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Comparison of Platelet Concentration Systems:

Emcyte AbsolutePRP GS60 GenesisCS Component Concentrating System

vs

Cellmedix Centrepid™ Platelet Concentrate Preparation System

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Executive Summary

This study evaluated the PRP products prepared using the **Emcyte AbsolutePRP GS60 GenesisCS Component Concentrating System** and the PRP products from the **Cellmedix Centrepid™ Platelet Concentrate Preparation System**. The EmCyte AbsolutePRP device use a 5 minute single spin centrifugation process. A robust platelet buffycoat is then siphoned from the top layer of red blood cells after centrifugation. The Cellmedix devices use a two-step centrifugation process. The first spin is a 'separation' step that removes the majority of red blood cells from an anticoagulated whole blood sample. The second spin is a 'concentration' step that facilitates enrichment of platelets suspended in plasma and the remaining red blood cells for a final concentrated platelet product. This report is a retrospective analysis that examines data from two independent 60-donor studies and compares critical parameters associated with platelet-rich plasma.

Results:
The mean platelet concentration for the Emcyte GS60 products was **~1500 million platelets/mL** of PRP, with an average of **~11 billion platelets** delivered in 7.2 mL of product. The Cellmedix Centrepid concentrate contained **~900 million platelets/mL** of concentrate, with an average of **6 billion platelets** delivered in 7mL of product. Platelet recoveries were 75% and 71%, respectively for the Emcyte and Cellmedix products. The platelet concentration factor for the Emcyte device was 6.2 times baseline (whole blood), while the platelet concentration factor for the Cellmedix device was 5.1. There was no evidence of significant device-dependent platelet activation in concentrates from either device.

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1. Introduction

The objective of this study was to test parameters associated with the platelet concentrates (PC) produced by the **AbsolutePRP GS60 GenesisCS Component Concentrating System** and the **Cellmedix Centrepid Platelet Concentrate Preparation System**.

2. Study Design

This was a single center study conducted by BioSciences Research Associates, Inc. (BSR). BSR provides custom contract research and laboratory services for product development, medical device testing and clinical trials support to Pharmaceutical and Biotechnology companies. All studies are conducted within BSR's Quality Systems and are cGXP compliant. BSR has extensive experience with development and testing of platelet concentration devices and product evaluation, including support for FDA CBER and CDER filings.

Up to 130 ml of human whole blood was obtained from each of 60 donors following informed consent. The consent form and blood collection protocols are approved by the New England Independent Review Board, Protocol number 04-14. Donors met the requirements of the American Association of Blood Banks (AABB), the FDA CBER and the Code of Federal Regulations: 21 CFR 606 and Title 45 Public Welfare – Department of Health and Human Services Part 46 Protection of Human Subjects. There were no specific exclusion specifications, other than the donor be healthy. There was no selection for age, sex or ethnicity. Donors are referenced only by assigned code numbers.

For the Emcyte GS60 device, peripheral whole blood was drawn to a final volume of 60mL into syringes containing Na Citrate anticoagulant (10% Na citrate). For the Cellmedix Centrepid device, whole blood was drawn to a final volume of 50mL into syringes containing ACDA anticoagulant (18% ACDA). Whole blood baseline measurements were also obtained from an unprocessed anticoagulated whole blood sample (Na Citrate – GS60; EDTA – Centrepid). For each device, anticoagulated whole blood was processed according to manufacturer's instructions for use to produce platelet concentrate product. Platelet concentrates were analyzed immediately following processing. The platelet concentrate samples were processed in a non-controlled environment simulating the worst case of the patient point of care. Donor samples were disposed of in biohazardous waste following testing.

Table I. Anticoagulant Protocol

Platform	Anticoagulant	Blood
EmCyte GS60	6 mL Na Citrate	54 mL
Cellmedix Centrepid	9 mL ACDA	41 mL

Table II. Centrifugation Protocols

Platform	First Spin	Second Spin
Emcyte GS60	4400 rpm, 5 min	NA
Cellmedix Centrepid	3500 rpm, 5 min	3500 rpm, 8 min

3. Study Parameters

3.1. *Hematology Parameters*

Complete blood counts (CBCs) were performed using a hematology analyzer for baseline and concentrate samples. The White Blood Cell (WBC), Platelet counts and Hematocrit were recorded for each sample. Complete blood counts (CBCs) were tested according to SOP: TM-076 Coulter Ac-T diff 2 Hematology Analyzer.

