

# Clinical Efficacy of Intercept-Treated Platelets, A Concise Review

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### Introduction

Current practices aimed at reducing transfusion-transmitted (TT) infections include screening donors for active infection or high-risk status for bloodborne pathogens, donor arm inspection for suspicious lesions, phlebotomy site preparation with povidone-iodine or chlorhexidine solutions, use of diversion pouch technology, and testing of donors and platelet units for evidence of bloodborne pathogens. Despite these measures, transmission of pathogens through blood transfusion continues to occur. In 2003, the AABB issued standard 5.1.5.1 [1]: “The blood bank or transfusion service shall have methods to limit and detect bacterial contamination in all platelet components” with an implementation date of March 1, 2004. At that time, two culture-based systems had received FDA clearance for quality control testing in apheresis platelets: Bact/ALERT (bioMerieux) and eBDS (Pall Corp) [2]. Current practices require that cultures are obtained from platelet units between 24 and 36 hours after collection and platelets released to inventory only when the culture systems demonstrate no growth after 12 or more hours of incubation and that monitoring for growth continue throughout shelf-life [3]. Despite current methodologies, the rate of confirmed-positive bacterial culture continues at a rate of approximately 1:1000 to 1:3000 units [4-6]. The rate of septic transfusion reactions (STRs) to apheresis platelets (e.g., false-negative cultures) is estimated between 1: 41,173 and 1: 193,305 and that of fatal STRs is estimated at 1: 498,711 units transfused [7, 8]. For red cells, which do not routinely undergo bacterial surveillance cultures, the rate of STRs has been estimated at 1: 250,000 [9]. Overall, 70% of cases of TT sepsis are due to gram-positive organisms, most of which are represented by skin commensals. However, 80% of fatalities are due to gram-negative organisms that often derive from asymptomatic bacteremic states, thus eluding skin preparation and diversion pouch interventions [10]. Testing only by bacterial culture does not eliminate all contaminated platelet units. A point-of-care immunoassay (Platelet PGD Test, Verax Biomedical), tested in a multicenter study [11] detected an additional 9 contaminated apheresis platelet units out of 27,620 released via negative prestorage culture results for a breakthrough rate of 1:3069 with current testing; these results were consistent with a prior study which determined at time of issue a detection rate of 1:3866 whole-blood derived platelet pools [12].

Septic transfusions were the fourth most common cause of transfusion-related fatalities between FY10 and FY14 [13], see Figure 1. Implicated pathogens included gram negative bacteria (*E. coli*, *M. morganii*, *K. pneumoniae*, *S. Marcescens*, *P. fluorescens*, and *acinetobacter sp*) in 7/15 (47%); *Babesia microti* in 4/15 (27%), gram positive organisms (*S. aureus*, *S. epidermidis*) in 3/15 (20%), and West Nile Virus in 1/15 (6.7%) deaths.

Increasingly sensitive methodology has reduced the risk (and respective window periods) for screened viral transmissions for HIV to 1:1,467,000 (9.0-9.1 days), HCV to 1:1,149,000 (7.4 days) [14] and HBV to 1:843,000-1:1,208,000 (18.0 – 26.5 days) [3, 15]. Improvements in these and other tests have resulted in very low residual transmission risk for tested units: 1:1,149,000 for HCV, 1:1,146,000 for HIV [16] 1:9,090,909 for HTLV I/II, [17], and 1:282,000 to 1:357,000 for HBV [18].

However, the specter of emerging pathogens persists. Such entities include (but are not limited to): *Dengue virus*, *Chikungunya virus*, *Malaria species*, *Trypanosoma cruzi*, *West Nile Virus*, and *Babesia*

*species*. In addition, travel deferrals for reducing TT-malaria risk cost the industry thousands of annual donor-deferrals [3].

During the 2007 Dengue outbreak in Puerto Rico, 29/15,350 donor specimens were positive for Dengue by transcription mediated amplification (TMA) for an incidence of 1/529 (0.19%) [19]. Out of three recipients tested, one of whom subsequently tested positive and developed dengue hemorrhagic fever [19]. In another report, a donor reported fever 1 day after donation of a whole blood unit in Singapore, where Dengue is endemic. Polymerase chain reaction (PCR) testing was positive for Dengue virus type 2. Both recipients – one of the donor’s red cells, the other of a plasma unit derived from the original collection – developed post-transfusion fever and subsequent tested positive for dengue [20].

