Present Cord Blood Testing Fails to Determine if the Stem Cells Used for Transplantation are of High Quality and Potency

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There is an aura that surrounds stem cells and their use. These rare cells are lifesaving, and are often the last resort, when transplanted into patients with blood malignancies. **Umbilical cord blood (UCB)** is a source of blood stem cells and their use to treat patients has been embraced worldwide since the first UCB stem cell transplant in 1988 [1].

Yet, despite this success, most people would be surprised to learn that when a UCB stem cell transplant is performed, neither the **quality** (the ability of the cells to proliferate) nor **potency** (a quantitative measure of biological function) of the stem cells are determined before they are given to the patient - in fact, the stem cell content is not measured at all [2].

**How public cord blood banks decide to store UCB**

When parents donate UCB to a public **cord blood bank (CBB)**, the decision of whether or not to store the UCB unit is based primarily on the number of cells collected before and after processing. The processing usually removes most of the red blood cells and plasma. However, the final UCB unit still contains red blood cells, granulocytes, platelets, lymphocytes and other cells, in addition to stem cells. Together, these cell types all contribute to the so-called **Total Nucleated Cell (TNC)** fraction.

Public CBB only save UCB donations that have a high TNC count. The first reason they do this is because the UCB unit must be big enough to transplant an adult. The second reason is economics: public CBB must sell units for transplants in order to reimburse their operating expenses, and doctors are most likely to request the biggest units for their patients [3].

The [FDA sets the minimum](http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/UCM357135.pdf) size threshold for UCB storage in a public CBB at 0.5 billion TNC per unit [4]. But today, most public CBB that participate in the [Be The Match](http://bethematch.org/) network will only store UCB that have a TNC over 1.2 billion [3]. Once stored, information about UCB in the Be The Match network is entered into a searchable database called EmTrax®.

This reliance on TNC will be important later, because the miscellaneous cells that contribute to the TNC act to dilute and mask the rare stem cells responsible for engraftment. Hence TNC counts can be a misleading way to characterize high quality UCB units.

Besides the TNC count, additional tests that are routinely used to characterize a UCB unit are: the CD34 assay, which is a test for a cell surface protein which is only present on a small proportion of stem cells [5]; a dye assay which determines whether the cells are alive or dead (ie: viability); and the Colony-Forming Unit (CFU) assay which tests the in vitro growth of progenitor cells [6,7].

**How to know if stored UCB are High Quality**

There are two major flawed assumptions associated with the "minimum guidelines" currently in use at public CBB [4]. First, stem cells are assumed to be present, but that is never proved. To date over 730,000 UCB units have been collected by public CBBs and more than 35,000 transplants performed from these units worldwide [8]. None of those units were characterized to ensure that the stem cells were of high-quality, let alone high potency.

The second flawed assumption is that the stem cells in the UCB unit will exhibit the same quality and potency when they are thawed and prepared for a patient, as they did when the unit was collected and tested prior to cryopreservation.

At [HemoGenix](http://www.hemogenix.com/), we have developed tests that measure the ability of cells to produce chemical energy in the form of adenosine triphosphate (ATP). All viable cells produce a certain amount of ATP. However, the amount of ATP produced can increase several fold when cells grow and proliferate. This fundamental relationship between ATP and proliferation allows both the quality and potency of stem cells to be quantified in a standardized and validated manner as recommended by FDA guidelines.

HemoGenix assays can measure stem cell quality before cryopreservation ([STEMpredict](http://www.hemogenix.com/shop.php?Product_Name=STEMpredict-LS)) and potency after thawing ([HALO-96 PQR](http://www.hemogenix.com/prod_Halo2.php?tab=2#TabbedPanels2)) [9]. HemoGenix is the only company that has developed a post-thaw stem cell potency assay that has the capability of predicting stem cell engraftment with over 90% accuracy [9].

**Our current quality tests have multiple flaws**

Unfortunately, the problems with our conventional cord blood transplant practices are even worse than described so far: not only are we not using the best tests available to measure UCB stem cells released for transplant, we also are not running those tests on the best samples to represent the UCB unit.

The current standard operating procedure is for CBB to perform quality assays on the small testing segments, each about 0.1mL in volume, that are sealed in strips of tubing attached to the main storage bag. It has been assumed that these test segments are both similar to each other and representative of the whole unit.

We conducted a [study](http://www.translational-medicine.com/content/13/1/94) that compared post-thaw test results obtained with the HemoGenix HALO-96 SPC-QC versus conventional assays, for 63 testing segments and 10 whole UCB units that were sourced from two different CBB. The peer-reviewed publication of our study results found [2]:

(1) Previous studies that demonstrated the correlation between traditional CFU colony counts and our ATP proliferation assay were confirmed.

