A Potency Assay for Mobilized Peripheral Blood, Umbilical Cord Blood and Bone Marrow Stem Cell Products

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SUMMARY and CONCLUSIONS.

Stem cells are the only cells responsible for engraftment and reconstitution. Stem cell potency is a measure of stem cell primitiveness or proliferation potential. Stem cell quality is proliferation ability. Potency is measured from the potency ratio by comparison with a reference standard of the same material. There is a direct correlation between stem cell potency and quality. These parameters define the release criteria of a hematopoietic stem cell product. In contrast to other proposed assays for potency, which do not comply with regulatory requirments for potency assays (e.g. TNC, viability, CD34 or even CFU), HALO®-96 PQR is not only complient, but is standardized and validated. HALO®-96 PQR has therefore been designed specifically as a potency assay. HALO®-96 PQR is a 3-step procedure. However, only the first step involves cell culture and the bioluminomics™ measurement of a minimum of two stem cell populations. Cells are culture for 5-7 days followed by measurement of the intracellular ATP (iATP) concentration, which then provides all the necessary information to measure stem cell potency, quality and release. The results from HALO®-96 PQR correlate with the ability of the stem cells to engraft and can be used for mobilized peripheral blood, cord blood or bone marrow (not shown). The iATP concentration directly correlates with both TNC and MNC showing that the greater the iATP the greater the chance of engraftment. HALO®-96 PQR and its counterpart assay for mesenchymal stem cells, LUMENESC™-96 PQR, are the only potency assays available. They are all simple, easy and fast to use and provide quantitative, reliable and reproducible results for the stem cell therapy processing laboratory.

INTRODUCTION

What is Potency and How is it Measured?

Potency is the quantitative measurement of biological activity of a product.

Potency is measured by performing a dose response of the sample and comparing it with a reference standard (RS) of the same material.

For drugs, growth factors, vaccines etc., the regression of the dose responses MUST be parallel with each other.

Only then can potency be measured as the POTENCY RATIO (PR).

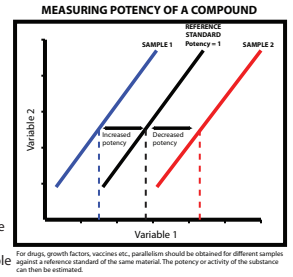
When:

PR > 1, potency of sample > RS; less of sample required to achieve same response as RS.

PR < 1, potency of sample < RS; more of sample required to schieve same response as RS.

Therefore:

POTENCY predicts the DOSE



For drugs, growth factors, vaccines etc., parallelism should be obtained for different samples against a reference standard of the same material. The potency or activity of the substance can then be estimated.

How is Cell Potency Measured?

Because cells are living entities and in a constant state of flux, the principle of parallelism can not apply as a general rule.

For cells, and STEM CELLS in particular, the potency ratio MUST be measured by comparing the slope of the cell dose response of the sample to that of the RS:

Potency Ratio = Slope of Sample Linear Regression / Slope of RS linear regression.

How the Potency of a Stem Cell Product is Measured

Stem cells are the ONLY cells responsible for engraftment and reconsitution.

Stem cell potency must be measured using one or more biological properties of the stem cells. Parameters such as total nucleated cell count (TNC), viability and/or flow cytometry (e.g. CD34, ALDH) are not specific and do not measure the biological properties of stem cells. Even the CFU assay is not a potency assay. These parameters do not and cannot measure potency because they do not conform to potency assay regulations.

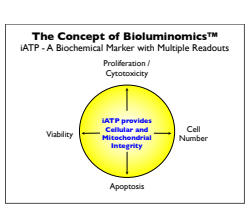
A property of stem cells is their capacity and ability to proliferate. Without proliferation, engraftment and reconstitution will not occur.

