# A NOVEL ASSAY to EVALUATE the FUNCTIONAL POTENTIAL of UMBILICAL CORD BLOOD PROGENITORS


## I. N. Rich[1], Karen M[1]. Hall, L.H. Gebru and J.A. Reems[2]

[1]HemoGenix, Inc. Colorado Springs, Colorado

[2]Puget Sound Blood Center, Seattle, Washington


### THE PROBLEM: A Question of Stem Cell Potency

The Colony Forming Cell (CFC) Assay was not designed as a potency assay for stem cell transplantation, but developed for investigational use. The CFC assay is not in accordance with article 21 CFR 610.10, which states that "tests for potency shall consist of either an *in vitro* or *in vivo* test, or both, which have been specifically designed for each product so as to indicate its potency".There are four fundimental problems with the CFC assay as used for potency testing of stem cell products:

**Subjectivity:** A potency assay should be based on an independent, instrument-based reading and not manual enumeration of colonies.

**Calibration and Standardization:** A potency assay should be calibrated against an external standard, thereby allowing the assay to be intra- and interlaboratory validated.

**Assay time:** According to the FDA, "product potency for living cell products may be compromised by extensive assay times". The 14 day culture period is too long to predict engraftment after transplantion which usually occurs between 14 and 21 days.

**Differentiation:** The CFC assay is a differentiation assay. Differentiation is the process whereby an undifferentiated cells, i.e. a stem cell (by definition), acquires the features of a specialized cell. Proliferation is defined as the expansion of cells by the continuous division into two identical daughter cells. Proliferation occurs prior to differentiation. Without proliferation, differentiation would not occur. And differentiation is a default program requiring prior proliferation. Since, by definition, stem cells are undifferentiated, the CFC assay measures differentiation potential and not the capability of stem cell to proliferate, which is an absolute requirement for engraftment and reconstitution. As such, not only was the CFC assay not designed to measure potency, but fails to provides the necessary information required as defined by a potency assay. The CFC assay is the incorrect assay to use for stem cell potency measurements.

### THE SOLUTION - HALO™-96 MeC SPC-QC

These, and many other aspects were taken into account in the development of the *HALO™* Stem and Progenitor Cell - Quality Control Platform. HALO™ (Hematopoietic Assays via Luminescence Output) is a colony-forming proliferation assay in methylcellulose. Instead of counting colonies, cell proliferation of individual cell populations is directly measured by virtue of the fact that, when cord blood cells are stimulated with growth factors, an increase in intracellular ATP (iATP) concentration occurs that directly correlates with proliferation. After only 7 days, the released iATP acts as a limiting substrate for a luciferin / luciferase reaction to produce bioluminescence in the form of light detected in a plate luminometer. HALO™ is calibrated and therefore standardized using an ATP standard dose response curve prior to measuring the samples. This allows intra- and inter-laboratory comparison of results. It also allows various procedures which rely of the potency of the sample, e.g. sample preparation, cryopreservation and thawing, to be compared and standardized between laboratories.

### THE STUDY

This study was performed at two geographically disinct locations; Puget Sound Blood Center (Site 1) and HemoGenix, Inc (Site 2). All samples were collected at Site 1. The cord blood units used in this study did not conform to one or more specifications. The samples were divided into two and one was sent by overnight courier to Site 2. On the day of arrival at Site 2, both sites performed the assays. The total nucleated cell count, viability, colony forming cell assay (CACA) and HALO™-96 MeC SPC-QC were performed at both sites. Only phenotypic analysis of CD34⁺ cells was performed at Site 1. The CFCA was performed at Site 1 using reagents from Stem Cell Technologies, while the CFCA at Site 2 was performed using the same HALO™-96 MeC SPC-QC Master Mix. Therefore, both CFCA and HALO™-96 MeC SPC-QC for the CFC-GEMM population were performed under exactly the same conditions.

NOTE: To distinguish between CFC-GEMM detect by CFCA and CFC-GEMM or other populations detected using HALO™-96 MeC, cell populations are referred to with the prefix (p), meaning "proliferative".

### THE RESULTS

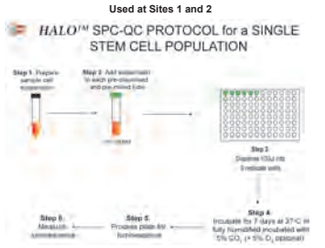
1. Detection of potency of cord blood samples using the single population (pCFC-GEMM) HALO™-96 MeC SPC-QC correlated between sites with an R = 0.94, P < 0.001).
2. There was a high correlation for TNC between sites (R = 0.98) despite the different methods used.
3. There was a correlation between sites for the CFC assay, despite the difference in technologies used (R = 0.79, P < 0.002).
4. There was a good correlation between the CFC assay and HALO ™-96 MeC SPC-QC for the CFC-GEMM population (R = 0.73, P < 0.005).
5. A correlation was found at Site 1 between CFC and CD34⁺ cells.
6. There was no correlation between the potency of pCFC-GEMM detected using HALO™-96 MeC SPC-QC and the presence of the CD34 membrane marker.
7. Using the 7-population HALO™-96 MeC SPC-QC, predicted and expected differences between the proliferation potential of stem and progenitor cells of the lympho-hematopoietic system can be rapidly assessed prior to and after transplantation.

