The “Null Hypothesis” and Methods

Virtually all cord blood unit (CBU) measurements are based on total nucleated cell (TNC) counts, from the decision to store a CBU, to release for transplantation and correlation with clinical outcome (time to engraftment). The “Null Hypothesis” states that measurement of stem cells in the UCB should not be dependent upon the purity of the UCB preparation. In short, there should not be a difference between measuring UCB stem cells in a TNC or mononuclear cell (MNC) fraction.

Essential Methods

- CBUs obtained from two cord blood banks, one using Sepax the other AXP technology.
- Total of 63 frozen individual segments (~0.1mL) and 10 units with segments tested.
- TNC and cell differential of thawed segments and units measured using Medonics.
- MNC count determined by 22 particle counter after density gradient centrifugation.
- Dye exclusion viability of 7-AAD performed by flow cytometry.
- CFU assay performed using CAMEO™-4 (HemoGenix).
- ATP bioluminescence assay performed using HALO®-96 SPC-QC & PQR (HemoGenix).

Stem Cell Populations Detected

CFC-GEMM: Primitive hematopoietic stem cell.
HPP-SP: Primitive lympho-hematopoietic stem cell.

Assay Readouts

HALO®: Slope of ATP dose response = Stem cell proliferation potential or primitiveness.

CFC-GEMM: Primitive hematopoietic stem cell.

TNC and cell differential of thawed segments and units measured using Medonics. Total of 63 frozen individual segments (~0.1mL) and 10 units with segments tested.

Stem cell “quality” (proliferation ability) and potential were analyzed from multiple segments prepared by two different cord blood banks (CBBs). CB1 used Sepax, while CB2 used the AXP processing system.

Results: Although it appeared that CB1 produced a slightly higher “quality” of both TNC and MNC, the TNC fractions from both CBs produced equally abysmal results as far as stem cell “quality” and potency were concerned.

Conclusion: Both CBs could not produce UCB samples that provided an adequate representation of stem cell “quality”.

3. The TNC Fraction Cannot Distinguish between Stem Cell “Quality” of Segments from the Same UCB Lot

Primitive hematopoietic and lympho-hematopoietic stem cell “quality” was analyzed in two segments from the same cord blood unit in TNC and MNC fractions.

Result: The TNC fractions from each of the two segments were statistically indistinguishable, whereas those from the MNC fraction could be shown to be statistically significant from each other.

Conclusion: The TNC fraction can produce a false interpretation indicating that segments of the same cord blood unit are similar, when in fact the opposite result occurs.

4. Comparison between UCB Samples from 2 Cord Blood Banks

Stem cell “quality” (proliferation ability) and potential were analyzed from multiple segments prepared by two different cord blood banks (CBBs). CB1 used Sepax, while CB2 used the AXP processing system.

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5. Is the Cord Blood Segment Representative of the Unit?

Using both CFU and HALO, the segment and unit were analyzed to determine if the segment was a true representation of the unit.

Results: This shows a single example of 10 segments and units tested. In each case, the TNC fraction from the segment and unit produced similar results, whereas the MNC fraction allowed the segments to be statistically distinguished from the unit.

Conclusion: Using CFU, a false interpretation occurs leading to the conclusion that the segment is representative of the unit. The MNC fraction produces the opposite conclusion.


For every segment and unit tested, viability by 7-AAD was tested before (TNC) and after (MNC) fractionation.

Results: Virtually all TNC samples produced viabilities of >85% after thawing. All MNC viabilities were near 100%. Metabolic viability using ATP bioluminescence of the stem cells did not correlate with 7-AAD.

Conclusion: Dye exclusion viability by 7-AAD produces a false positive result, which is interpreted as indicating that the cells are functional, when in fact they may not sustain proliferation or are metabolically dead.


Potency can only be measured using a quantitative and validated assay that measures the biological activity of the “active” components (in this case, stem cells), that are responsible for the intended response (in this case, engraftment). The measure of potency is the potency ratio that can only be estimated using a reference standard (potency = 1) of the same material, i.e. cord blood. 25% of samples have low potency and would be rejected; this corresponds to ~24% graft failure rate (see (11) below).

CONCLUSIONS & CONSEQUENCES OF CURRENT TESTING

1. Current tests show little metrology and are inaccurate and insensitive.
2. HALO ATP bioluminescence assay verified against CFU assay.
3. TNC fraction dilutes, masks and severely underestimates the functional capacity of the unit.
4. Current processing to the TNC fraction cannot be used to measure stem cell “quality” and potency. Threshold for storing or discarding CBUs is falsely determined.
5. The “Null Hypothesis” is rejected.
6. Segments do not compare to each other or the unit.
7. Dye exclusion viability produces false positive results leading to a misleading indication of functional ability.
8. Cord blood has to be further purified to MNC fraction.
9. A validated potency assay must be performed at least after cryopreservation and before release to ensure stability and consistency of the stem cells prior to use.
10. Lack of measurement have led to false assumptions and inferences have been made, i.e. assuming the presence, quality and potency of stem cells without measuring them.
11. High graft failure rate (1 in 5 patients) due to low or lack of potency.
12. Not a single unit tested for stem cell “quality” or potency out of >730,000 units collected and >35,000 CB transplants performed worldwide, and >206,000 units collected and >5,000 CB transplants in the U.S.
14. There is a dire need to change current UCB testing practices.