPT kit. Amotosalen (1) is added by gravity flow and the platelet mixture is illuminated with UVA light (2). Residual amotosalen and its photoproducts in the platelet mixture are reduced to low levels using a compound adsorption device (CAD) (3) before the platelets are transferred to the storage container.



## Aims

**Evaluate the efficacy of pathogen inactivation and maintenance of in vitro platelet function following INTERCEPT treatment of PCs suspended in 100% plasma.**

## Methods

Either apheresis or pooled whole blood-derived PCs containing 2.5-7.0 x10<sup>11</sup> platelets in 255-390 mL of plasma were used in a series of experiments. Inactivation experiments were performed by inoculating PCs with 10<sup>5</sup>-10<sup>6</sup> cfu/mL of bacteria or 10<sup>5</sup>-10<sup>6</sup> infectious units (TCID<sub>50</sub>)/mL of virus, adding 150 μM amotosalen, and illuminating with a 3.0 J/cm<sup>2</sup> UVA (320-400 nm) treatment. Samples taken before and after treatment were assayed for viable organisms and results used to calculate the level of inactivation (log-reduction). Four to ten replicate experiments using independent PCs for each organism were performed. For in vitro function experiments, seven double-dose apheresis platelet collections or twelve pooled whole blood-derived PCs in plasma were each split evenly into two identical units. The CONTROL was not treated. The TEST was treated with amotosalen and UVA. After 6 to 24 hours of incubation in a compound adsorption device (CAD) to reduce the amotosalen concentration, the treated PCs were stored under blood bank conditions and evaluated for platelet count, pH (37°C), pO<sub>2</sub>, pCO<sub>2</sub>, HCO<sub>3</sub><sup>-</sup>, glucose, lactate, LDH, swirling, MPV, morphology and soluble p-selectin etc.

## Results

The results showed significant inactivation of viral and bacterial pathogens in PCs suspended in 100% plasma following treatment with INTERCEPT (Table 1).

After treatment of apheresis PCs with INTERCEPT (n=7) and storage for 7 days, there were no statistically significant differences between the CONTROL and TEST PC units for lactate, LDH, MPV, and p-selectin. Swirling was observed for all units up to 7 days of storage. Differences were observed for measurements of platelet content, pH, blood gases, and glucose levels for most of the storage days. All units had pH values greater than 6.4 on Day 5 meeting Council of Europe requirements. On Day 7 all in vitro parameters indicate metabolically viable platelets with a mean pH of 6.91 (SD 0.3), however, pH of one TEST unit fell below 6.4 (pH=6.36) (Table 2).

Treatment of whole blood-derived PCs (n=12) resulted in slightly reduced pH in TEST pools compared to that of matched CONTROL pools after 5 days of storage. TEST and CONTROL pools had similar levels of pO<sub>2</sub> throughout 7 days of storage. Although glucose levels in TEST pools decreased slightly more than CONTROL during 7 days of storage, lactate levels in TEST and CONTROL were similar during storage. On day 5, TEST pools were associated with lower platelet counts, bicarbonate levels, and ESC values and higher pCO<sub>2</sub> levels, LDH release, CD62+ cells compared to those of CONTROL pools. Day 5 TEST pools maintained adequate discoid platelets based on morphology evaluation. These results suggest that TEST pools generally maintained adequate in vitro properties throughout 5 days of storage. The pH of TEST and CONTROL pools declined on Day 7, with 2 of 12 TEST pools having a pH value of less than 6.20, while all CONTROL pools had pH values of more than 6.66. On day 7, TEST pools showed further decreases in platelet count, pCO<sub>2</sub>, bicarbonate levels, HSR values, ESC values, and morphology scores, as well as greater levels of LDH and higher percentage of CD62+ cells compared to CONTROL pools (Table 3).

