A Stem Cell Potency and Release Criteria Assay Specifically Designed for Umbilical Cord Blood Transplantation that is Compliant with Regulatory Guidelines

Ivan N. Rich, Karen M. Hall and Holli Harper

HemoGenix®, Inc. Colorado Springs, CO,

POTENCY IS A MEASURE OF CHEMICAL OR BIOLOGICAL ACTIVITY. HOW IS POTENCY MEASURED?

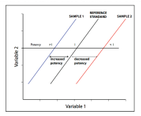
"All potency assays used for release testing of licensed biological drug products must comply with applicable biologics and cGMP regulations including:

1. Indicate potency (biological activity/activities) specific to the product.
2. Provide test results for release of the product.
3. Meet pre-defined acceptance and/or rejection criteria.
4. Include appropriate reference materials, standards, and/or controls.
5. Establish and document the accuracy, sensitivity, specificity, reliability, reproducibility and robustness of the test methods, employed through validation".

(FDA Draft Guidance for Industry: Potency Tests for Cellular and Gene Therapy Products", October 2008.

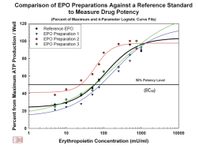
To measure potency, a reference standard is an absolute requirement. A dose response is performed for the reference standard and the sample(s). Comparison of the dose response lines should demonstrate statistical parallelism as seen in the example below. The horizontal displacement from the reference standard provides the potency ratio and therefore the activity of the sample.

POTENCY:
A Specific Measure of Standardized Chemical or Biological Activity



For drugs, growth factors etc., parallelism should be obtained for different samples against a reference standard of the same material. In this way, the specific activity of a substance is determined.

An Example of Measuring Drug Potency



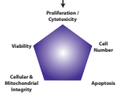
Erythropoietin potency measured using the HALO®-96 MeC Platform

NO test or assay (TNC, viability, CD34 or CFC) presently used by stem cell transplantation and cord blood storage processing laboratories complies with these criteria (FDA and EMEA) for a potency assay designed for the intended use of the product.

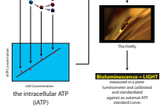
HALO®-96 PQR

An instrument-based, ATP bioluminescence proliferation assay that measures cord blood stem cell potency and defines release crietria.

ATP - A Powerful Biochemical Marker of Cellular Function



The HALO® Principle



HALO®-96 PQR utilizes a proprietary Suspension Expansion Culture (SEC) technology, rather than methyl cellulose. This allows greater ease of use, rapid 5 day assay completion, increased sensitivity and low coefficients of variation (see below). Unlike the CFC assay, HALO®-96 PQR (like all HALO® products) is standardized and validated according to FDA regulations and guidelines. All HALO® products show equivalency to the CFC assay and therefore can replace the CFC assay for most applications.

HALO®-96 PQR Precision: Part of the validation process to test reproducibility

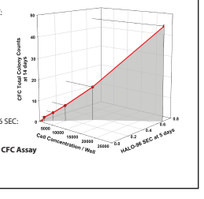
Background Controls				
Cells / Well	2,500	5,000	7,500	10,000
No. of Columns	24	24	24	24
Mean %CV	5.0	4.3	3.7	3.4

CFC-GEMM				
Cells / Well	2,500	5,000	7,500	10,000
No. of Columns	24	24	24	24
Mean %CV	16.6%	10.4%	9.4%	8.0%

HPP-SP				
Cells / Well	2,500	5,000	7,500	10,000
No. of Columns	24	24	24	24
Mean %CV	12.0%	9.7%	6.7%	5.7%

Equivalence and Validation of HALO®-96 PQR against the CFC Assay

Correlations:
Cell dose vs CFC: R = 0.99
Cell dose vs HALO®-96 SEC: R = 0.987
HALO®-96 SEC vs CFC: R = 0.986.



Combined Correlation:
Cell dose vs CFC vs HALO-96 SEC: R = 0.998.
Conclusion:
HALO®-96 can replace the CFC Assay

Umbilical Cord Blood Stem Cell Potency, Release and Engraftment Study

1. Performed in collaboration with the University of Colorado Cord Blood Bank (ClinImmune, Inc).

2. 56 Umbilical cord blood frozen pellet samples for which engraftment information was known.

3. Approx. 700µl / sample.

4. All samples tested randonly.

5. NycoPrep MNC, cell count, viability by 7-AAD.

6. 24 Samples tested for CFC-GEMM and 23 samples tested for HPP-SP at 2,500, 5,000 and 7,500 or 10,000 cells/well, when sufficient cells were available.

