Studies on Practical Issues for Cord Blood Banking: Effects of Ionizing Radiation and Cryopreservation Variables

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Abstract: As CB banks (CBB) become commonplace and seek to increase the numbers and diversity of their inventory, samples are being collected from off-site hospitals and shipped to the processing facility. CBB are concerned as to which variables may significantly influence the collection and banking of CB for future use in transplantation and regenerative medicine. Many CB samples are transported via airlines and questions have arisen as to whether samples may be negatively impacted by ionizing radiation encountered during transport or during airport security screening measures. Samples may arrive and be processed at different times during the work day, and concerns arise as to the effects of such delays in cryopreservation. Further, although many CBB store processed samples in multiple aliquots, the numbers of such aliquots are generally limited; raising the possibility that repeated rounds of freezing/thawing may be required for optimal use; which could affect sample utility. Analyses were performed to ascertain any effects of low dose radiation on CB utility, any changes in CB stem cells as a result of delays in cryopreservation, and to what end a CB sample could be frozen, thawed and refrozen before losing utility. It was observed that CB samples are able to tolerate normal delays and potential radiation exposures that might be routinely encountered during shipment to CBB. However, CB are only able to undergo limited rounds of freezing and thawing while maintaining stem/progenitor cell activity.

Keywords: Umbilical cord blood, stem/progenitor cells, freeze/thaw, banking, radiation.

INTRODUCTION

Work that was begun in the early 1980s revealed that cord blood was comparable to bone marrow in terms of its utility in stem cell transplantation [1-8]. Cord blood (CB) offers a number of advantages over bone marrow [8, 9] including a lower incidence of graft-versus-host disease (GVHD) and less strict HLA-matching requirements, which could increase its availability to transplant patients. During the past 20 years, clinical use of cord blood (with more than 20,000 transplants worldwide; [10]) has shown that it is a suitable alternative to bone marrow, and numerous public and private agencies have emerged to store cord blood for public or familial use. In addition to its use as a substitute for bone marrow, cord blood has recently been used in a variety of regenerative medicine applications. Work done by McGuckin and colleagues [11, 12], Rogers and colleagues [13], Kucia and colleagues [14], and Harris and colleagues [15, 16] has shown that cord blood contains a mixture of pluripotent stem cells capable of giving rise to cells derived from the endodermal, mesodermal, and ectodermal lineages. Thus, cord blood appears to be a readily available source of cells for potential use in tissue engineering and regenerative medicine. Recently, clinical trials have begun using family-banked cord blood stem cells to treat type 1 diabetes, cerebral palsy, and peripheral vascular disease, among others. (see [17-20]; and, Safety and Effectiveness of Cord Blood Stem Cell Infusion for the Treatment of Cerebral Palsy in Children; Medical College of Georgia. ClinicalTrials.gov identifier: NCT01072370; Cord Blood Infusion Plus Vitamin D and Omega 3 Fatty Acid Supplementation to Preserve Beta Cells in Children With Recent Onset Type 1 Diabetes; University of Florida. ClinicalTrials.gov identifier: NCT00873925).

Cord blood banking has become commonplace over the past 20 years, with nearly 1,000,000 samples having been collected nationwide in both public and family cord blood banks. However, many of the samples, particularly those harvested for private (familial) banking are collected at distant locations and sent by air transport to a centralized facility for processing and banking. In general, all samples used for transplant are also shipped by air to the transplant center. It has been questioned that air travel might in and of itself expose the stem cell samples to significant background ionizing radiation (i.e., “cosmic” radiation present at high altitudes) and samples that inadvertently passed through airport security screening procedures might be damaged to a point to be unusable. Further, as CB samples may arrive throughout the work day, and for economic and regulatory reasons be batched for later cryopreservation, the effect of such delays upon final stem/progenitor cell outcomes needs to be determined. This variable of cryopreservation delay may be of particular importance for facilities that process a limited number of samples each day.

Many investigators have postulated that expansion of the limited numbers of stem/progenitor cells contained in most CB units might be used to speed up engraftment, conserve samples with rare HLA haplotypes and enable more adult
patients to undergo transplant. Furthermore, the ability to expand stem/progenitor cells would enable cord blood banks to utilize banked samples for tissue engineering, regenerative medicine and gene therapy without fear of depleting the sample if it should be needed for use in a standard transplant setting (e.g., for the treatment of leukemia). Although many cord blood banks store samples in multiple aliquots, the numbers of aliquots are generally limited (two typically), and repeated rounds of freezing/thawing may be required. A previous study by Timeus et al. [21] examined the effects of successive rounds of freezing/thawing on CB hematopoietic progenitors. Their studies revealed that two rounds of freezing/thawing did not significantly affect the clonogenic potential of CB stem cells (as assessed by colony assays), in contrast to findings of other investigators who had examined peripheral blood stem cells (PBSC; [22, 23]). Thus, this question remains unresolved, especially as the previous results have not been confirmed.

Therefore, the goals of this study were to examine how everyday and commonplace variables might impact the collection and banking of cord blood stem cells. To that end we analyzed the effects of low dose radiation that might be encountered during unit transport, the effects of temperature and time on CB unit storage before cryopreservation, and the numbers of sample aliquots that should be used for storage during cryopreservation. Each of these variables could negatively impact the utility of cord blood samples for future use in transplantation and regenerative medicine.