For units donated between 1979 and 2009 and inclusive of subsequent cases diagnosed through 2010, there were 162 patients who developed transfusion-transmitted Babesiosis [21]. The majority of cases, 159/162 (98%) were due to *B. microti*, whereas 3/162 (2%) were due to *B. duncani*. During the study period, TT-Babesiosis became increasingly recognized in non-endemic states – mostly due to interstate travel of donors from endemic areas and interstate commerce of blood components. Among 84 patients with available data, the median (IQR) time from transfusion to symptom onset was 37 days (11-176 days) [18].

On May 13, 2015, the FDA’s Blood Products Advisory Committee (BPAC) specifically addressed transfusion-transmitted Babesia. Members of BPAC agreed that 1) “available scientific data and FDA analysis support the concept of nation-wide, year round testing of blood donations for Babesia-risk by an antibody-based test”, 2) “NAT-based testing should be performed in blood donations in certain high risk states” with the majority of members selecting 9 endemic states: CT, MA, RI, NY, JN, MN, WI, NH, and ME, and 3) “a deferral period [for donors with *B. microti*-positive test results] of at least two years and recommended that a re-entry algorithm should include antibody and NAT testing” [22]. Introduction of additional testing would obviously contribute incremental costs to blood component manufacture.

### **Pathogen Reduction (PR)**

Pathogen reduction, a term used interchangeably with pathogen inactivation, refers to technologies and processing methods that nonspecifically reduce infectivity of collected units. Pathogen reduction therefore represents a paradigm shift away from the current strategy of reactive testing and toward one of proactive risk mitigation. Such a strategy allows for risk reduction from known, emerging, and unknown pathogens even prior to availability of screening or testing methods.

Pathogen reduction technologies focus mainly around photochemical treatment (PCT) and solvent-detergent treatment, the latter being applicable only to plasma. Various PR platforms, summarized in Table 1 [23-29], either incite terminal membrane disruption or irreparable damage to nucleic acid material. This article will focus primarily on Intercept (Cerus Corporation, Concord, CA) as it has recently gained market approval in the US.

### **The Intercept Process**

The Intercept system for platelets incorporates a single-use, integrated, fluid path processing set, a UVA illumination device, amotosalen (S-59) solution container, and a compound adsorption device for reduction of residual amotosalen compounds following illumination. Each illumination device can accommodate two illumination containers simultaneously.

Following apheresis collection, tubing from the apheresis container is sterile-docked to the platelet processing set. Platelets flow through the amotosalen container into the illumination container – see figure 1 below – thereby acquiring a final amotosalen concentration of ~150 µmol/L. Amotosalen

intercalates between opposing nucleic acid base pairs, forming a monoadduct. Exposure to UVA light at 320-400 nm (at a dose of 3.0 J/cm<sup>2</sup>) induces photoactivation of the amotosalen, causing formation of a second adduct thus crosslinking the opposing chains [30]. The process produces cross-links at a rate of 1 amotosalen adduct per 83-89 base pairs [31] thus preventing replication of contaminating organisms and leukocytes. The process therefore reduces the risk of TA-GVHD and in some countries takes the place of gamma-irradiation [32, 33]. Studies have shown that for the majority of tested pathogens, Log<sub>10</sub> reduction in infectivity in general is in the range of >4.0-6.0 [34, 35]. Levels of residual amotosalen and its photoproducts are reduced in the ensuing 6-16 hour compound adsorption step prior to transfer into final storage bags for a standard 5-day outdate [31].

### **Clinical Experience**

The euroSPRITE trial [36] randomized 103 patients with counts <20 x 10<sup>9</sup>/L and requiring platelet transfusion support to receive leukoreduced buffy coat-produced platelet pools that were either amotosalen-UVA treated (n=311 transfusions) or untreated (n=256 transfusions) for up to 56 days. Platelets were gamma irradiated prior to transfusion. Exclusion criteria included pregnancy, presence of splenomegaly >18 cm, history of ITP, DIC, or refractoriness to platelet transfusion. Co-primary endpoints included one-hour platelet count increment and one-hour corrected count increments (CCI). The mean one-hour count increment for test and reference platelets was 27.5 ± 13.5 x 10<sup>9</sup>/L vs 35.8 ± 23.3 x 10<sup>9</sup>/L [mean difference 8.3 x 10<sup>9</sup>/L, 95% CI 0.9, 15.8; p=0.03]. The mean one-hour CCI was 13,100 ± 5400 vs 14,900 ± 6200, respectively – p=0.11.