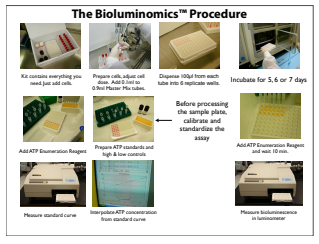
Stem cells exhibit different potentials for proliferation depending on their primitiveness or "stemness". This property has been used to develop a stem cell Potency, Quality and Release assay (PQR) for hematopoietic stem cells and mesenchymal stem cell products according to the following principle:

Primitiveness or "stemness" of stem cell = Proliferation Potential of Stem Cell Population (SCP)
Proliferation Potential of SCP = Slope of the SCP Cell Dose Response Linear Regression
Slope of SCP Cell Dose Response Linear Regression = Stem Cell Potency (when compared to RS)
Stem Cell Potency = Engraftment Potential (not Time to Engraftment)
Stem Cell Potency predicts Stem Cell Dose, which predicts Engraftment Potential

HALO®-96 PQR: An ATP Bioluminomics™ Potency, Quality and Release Assay for Hematopoietic Stem Cell Products

HALO® is an assay platform that was originally developed from the CFU assay and is therefore equivalent to the CFU assay. However, HALO® is an advanced CFU assay that contains no methylcellulose and measures stem cell proliferation using the most sensitive, non-radioactive signal detection system available; ATP bioluminescence. HALO® is non-subjective, fully standardized and validated according to regulatory requirements. HALO® is a bioluminomics™ assay system. The concept, principle and procedure of a bioluminomics™ assay are shown below.



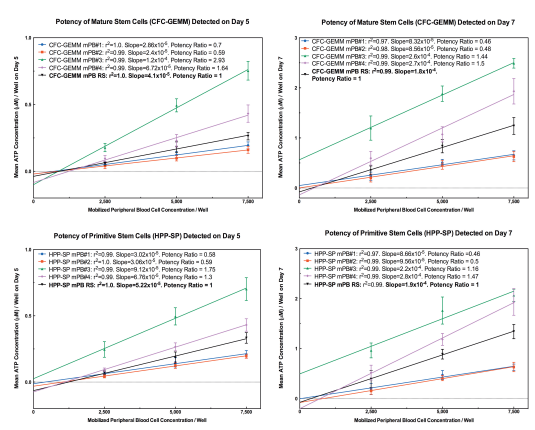


3-STEP PROCEDURE TO MEASURE POTENCY, QUALITY AND RELEASE OF STEM CELL PRODUCTS

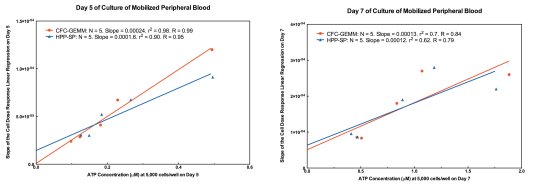
Mobilized Peripheral Blood

Step 1: Measuring the Potency Ratio of Two Hematopoietic Stem Cell Populations

The assay requires measurement of at least 2 hematopoietic stem cell populations, the primitive High Proliferative Potential Stem and Progenitor Cell (HPP-SP) and the more mature Colony-Forming Cell - Granulocyte, Erythroid, Macrophage, Megakaryocyte (CFC-GEMM).

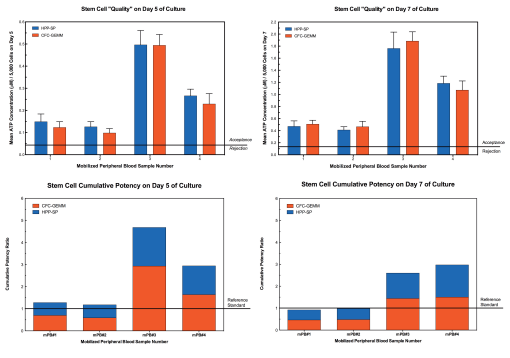


Step 2: The Relationship between Stem Cell Potency and Quality



There is a direct correlation between stem cell potency and quality. This means that both parameters must be considered for release of the product.

Step 3: Release Criteria for Stem Cell Products



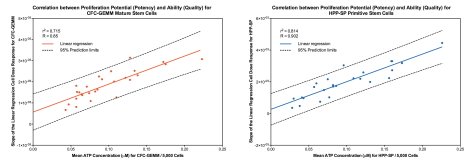
Although stem cell "quality" (proliferation at 5,000 cells/well) is in the "acceptable" region on days 5 and 7 for samples 1 and 2, the potency of these samples is below the RS and should not be considered for transplantation purposes.