**Table 1: Bacterial and Viral Inactivation**

Bacteria	Pre-treatment (cfu/mL) mean ± SD	Post-treatment (cfu/mL) mean ± SD	Log <sub>10</sub> Reduction mean ± SD
<i>S. aureus</i> (n=4)	2.1 ± 0.4 x10 <sup>6</sup>	< 0.05 ± 0	> 7.6 ± 0.1
<i>S. epidermidis</i> (n=4)	1.2 ± 0.1 x10 <sup>6</sup>	< 0.05 ± 0	> 7.4 ± 0.1
<i>E. coli</i> (n=4)	1.4 ± 0.34 x10 <sup>6</sup>	≤ 0.1 ± 0.1	≥ 7.3 ± 0.2
<i>K. pneumoniae</i> (n=4)	2.1 ± 0.25 x10 <sup>6</sup>	≤ 0.8 ± 0.3	≥ 6.7 ± 0.6
Virus	Pre-treatment (TCID <sub>50</sub> /mL) mean ± SD	Post-treatment (TCID <sub>50</sub> /mL) mean ± SD	Log <sub>10</sub> Reduction mean ± SD
HIV-1 (n=10)	6.5 ± 4.0 x 10 <sup>5</sup>	≤ 5.1 ± 1.8	≥ 5.0 ± 0.3
BVDV (n=8)	9.7 ± 7.1 x 10 <sup>5</sup>	≤ 3.0 ± 1.2	≥ 5.4 ± 0.2
PRV (n=7)	8.6 ± 4.5 x 10 <sup>5</sup>	≤ 16.0 ± 8.7	≥ 4.7 ± 0.4

**Table 2: Platelet in vitro Properties (Apheresis Platelets in 100% Plasma)\* (n=7)**

	Day 0	Day 1		Day 5		Day 7	
	Pre-treatment	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
Platelets (x10 <sup>11</sup> /unit)	4.1 ± 0.9	<b>3.8 ± 0.7</b>	<b>4.2 ± 0.8</b>	<b>3.3 ± 0.6</b>	<b>3.7 ± 0.7</b>	3.6 ± 0.7	3.3 ± 0.4
pH (37°C)	7.22 ± 0.04	7.22 ± 0.06	7.22 ± 0.09	<b>7.08 ± 0.15</b>	<b>7.16 ± 0.19</b>	6.91 ± 0.31	6.84 ± 0.23
pCO <sub>2</sub> (mm Hg)	39 ± 4	<b>29 ± 3</b>	<b>34 ± 3</b>	<b>14 ± 1</b>	<b>17 ± 2</b>	16 ± 3	13 ± 1
pO <sub>2</sub> (mm Hg)	143 ± 12	<b>161 ± 14</b>	<b>146 ± 13</b>	<b>160 ± 5</b>	<b>141 ± 13</b>	159 ± 12	164 ± 3
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	16.8 ± 1.3	<b>13.7 ± 1.8</b>	<b>15.1 ± 2.8</b>	<b>6.4 ± 3.7</b>	<b>9.2 ± 5.1</b>	4.5 ± 4.5	2.5 ± 3.8
LDH (U/L)	346 ± 54	375.5 ± 57	363 ± 60	420 ± 69	373 ± 56	373 ± 54	430 ± 81
MPV (μm <sup>3</sup> )	7.6 ± 0.7	7.3 ± 0.7	7.3 ± 0.7	7.3 ± 0.6	7.4 ± 0.7	7.3 ± 0.4	7.3 ± 0.4
Glucose (mg/dL)	322 ± 11	286 ± 9	300 ± 12	199 ± 16	222 ± 32	173 ± 45	159 ± 27
Lactate (mM)	2.5 ± 0.3	4.6 ± 0.7	5.0 ± 1.4	13.0 ± 2.2	12.2 ± 3.8	16.6 ± 4.9	16.9 ± 3.0
Swirling	+	+	+	+	+	+	+
p-selectin (ng/mL)	119 ± 15	159 ± 35	170 ± 23	210 ± 18	225 ± 19	261 ± 22	270 ± 39

\* Data are presented as mean ±SD, statistical significant differences between Test and Control with p-values <0.05 are indicated in bold.