Subjects randomized to test platelets required more transfusions overall than control subjects: 7.5±5.8 units vs 5.6±5.5 units (inclusive of on and off protocol transfusions). Test platelets were characterized by lower platelet doses (x10<sup>11</sup>) than control platelets: 3.9±1.0 vs 4.3±1.2, p<0.001). Authors attributed the lower platelet content to extra samples required for measurement of amotosalen levels as well as losses due to multiple transfers during processing. The rates of selected secondary endpoints including number of platelet transfusions during the period of platelet support, interval between transfusions, development of refractoriness, adverse reactions, and hemostatic efficacy (assessed prospectively by trained observers blinded to treatment arm) did not differ statistically [36].

The SPRINT study [37] randomized patients to photochemically treated (PCT, n=318) vs control (n=327) apheresis platelets for up to 28 days or transfusion independence. All platelets for the study were collected using the Amicus separator (Baxter Healthcare, Round Lake, IL) and stored in 30%-45% plasma and 70% to 55% platelet additive solution – Intersol, Baxter Healthcare, Deerfield, IL. Patients 6 years of age or above with thrombocytopenia requiring transfusion support were eligible. Patients with HLA alloimmunization (Panel Reactive Antibody >20%), TTP/HUS, acute promyelocytic leukemia, interleukin-11 therapy were excluded. The primary efficacy endpoint (assessed prospectively by trained observers blinded to treatment group) was occurrence of Grade 2 bleeding. Additional endpoints included Grade 3 or 4 bleeding, increments, development of refractoriness, and overall safety.

The proportion of patients with Grade 2 bleeding was equivalent between PCT and control subjects: 58.5% vs 57.5%, respectively – p<0.01 for non-inferiority. Grade 3 and 4 bleeding events also did not differ between groups, 4.1% vs 6.1%, respectively, p<0.01 for non-inferiority. PCT-related processing resulted in a lower mean platelet dose (x10<sup>11</sup>) 3.7 PCT vs 4.0 control, p<0.001) and greater proportion receiving low-dose (<3.0 x 10<sup>11</sup> platelets/unit) apheresis units between PCT (60%) and control (36%) groups. These differences likely explained the greater degree of platelet support required by the PCT group 8.4 platelet units overall and 0.74 units per day compared to 6.2 platelet units overall and 0.65 units per day in the control group (p<0.001 for both comparisons).

Even when longitudinal regression was used to control for platelet dose, the PCT group 1 hour post-transfusion platelet count was estimated to be  $10.4 \times 10^9/L$  lower than for control and the time to next transfusion was shorted by 0.4 days compared to control. Factors contributing to lower increments – such as splenomegaly, fever, sepsis, and amphotericin, were comparable between groups. Mean one-hour post-transfusion counts ( $\times 10^9/L$ ), count increment ( $\times 10^9/L$ ), and CCI's ( $\times 10^9$ ) were lower for PCT vs control: 36.5 vs 49.5, 21.4 vs 34.1, 11.1 vs 16.0 as were 24 hour post-transfusion counts ( $\times 10^9/L$ ), count increments ( $\times 10^9/L$ ) and CCIs ( $\times 10^3$ ): 27.9 vs 36.1, 13.2 vs 21.5, and 6.7 vs 10.1, respectively.

One hour CCIs of  $<5 \times 10^3$  were more common among PCT vs control patients: 27.4% vs 12.7%,  $p < 0.001$ ). Episodes of clinical refractoriness (defined as 1-hour CCI  $< 5 \times 10^3$  following each of two consecutive platelet transfusions) were observed in 21.4% of PCT vs 7.0% of control patients ( $p < 0.001$ ). Many events were transient and long-term refractoriness persisted in 6% in the PCT and 9% in the control patients (still favoring PCT over control for development of refractoriness).