7. All remaining samples tested at 5,000 cells/well.

Characteristics of the Study Population

Time from freezing to transplantation: 91 days to 9 years and 311 days.
Time from transplantation to ANC recovery (> 500 cells/µl): 5 - 114 days.
Time from transplantation to platelet recovery (> 50k/µl): 2 - 237 days.

Cord Blood Characteristics

Pre-cryopreservation total nucleated cell count: 15.7 x 10^6 - 178 x 10^6.
Post-thaw total nucleated cell count: 20,000 - 5.55 x 10^6.
Post-thaw viability (7-AAD): 49% - 100%.

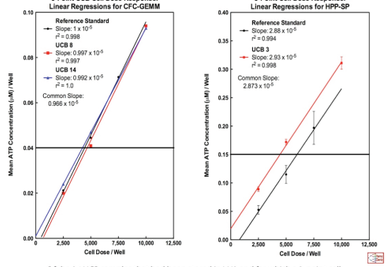
Cord Blood Storage, ATP Values and Engraftment

32 of 56 UCB samples stored at -80°C (but units stored in LN2).
17 out of these 32 units engrafted, but ALL 32 samples tested exhibited ATP values <0.04µM.

24 of 56 samples (and units) stored in LN2.
ALL 24 engrafted (100%) and ALL exhibited ATP values >0.04µM for CFC-GEMM or >0.05µM for HPP-SP or both.

Measurement of Cord Blood Stem Cell Potency

Measurement of Stem Cell Potency by Statistical Parallelism



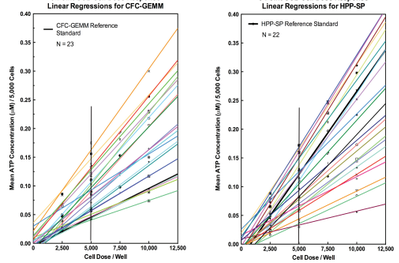
Of the 24 UCB samples that had been stored in LN2 and for which a 3-point cell dose response could be performed, only 3 UCB samples produced dose response curves statistically parallel to the UCB reference standard. The common CFC-GEMM slope for UCB 8 an 14 was 0.97 x 10^{-5}, while that for HPP-SP was 2.93 x 10^{-5}. The CFC-GEMM potency ratio for UCB 8 was 0.94 and that for UCB 14 was 1.05. The HPP-SP potency ratio for UCB 3 was 1.36. A potency ratio greater than 1, indicates a greater potency than the reference standard.

What does it mean when the slopes are parallel?

1. The samples contain a greater or lower number of stem cells than the reference standard.

2. But, their "stemness" or "primitiveness" is the same as the reference standard.

Measurement of Stem Cell Potency by the Slope of the Dose Response



When potency is measured by parallelism, the slope of the dose response must be the same as that of the reference standard. As seen here, this is the exception. The rule is shown above for the remaining UCB samples. In this case, non-parallelism was obtained. The cell potency ratio of the CFC-GEMM and HPP-SP populations is then obtained by comparing the slope of the UCB sample to that of the reference standard.

What does the correlation of slope with iATP concentration mean?

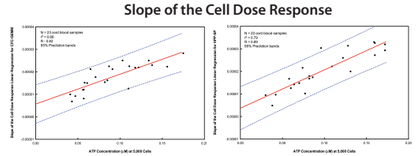
The steeper the slope of the cord blood dose response:
1. The greater the proliferation potential.
2. The more primitive the stem cells in the sample.
3. The greater the stem cell potency, and
4. The greater the probability of engraftment.

REFERENCES

1. Developemnt of a Novel Assay to Evaluate the Functional Potential of Umbilical Cord Blood Progenitors, Reems, Hall, Luladay, Taber, Rich. Transfusion, 48:620-628 (2008).
2. Cell Potency Assays for the 21st Century Stem Cell Transplantation and Cord Blood Bank Processing Laboratories. HemoGenix® White Paper. Please download this article from the HemoGenix® website at www.hemogenix.com.
3. "Validation and Development of a Predictive Paradigm for Hemotoxicity Using a Multifunctional Bioluminescence Colony-Forming Proliferation Assay". Rich IN, Hall KM. Tox Sci 87: 427-441 (2005).
4. In vitro to in vivo concordance of a high throughput assay of bone toxicity across a diverse set of drug candidates. Olaharski et.al. Tox. Lett. 2009. Science Direct Online.

