

Analysis of Accelerate™ Platelet Concentrating System: Preparation of Concentrated Platelet Product — OCTOBER 1, 2006

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Verification studies have been performed at BioSciences Research Associates, Inc. (Cambridge, MA) to evaluate the use of the Accelerate™ Platelet Concentration System for the preparation of a concentrated platelet product (CPP). Testing was performed to evaluate platelet yields achieved following processing, according to protocol provided by the manufacturer and system's Operator Manual. Platelet in vitro characteristics (i.e., pH, p-selectin, platelet aggregation and hypotonic stress) were evaluated at time 0 (immediately after processing) and at time +4 hours (4 hours post-processing), in order to assess in vitro quality.

IN VITRO TESTING

1.1 TEST OBJECTIVE:

Verification studies have been performed at BioSciences Research Associates, Inc. (Cambridge, MA) to evaluate the use of the Accelerate Platelet Concentrating System for the preparation of a concentrated platelet product (CPP). Testing was performed to evaluate platelet yields achieved following processing according to protocol provided by the manufacturer and system's Operator Manual. Platelet in vitro characteristics (i.e. pH, p-selectin, platelet aggregation and hypotonic stress) were evaluated at time 0

(immediately after processing) and at time +4 hours (4 hours post-processing) in order to assess in vitro quality.

1.2 EXPERIMENTAL DESIGN:

Informed consent was obtained, and all blood collection protocols and donors met requirements of the American Association of Blood Banks (AABB) and the FDA-CBER. Donors are referenced only by assigned code numbers. Donors that had taken aspirin one week prior to blood donation or NSAIDS three days prior to donation were excluded from the study. Approximately two-thirds of donors were female. There was no selection for age or ethnicity. Blood was collected from twelve donors. Blood was collected from each donor into two pre-loaded syringes each containing 8ml of acid-citrate-dextrose formula A (ACD-A) anticoagulant (Baxter Health Care) and drawn to 60ml. A 12ml syringe loaded with 1.6ml of ACD-A was collected from each donor to serve as baseline whole blood control. The blood draw was performed using 16g needle, and all samples were kept at room temperature from time of draw through processing and analysis. All donors signed an IRB approved research donation consent form.

Blood samples from each donor were processed to produce platelet concentrate in the Accelerate Platelet Concentrating System (centrifuge settings: 2400 rpm for 12 min. with zero brake). Two process disposables were used for each donor to produce two CPPs. One CPP was analyzed at 0 hour (immediately after processing) and the other was analyzed at 4 hours post-processing. Volume of each CPP was recorded.

1.2.1 Post-process parameters:

The following table summarizes the parameters measured and the assay methods employed with reference to attached SOP in Appendix 1.

Parameter	Samples	Assay method	SOP Number
CBC	12 x 3	Automated Hematology Analyzer	BSR TM-017
pH	12 x 3	Blood Gas Analyzer	BSR TM-018
p-Selectin	12 x 3	Cytometric with Mab (CD41a and CD62p)	BSR TM-003
Hypotonic Stress Response (HSR)	12 X3	Optical Transmission	BSR TM-016
Platelet Aggregation (Collagen)	12 x 3	Optical aggregometer	BSR TM-001

References

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CBC, pH and p-Selectin measurements were made on whole blood samples and on CPP products without further processing. For optical methods (HSR and aggregation) interfering red cells were removed by centrifugation (soft spin).

1.2.2 Calculations:

Total platelet recovery was calculated based on the platelet counts obtained with the whole blood sample.

$$\text{Total Platelet Recovery} = \frac{\text{Plt} \times \text{Vol}}{\text{Plt}_0 \times \text{Vol}_0} \times 100$$

Where: Plt = CPP platelet count
Vol = Volume of CPP
Plt₀ = Whole blood platelet count
Vol₀ = 60 ml processed blood

1.3 RESULTS:

(Data values provided in Tables in Appendix 2 to this section)

1.3.1 Hematological values

Table I: provides whole blood, pre-process and CPP, post-process values for all samples.

Table I: Hematological Values

Mean ± SD (Range)

Parameter	Whole Blood	CPP @ 0 hr	CPP @ 4 hr	P-Values (0 hr vs. 4 hr)
Hct (%)	31± 4.6 (20-40)	37± 6.6 (26-45)	42± 7.7 (31-56)	p=0.03 (n=11)
WBC x 10 ⁹ /L	4 ± 1.5 (2-7)	20 ± 7.4 (12-41)	23 ± 8.9 (12-44)	NS* (n=11)
Platelet x 10 ⁹ /L	180 ± 58.7 (58-275)	1643±421 (926-2199)	1567 ± 350 (834-1929)	NS* (n=10)

*NS = Not Significant, p>0.05, paired Student's t-Test

1.3.2 Post-Process Platelet Yields

Table II provides summary data for platelet recovery in processed samples.