### CONCLUSIONS

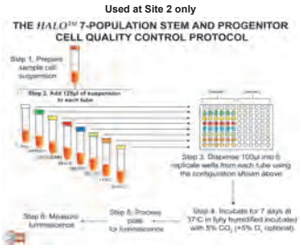
**Our results support the notion that HALO™-96 MeC SPC-QC is a reasonable approach for measuring the potency of hematopoietic progenitors within a unit of UCB. Moreover, because the final read-out is instrument-based, unlike the CFC assay, which requires a subjective enumeration of colonies, HALO™-96 MeC SPC-QC will be more amenable to standardization and validation among UCB banks.**

## THE ASSAYS

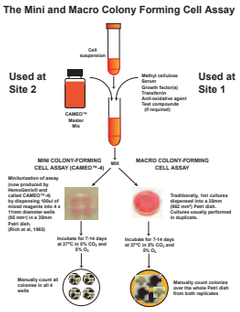
**Used at Sites 1 and 2**




**Used at Site 2 only**

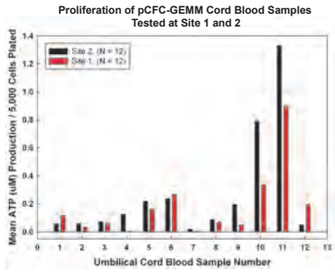



**The Mini and Macro Colony Forming Cell Assay**

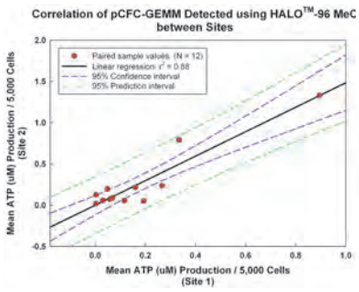



## CORRELATION of PROLIFERATION of pCFC-GEMM and other PROLIFERATING CELL POPULATIONS DERIVED from 12 CORD BLOOD SAMPLES as a DIRECT MEASURE OF STEM and PROGENITOR CELL POTENCY

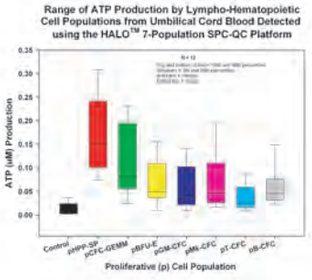
**Proliferation of pCFC-GEMM Cord Blood Samples Tested at Site 1 and 2**




**Correlation of pCFC-GEMM Detected using HALO™-96 MeC between Sites**



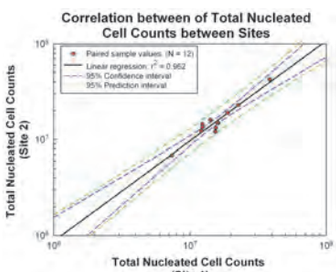

**Range of ATP Production by Lympho-Hematopoietic Cell Populations from Umbilical Cord Blood Detected using the HALO™ 7-Population SPC-QC Platform**



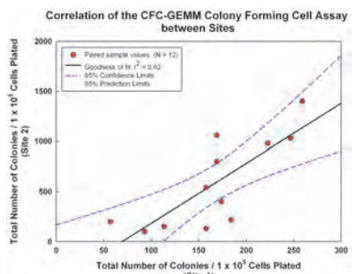

## CORRELATION of ASSAYS BETWEEN SITES and RELATIONSHIP between HALO™ SPC-QC, the COLONY-FORMING CELL ASSAY and ABSOLUTE CD34⁺ NUMBERS DERIVED from 12 CORD BLOOD SAMPLES

**(All assays performed at both sites, except absolute CD34⁺ determinations which were only performed at Site 1).**

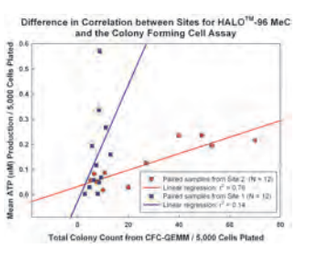
**Correlation between of Total Nucleated Cell Counts between Sites**



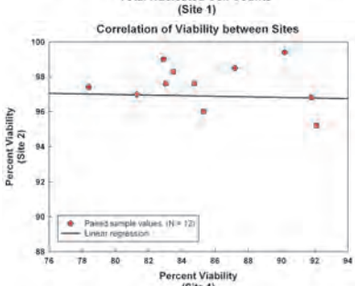

**Correlation of the CFC-GEMM Colony Forming Cell Assay between Sites**



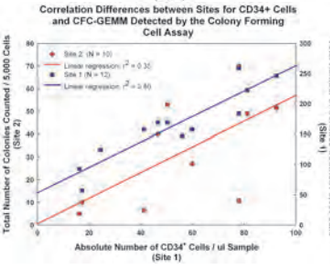

**Difference in Correlation between Sites for HALO™-96 MeC and the Colony Forming Cell Assay**




**Correlation of Viability between Sites**




**Correlation Differences between Sites for CD34+ Cells and CFC-GEMM Detected by the Colony Forming Cell Assay**




**Lack of Correlation between CD34⁺ Cells and pCFC-GEMM Detected using HALO™-96 MeC**