UMBILICAL CORD BLOOD STEM CELL POTENCY, QUALITY AND RELEASE CRITERIA: CORRELATION WITH ENGRAFTMENT

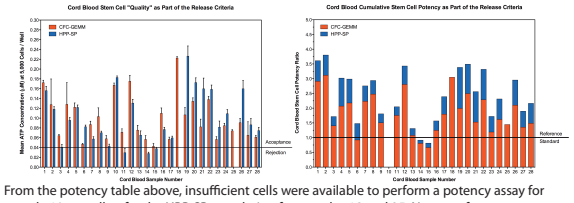
Step 1: Calculated Potency Ratios of Cord Blood Samples and Time to Engraftment

Sample Number	Slope for CFC-GEMM	Potency Ratio for CFC-GEMM	Slope for HPP-SP	Potency Ratio for HPP-SP	Days to Neutrophil Engraftment (>500/ul)	Days to Platelet Engraftment (>50k/ul)
1	2.94E-05	2.91	2.23E-05	0.70	28	237
2	3.14E-05	3.12	2.19E-05	0.69	14	2
3	1.41E-05	1.40	1.01E-05	0.31	6	45
4	2.09E-05	2.07	3.04E-05	0.95	30	49
5	2.19E-05	2.18	2.59E-05	0.81	17	39
6	9.28E-06	0.92	1.79E-05	0.56	12	9
7	2.25E-05	2.23	1.67E-05	0.52	17	45
8	2.50E-05	2.48	1.48E-05	0.46	22	39
9	1.52E-05	1.50	9.70E-06	0.30	56	13
10	IE	-	IE	-	43	103
11	1.77E-05	1.75	9.39E-06	0.29	34	7
12	2.83E-05	2.81	1.99E-05	0.62	20	26
13	1.09E-05	1.08	7.43E-06	0.23	NE	NE
14	8.12E-06	0.81	3.55E-06	0.11	19	183
15	6.69E-06	0.66	4.77E-06	0.15	31	122
16	1.26E-05	1.25	1.02E-05	0.32	13	39
17	1.81E-05	1.80	1.90E-05	0.60	5	40
18	3.07E-05	3.05	IE	-	NE	NE
19	2.01E-05	1.99	4.45E-05	1.39	27	38
20	2.52E-05	2.50	3.18E-05	1.00	22	62
21	1.54E-05	1.53	3.30E-05	1.03	29	55
22	2.31E-05	2.29	3.30E-05	1.03	18	23
23	1.20E-05	1.19	1.70E-05	0.53	28	70
24	1.63E-05	1.62	2.22E-05	0.70	15	46
25	1.46E-05	1.45	IE	-	26	39
26	2.12E-05	2.10	2.73E-05	0.85	28	61
27	1.37E-05	1.35	1.74E-05	0.54	37	126
28	1.50E-05	1.49	2.16E-05	0.68	114	113

Step 2: Correlation between Stem Cell Potency and Quality



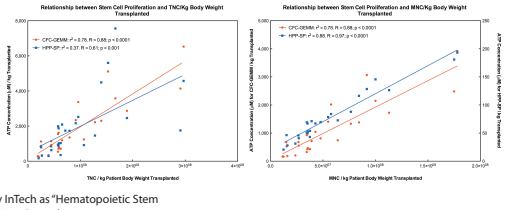
Step 3: Release Criteria for Cord Blood Stem Cell Products



From the potency table above, insufficient cells were available to perform a potency assay for sample 10 as well as for the HPP-SP population for samples 18 and 25. No engraftment was observed for samples 13 and 18. HALO®-96 PQR predicted that >85% of the samples tested contained sufficient stem cell potency and quality to achieve engraftment.

Correlation between ATP Concentration and TNC, MNC, Viability or CD34⁺ Cells

No correlation existed between the ATP concentration and viability or CD34⁺ cells. This is to be expected since these parameters do not measure stem cell proliferation. A correlation did exist between ATP and TNC and ATP and MNC, but on a per kilogram body weight basis. Notice that the correlation is greater between ATP and MNC than with TNC. This is because the MNC contains the stem cells, while the TNC dilutes the stem cells and contains other cells that do not contribute to engraftment. This correlation demonstrates that the larger the number of cells transplanted the greater the number of stem cells and the greater the probability that these stem cells exhibit high potency and quality for engraftment. Notice that ATP does not correlate with time to engraftment, since the latter is not measured by proliferation, but rather differentiation of GM-CFC into neutrophils and Mk-CFC into platelets.



 The results shown here will be published by InTech as "Hematopoietic Stem Cell Potency for Cellular Therapeutic Transplantation" online on November 29, 2011 and as hardcopy in "Hematopoietic Stem Cells", (ISBN 978-953-307-746-8), Ed. Rosana Pelago Camocho.