**Table 3: Platelet in vitro Properties (PRP Platelets in 100% Plasma)\* (n=12)**

	Day 2		Day 5		Day 7	
	TEST	CONTROL	TEST	CONTROL	TEST	CONTROL
Count (x10 <sup>9</sup> /mL)	<b>1359 ± 165</b>	<b>1460 ± 159</b>	<b>1300 ± 163</b>	<b>1449 ± 162</b>	<b>1265 ± 177</b>	<b>1442 ± 168</b>
pH (22°C)	<b>7.27 ± 0.04</b>	<b>7.33 ± 0.06</b>	<b>6.93 ± 0.11</b>	<b>7.11 ± 0.10</b>	<b>6.55 ± 0.24</b>	<b>6.90 ± 0.12</b>
pCO <sub>2</sub> (mm Hg)	46.9 ± 3.5	45.8 ± 4.9	<b>33.3 ± 5.6</b>	<b>30.1 ± 7.3</b>	<b>23.5 ± 5.2</b>	<b>35.6 ± 6.0</b>
pO <sub>2</sub> (mm Hg)	100 ± 12	104 ± 36	111 ± 12	115 ± 35	129 ± 18	114 ± 28
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	<b>13.4 ± 0.8</b>	<b>15.2 ± 0.8</b>	<b>4.6 ± 0.9</b>	<b>8.0 ± 0.8</b>	<b>1.7 ± 0.8</b>	<b>5.0 ± 1.1</b>
Glucose (mg/dL)	<b>614 ± 26</b>	<b>640 ± 29</b>	<b>484 ± 20</b>	<b>541 ± 19</b>	<b>423 ± 35</b>	<b>499 ± 25</b>
Lactate (mmol/L)	<b>8.4 ± 0.7</b>	<b>9.1 ± 0.7</b>	16.6 ± 3.6	17.7 ± 2.9	<b>24.3 ± 5.2</b>	<b>22.4 ± 4.2</b>
ESC (%)	<b>21.8 ± 3.7</b>	<b>26.3 ± 4.9</b>	<b>13.2 ± 2.5</b>	<b>19.4 ± 4.2</b>	<b>8.1 ± 3.6</b>	<b>16.2 ± 4.2</b>
HSR (%)	72.9 ± 7.8	72.5 ± 7.9	<b>60.0 ± 7.0</b>	<b>65.5 ± 6.6</b>	<b>44.1 ± 10.8</b>	<b>60.5 ± 7.7</b>
LDH (% release)	<b>7.41 ± 1.30</b>	<b>6.18 ± 1.16</b>	<b>7.88 ± 1.39</b>	<b>6.83 ± 1.21</b>	<b>8.05 ± 1.54</b>	<b>7.18 ± 1.56</b>
Morphology (% disks)	<b>76.2 ± 4.7</b>	<b>77.7 ± 5.2</b>	<b>51.1 ± 10.5</b>	<b>60.7 ± 9.0</b>	<b>35.4 ± 8.3</b>	<b>50.3 ± 7.7</b>
CD62+ (%)	<b>44.3 ± 7.1</b>	<b>40.0 ± 8.7</b>	<b>60.3 ± 11.7</b>	<b>47.4 ± 11.3</b>	<b>79.3 ± 7.9</b>	<b>60.1 ± 7.2</b>

\* Data are presented as mean ±SD, statistical significant differences between Test and Control with p-values <0.05 are indicated in bold.

## Conclusions

- INTERCEPT Blood System can be used to inactivate viruses and bacteria in platelet components suspended in 100% plasma without the addition of platelet additive solution.
- INTERCEPT platelet components suspended in 100% plasma maintained good function with storage up through at least 5 days.
- These results provided the basis for CE Mark approval to expand the INTERCEPT treatment guardband range to include PCs suspended in 100% plasma without additive solution.