Reasons for lower overall platelet doses among the PCT arm included use of a nonintegrated processing set and prototype compound adsorption device and sampling losses incurred by the PCT arm but not the control arm (i.e., samples for amotosalen assay). Also, to avoid off-protocol transfusions, low-dose PCT units were transfused when a higher dose unit was unavailable.

The issue of the processing kit leading to reduced platelet content per unit in both euroSPRITE and SPRINT was addressed in a separate, smaller randomized, double-blind study [38] comparing the finalized production-ready Intercept system to conventionally prepared products. The intention to treat population included 43 patients, 22 receiving Intercept platelets and 21 receiving reference platelets. Subjects were followed until 7-days transfusion-free or up to a maximum of 28 days. Results for the primary clinical endpoints of 1 hour absolute and corrected count increments for the first eight study transfusions were similar and are summarized in Table 2.

### **Post-Marketing Experience and Safety**

A prospective, active hemovigilance study with combined cohort analysis of 4067 patients who received 19,175 Intercept-treated platelet components was recently published [39]. After implementation at each participating center, Cerus initiated an online hemovigilance reporting system to collect transfusion outcomes data from October 2003 through December 2010. A standardized data capture form was utilized by participating centers; adverse events occurring within 24 hours and all serious adverse events within 7 days of transfusion were recorded. Platelets were prepared by various methods – including apheresis and buffy coat method – all were leukoreduced and re-suspended in 35% plasma and 65% Intersol (Fenwal, Fresenius-Kabi, Lake Zurich, IL) and stored for 5-7 days depending upon the regulations of each country.

Of interest, the majority of centers (97%) allowed the Intercept process to take the place of gamma irradiation for recipients at risk for TA-GVHD (only 1.9% of platelets were HLA matched). On a per-patient basis, the proportion of patients who experienced any adverse event was 126/4067 (3.1%); of these, 94 (2.3% of the study population) were considered to be transfusion reactions. On a per-transfusion basis, 167/19175 (0.9%) transfusions resulted in an adverse event and of those, 123/19175 (0.6%) were considered to be transfusion reactions. Transfusion reactions were most commonly comprised of chills (1.5% of patients, 0.4% of transfusions) and urticaria (0.9% of patients, 0.2% of transfusions). There were no cases of TRALI, TA-GVHD, TT-Infection, or death attributable to Intercept treated platelets.

## **Cost Considerations**

McCullough and colleagues [40] sought to estimate potential cost implications of adopting Intercept processes. The authors accounted for various costs such as those for avoidance of selected infectious disease testing, irradiation, and estimated impact of test anticipated avoidance of potential Babesia and Dengue testing, the potential for 7 day-shelf life with avoidance of point-of-release testing, and others. Using data apheresis platelet collection and processing data from several U.S. centers, a proposed cost savings of \$141.65 per unit was published.

Girona-Llobera and colleagues [41], after implementing Intercept platelets at the Balearic Islands Blood Bank (BIBB) performed a before-after single-center cost analysis. As opposed to McCullough, et al, Girona-Llobera took into account up-front implementation costs. Implementation at BIBB of the Intercept process (which was applied to both apheresis and whole-blood derived platelet products) took the place of irradiation and did not incur additional overall labor. The 3 year timeframe (Jan 2008 through Dec 2010) covered by the post implementation phase was associated with an 85.5% increase in platelet production costs – the greatest proportion (69.6%) was comprised of costs related to the PI process itself. The pre-implementation period (Jan 2005 through Dec 2007) was associated with a 5 day platelet expiration rate of 16.8%. Implementation of Pathogen Inactivation process allowed extension of platelet outdate to 7 day, thus resulting in reduction in expiration rate to 2.7% during the post-implementation period. The savings associated with reduced outdating and greater availability reduced by 13.7% the initial costs. Costs related to elimination of gamma irradiation, bacterial testing, and CMV testing were not included in the analysis. Demand for platelet products increased during the post-implementation period, which contributed 9.4% proportionally to the overall increase in production costs. The increase in production costs associated with implementation were partially offset by its benefits – primarily conversion to 7-day shelf-life – leading overall to 71.7% increase rather than 85.5%. The rate of febrile and allergic reactions declined after implementation 2.50% Pre to 0.90% Post and 1.02% Pre to 0.56% Post, respectively. These reductions could be attributable to displacement of 65% of autologous plasma by platelet additive solution during the post-implementation phase.

