# Test Report 1701

## Research Study
## Human Bone Marrow Aspirate Concentration: PUREBMC®

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## 1. Introduction

The objective of this study was to evaluate the bone marrow concentrates (BMC) produced by the EmCyte PUREBMC® Device.

## 2. Study Design

This was a single center paired sample study conducted by BioSciences Research Associates. (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 were conducted within BSR's Quality Systems and are cGXP compliant. BSR has extensive experience with development and testing of platelet and bone marrow aspirate concentration devices, including support for FDA Center for Biologics Evaluation and Research (CBER) and CDER filings.

Up to 70 ml of human bone marrow aspirate were obtained from each of 5 donors following informed consent. Marrow collection protocols and consent documents were approved by the LeukoLab Institutional Review Board, Protocol number 7000-SOP-078 expiration date 8 Feb 2017 and Donors met the requirements of 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 and donors were referenced only by assigned code numbers. Marrow aspirates were drawn with sodium heparin rinsed aspiration needles and placed in a 150ml transfer bag with a final concentration of 80 IU of Na Heparin per ml of BMA. Twelve mL of Na Citrate was added immediately prior to processing to give a final concentration of ~15% Na Citrate.

A bone marrow aspirate concentrate was prepared, from each donor sample, according to manufacturer's instructions for use. BMA samples were processed within approximately 24hr of collection.

**Parameters evaluated:**

*Nucleated Cells, Erythrocytes and Platelet Counts*

Complete blood counts (CBC) were performed using a Beckman Coulter Model A$^{c}$Tdiff2 hematology analyzer for baseline samples and marrow concentrates. The Nucleated Cells (NC), Erythrocyte (RBC) and Platelet concentrations were recorded for each BMA and BMC sample.

*CD34 Positive Stem/Progenitor cells*

The concentration and the yield of CD34 Positive Stem/Progenitor cells was determined for each BMC product. The cells represent proangiogenic and hematopoietic progenitor cells.

*Colony forming units-fibroblast*

The yield of fibroblast colony forming cells (MSCs) was calculated for each BMAC product. MSC cells are support cells in the marrow and can be shown to differentiate into osteogenic, chondrogenic and adipogenic cell morphology in vitro.

*Product pH*

The pH of each product sample was measured with a Blood Gas analyzer, Stat Profile Prime, Nova Biomedical.

**Results:**

Total nuclear cells (TNC) are all cells except mature RBC.
The yields, defined as the percent of cells in the start samples that were recovered in the products are displayed in Table I.

Table I: Total Nucleated Cells:

| Donor | Cell Count x $10^6$/mL | % Yield | Cell Viability |
|---|---|---|---|
| 701 | 99.0 | 78 | 96% |
| 702 | 181.5 | 87 | 98% |
| 703 | 154.8 | 79 | 97% |
| 704 | 147.3 | 75 | 95% |
| 705 | 147.3 | 81 | 97% |
| **Mean (STDEV)** | 146.0 (29.9) | 80.0 (4.5) | 97 (1.1) |

The concentration, in the BMA, of two cell types with multi-lineage differential potential, CD34 positive cell and Mesenchymal Support Cells (MSC) are shown in Table II. The yields of CD34+ cells and MSC are shown in Table III.

Table II. Hematopoietic Progenitor ($CD34^+$) and Mesenchymal Support (MSC) Cells:

| Donor | $CD34^+$ Stem/Progenitors | CFU-f (MSC) |
|---|---|---|
| 701 | 474,089 | 5,445 |
| 702 | 919,824 | 1,634 |
| 703 | 734,433 | 1,703 |
| 704 | 635,897 | 6,187 |
| 705 | 992,867 | 23,568 |
| **Mean (Standard deviation)** | 751,422 (210,478) | 7,707 ( 9,109) |

Table III. Hematopoietic Progenitor ($CD34^+$) and Mesenchymal Support (MSC) Cells: % Yield

| Donor | $CD34^+$ Stem/Progenitors | CFU-f (MSC) |
|---|---|---|
| 701 | 93% | 107% |
| 702 | 90% | 78% |
| 703 | 92% | 138% |
| 704 | 88% | 64% |
| 705 | 77% | 61% |
| **Mean (Standard deviation)** | 88% (6) | 90% (34) |

The Platelet concentration, RBC concentration, hematocrit and pH are listed in Table IV.

Table IV. Platelet and RBC Concentration, Hematocrit and Product pH.

| | Platelet x $10^6$/ml | RBC x $10^9$/ml | Hct (%) (St. Dev.) | pH** |
|---|---|---|---|---|
| 701 | 1278 | 2.1 | 19.5 | 7.25 |
| 702 | 908 | 2.6 | 24.6 | 7.19 |
| 703 | 732 | 3.1 | 26.7 | 7.20 |
| 704 | 1440 | 2.5 | 22.8 | 7.24 |
| 705 | 621 | 1.6 | 15.6 | 7.17 |
| **Mean (STDEV)** | 996 (352) | 2.4 (0.6) | 21.8 (4.4) | 7.2 (.03) |

Table V. Cell concentration factor over baseline concentration

| | TNC | Platelet | CD34+ | MSC |
|---|---|---|---|---|
| 701 | 6.5 | 6.5 | 7.7 | 8.9 |
| 702 | 7.3 | 7.3 | 7.5 | 6.5 |
| 703 | 6.3 | 6.3 | 7.6 | 11.5 |
| 704 | 5.6 | 5.6 | 7.3 | 4.8 |
| 705 | 6.5 | 6.5 | 6.4 | 5.1 |
| **Mean (STDEV)** | 6.4 (0.6) | 6.4 (0.6) | 7.3 (0.5) | 7.4 (2.8) |

The ability of BMC product to deliver growth factors was evaluated. Table VI shows the concentration of Platelet Derived Growth Factor (PDGF) in the baseline sample and in the BMC.

Table VI. Growth Factor in baseline sample and BMC: PDGF-AB pg/mL

| | Baseline | BMC | Fold increase + |
|---|---|---|---|
| 701 | 5,948 | 44,404 | 7.4 |
| 702 | 8,920 | 28,612 | 3.2 |
| 703 | 4,532 | 21,880 | 4.8 |
| 704 | 7,996 | 31,399 | 3.9 |
| 705 | 3,995 | 10,008 | 2.5 |
| **Mean (STDEV)** | 6278 (2139) | 27,261 (12,644) | 4.4 (1.9) |

**Discussion:**

This study evaluated the BMC product produced by the EmCyte centrifuged based bone marrow aspirate concentrators. The cell viability both pre- and post-processing exceeded 95%. Nucleated cells recovery averaged 80%. The stem/progenitor cells in the mononuclear fraction were recovered in high percentages averaging 88% yields and enrichment >700%.
It was also demonstrated that BMC is a source of growth factors.

**Conclusion:**

The EmCyte PUREBMC® is convenient to use and produces approximately 7.5 mL of concentrated bone marrow aspirate containing most of the nucleated cells and nearly all of the stem/progenitors cells in the mononuclear fraction of the aspirate. In addition to cellular components that contribute to healing, it is demonstrated that significant levels of growth factor are also present in the BMC product.