(2) Paired UCB testing segments produced highly variable results, and the **UCB testing segment did not produce similar results to the whole unit**. This calls into question our reliance on testing segments when deciding whether a unit is of adequate size and quality for a patient.

(3) The TNC fraction produces variable results and **TNC underestimates the stem cell quality and potency** of both the testing segments and the whole UCB unit.

(4) Given that the TNC fraction is unreliable and tends to underestimate stem cells, our reliance on TNC count as a threshold for keeping or discarding CBU is misplaced. We may be discarding too many CBU from minority donors that have rare HLA types that are desperately needed for patients. In addition, a CBU with low TNC counts will not be used, even though the stem cells might be of higher quality and potency than a CBU with high TNC counts.

(5) Dye exclusion assays have been used to provide a rapid and reliable measure of live/dead cells (viability), but we did not find them to correlate with stem cell metabolic viability (e.g. ATP assay).

(6) The possibility that **accepted viability measurements may produce false positive results** is of particular concern, because this could lead to graft failure if a patient is transplanted with a UCB unit that is not in fact viable.

(7) **All of the above points raise serious concerns about the accuracy of previous studies** that compared the efficacy of cord blood transplants to other therapeutic modalities (such as haploidentical bone marrow transplants, etc.).

**Call to Action**

According to Be The Match, about 206,000 UCB units have been collected in the U.S. and some 5,000 of them have been transplanted. Of these, about 1,200 patients or about 24% have succumbed to [graft failure](http://www.ncbi.nlm.nih.gov/pubmed/24972767) [10,11]. Yet we are still using UCB testing practices that are inadequate to measure the quality and potency of the UCB stem cells that are given to patients.

In 2005, the [Stem Cell Therapeutic and Research Act of 2005](https://www.govtrack.us/congress/bills/109/hr2520/text) [12] required UCB stem cells to be of "high-quality" when stored. It also provided funding to establish [HRSA's Advisory Council](http://bloodcell.transplant.hrsa.gov/about/advisory_council/) on Blood Stem Cell Transplantation. One of its charges is to identify the parameters that define a high quality CBU. To date, the meetings of this committee have not led to any official conclusions.

In 2009, the United States [FDA designated](http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM187144.pdf) transplants of unrelated UCB as a "drug" [13]. Consequently, the public CBB that produce UCB are subjected to rigorous BLA licensure, yet the UCB units themselves are still governed by the FDA's old "minimum guidelines" that are "nonbinding recommendations" [4,13]. Not surprisingly, voluntary accrediting agencies have been reluctant to apply higher standards than these nonbinding FDA guidelines.

Recently, in 2014 a new industry trade association has formed called the [Cord Blood Association](http://www.parentsguidecordblood.org/newsletter_archive/newsletters_2015-01.php#CBA) [15]. One of its stated priorities is the "rapid adoption of novel technology and therapies". The Association has to prove itself, since no new technology has been adopted in more than 25 years.

How can the FDA, standards organizations, and the cord blood community legitimize their present UCB testing practices, when they never measure the quality and potency of the stem cells they are purporting to give patients? ***At present 1 in 4 patients undergoing a cord blood transplant experiences graft failure. It is in everyone's best interest to ensure that patients receive the highest quality and most potent stem cell product available. Failure to do so is a betrayal of the patient's and the public's trust.***

The average cost of a single cord blood unit is now estimated to be about $50,000 [16]. For that price, the patient and his/her healthcare provider are entitled to accept only those UCB units that have been scientifically demonstrated to be of high quality and potency.***Public CBB that do not accurately certify the quality and potency of their UCB drug product should not receive government subsidies or health insurance reimbursements.***

Ivan N. Rich, PhD, is Founder and CEO of [HemoGenix](http://www.hemogenix.com/), a life sciences company located in Colorado Springs, USA. He has over 40 years experience in the fields of stem cell biology, hematology, in vitro toxicology and entrepreneurship. His company, HemoGenix, is the world leader for in vitro hemotoxicity testing and a pioneering company for developing stem cell quality control and potency assays for cell therapy and regenerative medicine products. Prior to starting HemoGenix in 2000, Dr. Rich was Director of Basic Research in the Division of Bone Marrow Transplantation and Professor at the University of South Carolina. Dr. Rich has written over 100 peer-reviewed research and review articles and has edited three books, the last being Stem Cell Protocols, published by Springer.

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