Bell and colleagues [42], performed a comprehensive economic analysis of using Intercept Blood System (IBS) for apheresis platelets (AP). Economic costs concerning platelet utilization in key clinical conditions, as well as infectious risks were incorporated into their decision-analytic model. The incremental cost per quality-adjusted life-year (QALY) gained for AP+IBS, provided bacterial testing and irradiation could be precluded (and taking into account an emerging HCV-like virus), was \$0.178 to \$1.06 million.

To place this figure into perspective, Davidson and colleagues [43] estimated the cost-effectiveness of individual-donor, nucleic acid amplification testing (ID-NAT) in addition to serologic tests alone for detection of HBV, HCV, and HIV among blood donors in Sweden. Using a health-economic model to estimate lifetime costs and effects from a societal perspective, the cost of ID-NAT plus serologic testing compared to serologic testing alone was \$12.7 million per avoided viral transmission. The quality per QALY gained was much lower, however, if in the setting of younger recipients, declining to \$1.8 million and \$2.0 million for neonatal and 30 year old female recipients, respectively. These latter estimates were comparable to those derived by Marshall and colleagues [44], who also examined cost effectiveness for the addition of mini-pool NAT testing (for HBV, HCV, and HIV). The incremental cost effectiveness ratio (over serologic testing alone) was estimated at \$1.5 million per QALY gained.

## **Conclusions**

Prior to U.S. approval of the Intercept Blood System, reducing infectious risks associated with platelet transfusion relied upon screening and testing with new tests introduced after an emerging pathogen

had already caused transfusion-transmitted disease. Pathogen reduction technologies provide a new option of proactive risk mitigation in a manner efficacious against known and unknown pathogens. The Intercept process for pathogen reduction in platelet products (also available for plasma) has been shown to be effective in reducing residual infectivity of pathogen-spiked units [35]. Implementation requires investment in infrastructure, disposables, and human resources. Girona-Llobera estimated the incremental cost per unit at €97, or about \$105 [41]. Initial clinical trials with Intercept platelets reported reduced platelet yields, post-transfusion increments, and greater number of units required per patient [36, 37]. However, these studies were performed using prototype systems and a smaller study demonstrated reduced impact on these variables with the production system [38]. Calculation of estimated cost benefits requires many assumptions – chief among them being conversion to seven day shelf-life. While implementation in European countries may allow this change [41], the FDA has not yet rendered a final decision on this or other changes U.S. implementation might allow, such as relaxation of infectious disease testing requirements or removal of irradiation requirements. Displacement of donor plasma logically provides donor safety benefits. In the 3 years following implementation, Girona-Llobera reported a significant reduction in both febrile and allergic reactions to Intercept platelets [41]. Increased availability of product owing to extended shelf-life may also be viewed as a safety benefit both for donors and recipients. In terms of societal cost, Bell and colleagues estimate the upper end for incremental cost per QALY gained with Intercept treated apheresis platelets to be around \$1.06 million, which is similar in scope to the costs associated with NAT testing [43,44]. Ultimately, society expects a risk-free transfusion product and historically has been willing to pay for it. The threat of emerging pathogens is constantly with us, and pathogen reduction technologies offer an effective and proactive option for additional risk mitigation.

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Figure 1: Causes of transfusion-related fatalities reported to FDA between FY'10 and FY'14 (n=176): TRALI = Transfusion Related Acute Lung Injury, TACO = Transfusion Associated Circulatory Overload, HTR = Hemolytic Transfusion Reaction.