Important Correlations (or lack of) Associated with ATP Concentration and Stem Cell Potency

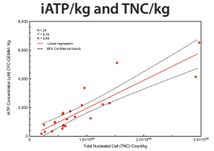
Correlation between Intracellular ATP and the Slope of the Cell Dose Response



If a vertical line is placed across all dose responses at any cell concentration, it will be seen that as the iATP concentration increases, so does the slope. This means that there is a direct correlation between iATP and the slope of the dose response, as shown above for CFC-GEMM and HPP-SP.

This correlation validates the use of iATP to determine stem cell potency.

Correlation between iATP/kg and TNC/kg

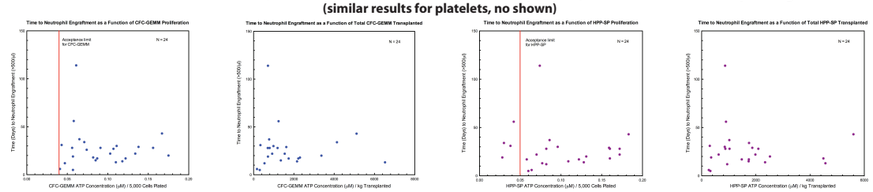


The correlation between iATP/kg and TNC/kg is to be expected, since the greater the number of cells, the greater the iATP concentration.

There is NO Correlation between:

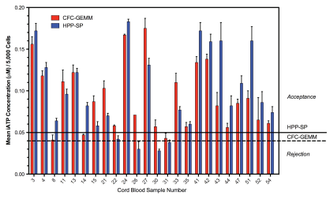
Pre-Cryo TNC vs iATP
Pre-Cryo TNC vs ANC (>500)
Post-thaw MNC vs iATP
TNC vs % viability
% Viability vs iATP
iATP vs ANC (>500)
iATP vs Platelets (>50k)
iATP/kg vs CD34/kg
TNC/kg vs ANC (>500)
TNC/kg vs CD34/kg
iATP/kg vs ANC (>500)
CFC-GEMM potency vs TNC/kg
HPP-SP potency vs TNC/kg
CFC-GEMM Potency vs ANC (>500)
HPP-SP Potency vs ANC (>500)

Neither the CFC-GEMM nor HPP-SP (as concentration or per kilogram transplanted) Correlates with Neutrophil Engraftment (similar results for platelets, no shown)



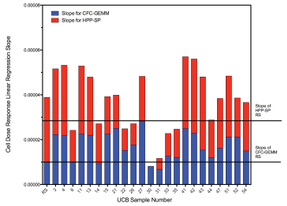
Relationship between iATP, Release Criteria, Stem Cell Potency and Engraftment

Intracellular ATP Concentrations Define Release Criteria



Those UCB samples exhibiting a CFC-GEMM iATP value <0.04µM were aided by a HPP-SP iATP value >0.05µM.
Those UCB samples exhibiting a HPP-SP iATP value of <0.05µM, were aided by a CFC-GEMM iATP value of >0.04µM.
Only 1 sample (UCB 31) might have been rejected.

The Slope of the Cell Dose Response Defines Proliferation Potential

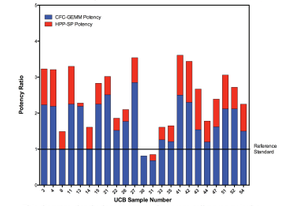


ALL 24 SAMPLES ENGRAFTED

The greater the iATP concentration, the greater the slope of the cell dose response. The greater the slope, the greater the proliferation potential. The greater the proliferation potential, the greater the contribution by primitive stem cells and the greater the overall stem cell potency.

Intracellular ATP and Slope Define Stem Cell Potency, which, in turn, can Predict Engraftment

(but whether engraftment occurs, depends upon the patient)



This diagram clearly demonstrates that stem cell potency is the cumulative effect of both the mature (CFC-GEMM) and primitive (HPP-SP) stem cells. No HPP-SP results were available for sample 30. The lower potency exhibited by sample 31 would reject it. However, all units engrafted. It must therefore be assumed that engraftment was contributed by other stem cells not measured. The results suggest that potency is not determined by a single stem cell population.

SUMMARY

1. TNC, viability, CD34 and CFC cannot be used as potency assays because they are not compliant with the regulations for potency.
2. iATP concentrations can distinguish between proliferative and non-proliferative cells.
3. Cellular potency must take the biology and physiology of the cells into account.

CONCLUSIONS

1. HALO®-96 PQR is a rapid, reference standard-based stem cell potency assay for umbilical cord blood that can help define acceptance limits for release criteria.
2. HALO®-96 PQR is validated and fully compliant with FDA and EMEA regulations and guidelines.
3. Stem cell potency is determined by the cumulative stem cell proliferation potential.