3.2 *Platelet Concentration Factor*

Complete blood counts (CBCs) were performed using a 3-part differential hematology analyzer to quantify the platelets contained within baseline and concentrate samples. The platelet concentration factor, which is the ratio of the concentration of platelets in the platelet concentrate product to the concentration of platelets in anticoagulated baseline sample, was determined for each device. Complete blood counts (CBCs) were tested according to SOP: TM-076 Coulter Ac-T diff 2 Hematology Analyzer.

3.3. *Platelet Yield*

Complete blood counts (CBCs) were performed using a hematology analyzer to quantify the platelets contained within baseline and concentrate samples. The platelet yield, which is the ratio of the number of platelets in the platelet concentrate product to the number of platelets in the anticoagulated start sample, was determined for each device. Complete blood counts (CBCs) were tested according to SOP: TM-076 Coulter Ac-T diff 2 Hematology Analyzer.

3.4. *pH*

Sample pH was measured in platelet concentrate samples. The minimal acceptable pH is 6.2. Testing was conducted according to SOP: TM-128 Blood pH.

3.5. *Platelet Activation*

Measurement of platelet surface p-selectin glycoprotein by flow cytometry to assess the degree of process-dependent platelet activation was performed with baseline and platelet concentrate samples. Samples were also analyzed for p-selectin following the addition of adenosine diphosphate (ADP) agonist to evaluate platelet function[1]. Resting platelets in citrate anticoagulated whole blood typically have less than 13% activated platelets. ADP stimulated whole blood typically results in greater than 70% activated platelets[2]. The testing method was conducted according to SOP: TM-003: Cytometric Analysis of the P-Selectin.

4. Calculations and Statistical Methods

Data tables and descriptive statistics are shown for each parameter. Mean values and standard deviation results for each parameter are shown in summary tables. Individual donor data is shown for each parameter.

Calculations

4.1. Platelet Concentration Factor

The platelet concentration factor (PCF) was derived as the ratio of the platelet count in the platelet concentrate (PCPRP) to the platelet count in baseline sample (PCWB):

$$PCF = PCPRP/PCWB$$

4.2 Platelet Yield

The platelet yield (PY) was derived as the ratio of the platelet count in the platelet concentrate (PCPRP) times the volume of the platelet concentrate (VPRP) to the platelet count in the baseline sample (PCWB) times the volume of the sample processed (VWB):

$$PY = (PCPRP*VPRP)/(PCWB*VWB)$$

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7. Conclusions

The total platelet deliverable on average in an Emcyte PRP preparation was ~11.1 billion, compared to nearly 40% less, for the Cellmedix product (~6.3 billion). Mean platelet concentrations were also substantially higher for the Emcyte device at 1533 x 10^6 platelets/mL, and 909 x 10^6 platelets/mL for the Cellmedix device.

The average Platelet Yields were significant at greater than 70% recovery for both devices. The average platelet concentration factor was 6.2 times baseline for the Emcyte device and 5.1 times baseline for the Cellmedix device. The pH of the PRP products from both devices were ~7.0.

With respect to Resting Platelet Activation, there was no evidence of significant device-dependent platelet activation. Both concentrate products had resting p-selectin expression of ~7%. Robust platelet responses to ADP-stimulation was measured in both products (97% - Emcyte; 93% - Cellmedix), indicating viability and functionality of recovered platelets[1].

8. References

1. Michelson A. Flow Cytometry: A Clinical Test of Platelet Function. Blood. 1996; 87: 4925-36.
2. CD62P-FITC REF A07790 Product Insert.

Summary Tables:

Table 5.1 Platelet Concentration (x 10^6/mL**), Total Platelets Delivered*** (x 10^6/mL)

	Emcyte	Cellmedix	Emcyte	Cellmedix
Mean	1533	909	11,078	6,363
St Dev	366	197		

**Total PLTs delivered in ~7mL PRP; N=60*

Table 5.2 Platelet Concentration Factor (x baseline) **and Platelet Yield** (% recovery)

	Platelet Concentration Factor		Platelet Yield	
	Emcyte	**Cellmedix**	**Emcyte**	**Cellmedix**
Mean	6.2	5.1	75	71
St Dev	1.2	0.6	14	8

N=60

Table 5.3 pH

	Emcyte	Cellmedix
Mean	7.1	6.9
St Dev	0.1	0.1

Emcyte: N=30; Cellmedix: N=60

Table 5.4 Platelet Activation, Resting and ADP-Activated (%p-selectin expression)

	Resting, PLT Activation		ADP-Stimulated, PLT Activation	
	Emcyte	**Cellmedix**	**Emcyte**	**Cellmedix**
Mean	7	7	98	93
St Dev	3	2	1	2

N=12