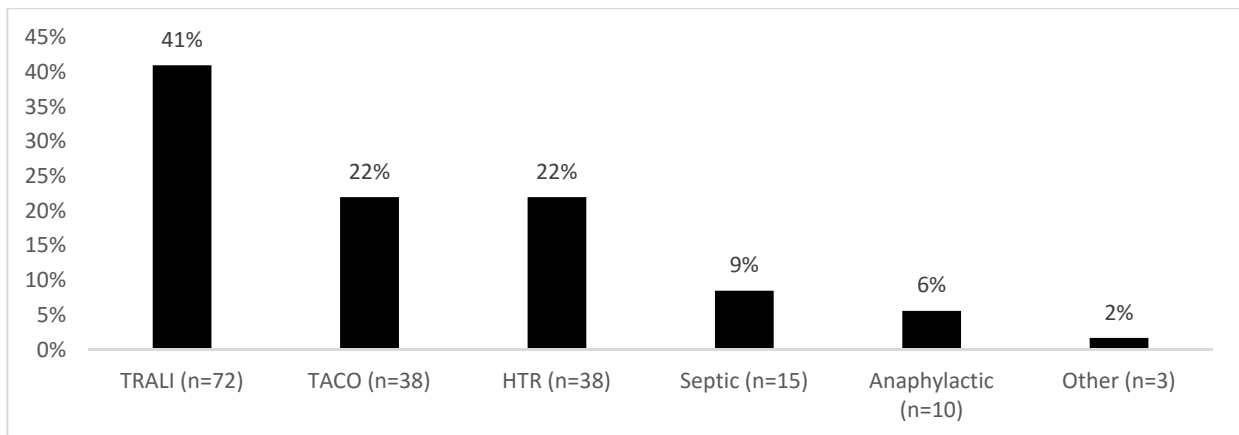


Table 1: Summary of Selected Pathogen Reduction Technologies [REF].

Technology	Mechanism	Product	Extraction/Notes
Solvent Detergent (Octapharma)	After filtration through a 1 µm pore size membrane, the plasma pool (limited to 390 kg comprising 630-1,520 individual donors) contain Parvovirus B19 DNA ) is treated with organic Solvent (1% Tri-N-Butyl phosphate) in combination with virucidal detergent (1% octoxynol) for 1-1.5 hours to inactivate enveloped viruses. Protein S and alpha 2 antiplasmin, which are labile to S/D treatment, are controlled to ensure levels $\geq 0.4$ IU/mL.	Plasma (Octaplas)	Agents later extracted by oil and removed by chromatographic adsorption. Non-lipid enveloped viruses like Parvovirus B19 and Hepatitis A Virus resistant to process but infectious risk low due to presence of neutralizing antibodies. In addition, the manufacturing plasma pool may not contain Parvovirus B19 DNA > 10 IU/µL and must have a negative result for Hepatitis E Virus by NAT PCR with a sensitivity of $\leq 2.5 \log_{10}$ IU/mL.
Theraflex-MB (Macopharma, Mouveaux, France)	Plasma unit sterile docked to processing kit. Plasma flows first through a 0.65 µm filter, then through tubing containing a plasma a dry pill of 85 µg anhydrous Methylene Blue chloride. The MB is dissolved into the plasma to a final concentration of 0.8 to 1.2 µM (235-330 plasma volume) as it enters the illumination bag. MB intercalates into viral nucleic acids – with affinity for guanosine-cytosine base pairs. Within the illuminator device, visible light (630 nm, 15 minute illumination time for 2 units) induces MB photoactivation generating singlet oxygen with subsequent guanine oxidation. Following illumination, the product is gravity filtered through the BlueFlex filter into the final storage container.	Plasma	Blueflex filter effects 10 log reduction of residual MB and its photo-degradation products (ie, Azur B). Disposables for UVC illumination produced from ethylene vinyl acetate allowing increased UV permeability compared to polyvinyl chloride. Hemovigilance data suggest a lower rate of adverse and allergic reactions vs quarantine plasma (1:24,593 MB vs 1:3620 Q and 1:24,593 MB vs 1:7489 Q, respectively*)
Intercept (Cerus Corp, Concord, CA)	Amotosalen (S59HCl) binds and inactivates ribosomal RNA thus inhibiting protein synthesis; Photoactivation (UVA 320-400nm, 3J/cm <sup>2</sup> ) causes extensive nucleic acid crosslinking leading to fusion of DNA/RNA strands hence preventing access to nuclear material.	Platelets Plasma	Compound Adsorption Device (CAD) 4-16 hours at room temperature on continuous agitation; platelets then transferred to final storage bag
Mirasol (Terumo BCT, Lakewood, CO)	Upon photoactivation with UVB (280-400 nm, 6.24 J/mL for 10 mL) illumination on continuous agitation Riboflavin (Vitamin B2), which associates with nucleic acids, mediates an oxygen-independent electron transfer process – primarily directed at guanine residues - thus introducing single-strand breaks (1 every 350 base pairs)	Platelets Plasma	None Needed