Table II: Post –Process Platelet Yields

Mean ± 1SD (Range)

CPP @ 0 hr	CPP @ 4 hr	P= Value 0 hr vs. 4 hr	
Yield (%)	68 ± 17 (48-106)	65 ± 16 (44-96)	NS* (n=10)

*NS=Not significant, p>0.05 (Student's t-test, paired, 2 tailed)

1.3.3 Platelet concentration (times baseline) in time 0 and Time +4 hr CPP

Table IV lists the calculated increase in platelet concentration in CPP samples above baseline in both Time 0 and 4 hr post-processing samples.

Table III: Post –Process Platelet concentration

Mean ± 1SD (Range)

CPP @ 0 hr platelet (Times baseline)	CPP @ 4 hr (Times baseline)	
Correlation Coefficient	10 ± 3 (5.8-14)	9 ± 3 (6.6-14)

CPP volume approximately 4ml

1.3.4 In Vitro Characteristics of Concentrated Platelets

Table IV provides summary of the in vitro characteristics of the concentrated platelets. Platelet function/integrity studies were performed at time 0 and +4 hr post-processing.

1.4 DISCUSSION

The Accelerate Platelet Concentrating System was developed to provide a reproducible method for the preparation of platelet concentrate from small volumes of patient's blood. Data from this qualification study has demonstrated that platelet concentrations in the platelet concentrate product were high with an average of greater than nine times baseline. The platelet yield was also high, averaging greater than 60 percent over a varying range of baseline platelet counts. The platelet yield was not correlated with the hematocrit of donor blood over a hematocrit range from 26-46 percent (adjusted for the dilution from ACD-A anticoagulant). The yields and platelet concentrations achieved with the Accelerate compare favorably with those achieved with other commercial CPC systems.^{1,2}

Table IV: In Vitro Characteristics of Platelets Collected with the GenesisCS System

Mean ± 1 SD (Range)

Parameter	Whole Blood	Time 0 hr	Time 4 hr	P-Value (0 hr vs. 4 hr)
pH	6.78±0.07 (6.61-6.84)	6.74±0.05 (6.64-6.85)	6.70±0.03 (6.63-6.74)	.03
p-Selectin (%)				NS*
Direct Measurement	1±4 (-2-10)	14±8 (1-24)	16±11 (4-33)	NS*
ADP (20 µM) Activation	63±7 (51-76)	64±10 (54-83)	69±10 (50-82)	
Platelet Aggregation (%)	80±7 (68-91)	84±9 (66-97)	81±6 (66-87)	NS*
Collagen agonist (190 µg/mL)				
Hypotonic Stress Response	85±17 (43-107)	90±12 (64-110)	77±13 (55-95)	NS*

*NS=Not significant, p>0.05 Student's t-Test (paired, 1 tail)

1.4.1 In vitro data from platelet integrity testing support that the separation and concentration process does not adversely affect the platelets.

1.4.1.1 pH:

There were no pH values less than 6.6 for any CPP at Time 0 or +4 hr. These values are within acceptable range for platelet concentrations. pH 6.2 correlates well with platelet survival and function³. While there was a statistically significant difference between the means for Time 0 CPP (6.74) and Time +4 hr CPP (6.70) the difference is not clinically significant.

1.4.1.2 P-selectin:

The in vitro p-selectin test is used to evaluate the quality of platelet products. Detection of p-selectin on platelet membranes correlates with platelet activation. High percentage of p-selectin positive platelets measured direct (unactivated) is associated with loss of viability. For comparison, the values of p-selectin for day 1 apheresis platelet concentrates collected on centrifugal equipment is approximately 8-23 percent⁴. The direct p-selectin values (averaging 14 percent, Table IV) observed for the Time 0 and +4 hr CPP from the Accelerate were consistent with these values.

Functional reactivity of the platelets is demonstrated by adding an exogenous platelet agonist (ADP). The ADP-stimulated p-selectin values for Time 0 and +4 hr CPP were similar to ADP-stimulated values for paired whole blood samples. The low direct p-selectin values observed for the Accelerate prepared CPP and the increase in p-selectin expression following exposure to ADP (averages greater than 60 percent) demonstrate the functional activity of the platelets is preserved.

1.4.1.3 Collagen-dependent Platelet Aggregation:

Platelet aggregation studies were performed using a collagen agonist. Accelerate prepared CPP samples and their paired whole blood samples all had normal aggregation response (greater than 60 percent of maximum) with average values greater than or equal to 80 percent.

1.4.1.4 Hypotonic Stress Response:

The hypotonic stress response assay measures the ability of platelets to recover their resting volume after exposure to a hypotonic environment and demonstrates platelet membrane integrity⁵. The optical method used in this study is that of Valeri et al⁶ as modified by Farrugia et al⁷. The reported values are the percent of recovery of platelet volume (assessed by change in light transmission) in platelets diluted in water as compared to control platelets diluted in isotonic buffer. The observed hypotonic stress values for Accelerate-prepared CPP were similar for paired, whole blood samples.

CONCLUSION:

These data have established that the Accelerate system is capable of preparing a platelet concentrate suitable for the purpose intended. Testing from in vitro studies, intended to evaluate the quality of the platelets have demonstrated that the functional characteristics are compatible to those using predicate devices or standard blood bank techniques. The Accelerate system provided consistent concentrated platelet product with predictable platelet yields and concentration factors.