Figure 2: Intercept Dual-Stage Processing Set (From Package Insert)

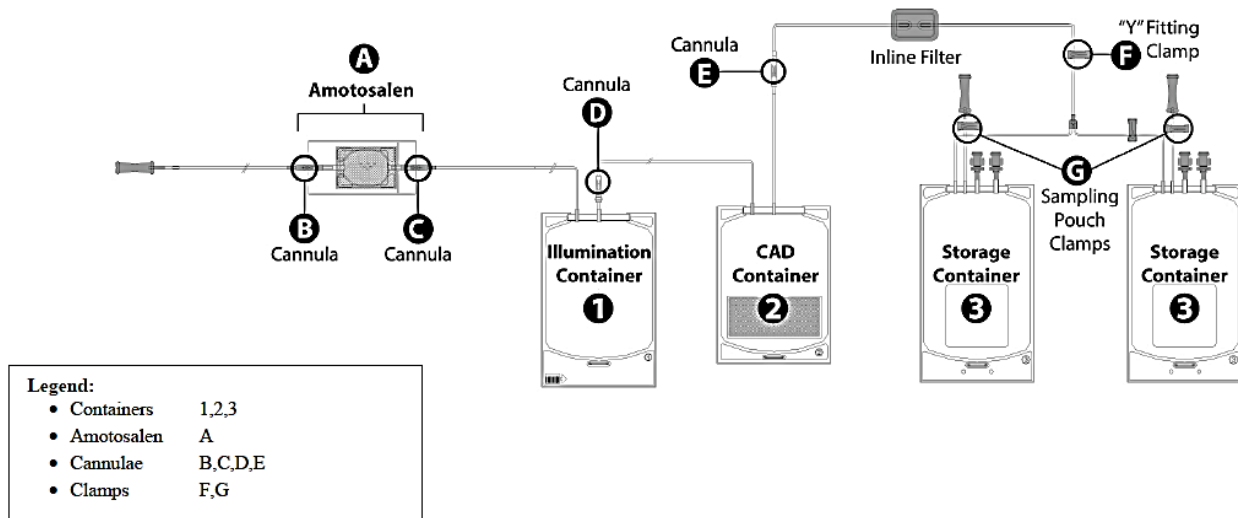


Table 2: Clinical Efficacy

Study	Post-Transfusion Increments								Platelets/Unit (x10 <sup>11</sup> )		Units/Patient		Notes
	1 Hour				24 Hour				PCT	Control	PCT	Control	
	PCT		Control		PCT		Control						
Abs	CCI	Abs	CCI	Abs	CCI	Abs	CCI	PCT	Control	PCT	Control		
euroSPRITE	27.5±13.5	13.1±5.4	35.8±23.3	14.9±6.2	16.4±9.5	7.4±5.5	24.7±17.6	10.6±7.1	3.9±1.0	4.3±1.2	7.5±5.8	5.6±5.5	1hAbs: 8.3 (95% CI 0.9 to 15.8), p=0.03 1hCCI: 1.8 (95% CI -0.4 to 4.1), p=0.11
SPRINT	21.4	11.1	34.1	16.0	13.2	6.7	21.5	10.1	3.7	4.0	8.4	6.2	1hAbs: p value for difference <0.001 1hCCI: p value for difference <0.001
Janetzko	23.8±18.5	11.6±7.3	31.2±15.5	15.1±6.4	16.3±14.4	7.3±6.2	21.3±14.4	10.4±6.5	4.1±1.2	3.8±0.4	4.7±3.3	5.5±4.7	1hAbs: 7.4 (95% CI, -3.1 to 17.9), p=0.16 1hCCI: 3.5 (95% CI, -0.8 to 7.8), p=0.11

Abs x 10<sup>9</sup>/L, CCI x 10<sup>3</sup>. 1hAbs = mean difference in 1 hour absolute platelet increment (x10<sup>9</sup>/L). 1hCCI = mean difference in 1 hour CCI (x10<sup>3</sup>).