**MATERIALS AND METHODS**

**Cord Blood Collection and Processing**

All patients were required to sign an informed consent prior to collection of cord blood in compliance with IRB regulations at University Medical Center, University of Arizona. Specialized collection kits provided by the cord blood bank were used for harvesting the cord blood. The cord blood samples were obtained under the auspices of the patient’s caregiver (i.e., physician or midwife). The collections were made after delivery of the infant and prior to expulsion of the placenta (in utero collections). All cord blood collections were red blood cell (RBC)-depleted prior to cryopreservation by the method of Harris [24], a previously validated density gradient method used to obtain enriched mononuclear cells prior to cryopreservation and storage. The density gradient methodology achieves a RBC depletion of >90% that permits cord blood banking in (2.0 and 4.5cc) cryovials.

Samples that were to be incubated prior to cryopreservation were re-suspended in freeze buffer (minus DMSO, see below) and incubated on ice post-processing. Such samples were then frozen, stored and later thawed for evaluation. Samples that were to be irradiated were exposed using a Co60 gamma irradiation source prior to processing. In the ionizing radiation experiments all samples were assessed 1-2 hours after exposure (i.e., the length of processing time).

**Cord Blood Cryopreservation, Storage and Thawing**

Cord blood cells were resuspended in ice-cold (9°C) autologous plasma (freeze buffer) to prevent introduction of foreign proteins and the subsequent concerns of infectious disease contamination. Cells were frozen at 300x10^6 cells/ml in both 2.0 and 4.5ml cryovials. An equal volume of cryopreservative solution containing autologous plasma and Dimethyl Sulfoxide (20% DMSO concentration) was then added drop-wise to the cell solution to obtain a final DMSO concentration of 10%. Cells were frozen using a programmable, controlled-rate freezer. At the end of the freezing procedure the samples were stored in a liquid nitrogen freezer in the liquid phase to minimize temperature fluctuations. All cryovials were sheathed in a plastic, impermeable “jacket” to prevent cross-sample contamination during storage. All samples were stored for 1 week between thaws. All samples for a particular time point were thawed at the same time to minimize variability. Samples were rapidly thawed at 37 C using the method of Rubinstein et al. [25].

Samples were repeatedly thawed (washed 1X with PBS-HSA and refrozen after 1h, using a controlled-rate freezer as described above) in a 37°C water bath. A minimum of 5 days between rounds of freezing/thawing was utilized. No additional processing was performed between cycles. Initial and subsequent numbers of CD34+ cells and colony forming units (CFU’s) were assessed by flow cytometry (FACS) and methylcellulose assays, respectively, using 0.1-0.2ml of sample. Viability was assessed at each stage by Trypan blue dye exclusion.

**Viability Determination**

Cell numbers and viabilities were determined by Trypan blue dye staining 30min after the last cell wash. Cell numbers used in the functional assays were based on viable cell numbers.

**CD34 Analysis**

The percentage (and numbers) of viable CD34+ cells in each CB upon isolation and after thawing was determined by flow cytometry using the ISCT-approved protocol for CD45/CD34 staining [26]. Only viable CD34+ cells are reported herein, based on light scatter (forward and 90°) gating.

**CFU Analysis**

 Colony-forming unit (CFU) assays were performed using methylcellulose-based media [24]. The media (MethoCult, Stem Cell Technology, Vancouver, Canada) was designed to support the formation of colonies for 10-14 days. The media contained 50ng/ml of recombinant stem cell factor, 10ng/ml of recombinant IL-3 (rIL-3) and 10ng/ml of recombinant human IL-6, among other components. After 14 days of incubation at 37°C in a humidified 5% CO2 atmosphere, total GM (G, M, G+M) colonies (per 10,000 cells plated) were scored using an inverted microscope applying standard criteria for identification.

**RESULTS**

**Transportation Variables after CB Collection**

Both public and private cord blood banks utilize sample collection at distant sites as a means to increase unit diversity.
Cord Blood Banking Variables

and numbers. It has been postulated that during transport CB samples may be exposed to inadvertent X-ray exposure during airport security screening, as well as to cosmic ionizing radiation during high-altitude flight. To examine these possibilities we subjected three (3) CB samples to varying doses of gamma (Co60) radiation and assessed any effects on sample utility. As shown in Fig. (1), exposure to ionizing radiation doses as high as 500 rads had no effect on sample viability (100% viable). However, exposure to radiation doses as low as 50 rads resulted in significant losses of viable CD34+ cells (72% of control, no radiation exposure) as assessed by flow cytometry (p<0.05). Measurement of stem/progenitor cell biological activity by determination of total CFU-GM content demonstrated an initial resistance of CB samples to low dose radiation (50 rads; 101% of control), followed by a progressive decrease in activity thereafter (50% to 26% to 15%; p<0.05).

CB Processing Variables

In order to conserve resources and funds, CB samples are processed upon arrival at the facility but are batched for later cryopreservation and storage. Thus, samples may wait several hours prior to final disposition. To investigate any detrimental effects such practices might have upon the final utility of the CB units, six (6) samples were processed and then incubated on wet ice (9C) for varying periods of time before addition of the cryoprotectant DMSO and the initiation of freezing. As shown in Fig. (2), “wait” times of up to 24 hours prior to freezing had no effect upon sample viability, with all samples demonstrating 100% viability at

![Fig. (1). Effects of low dose radiation on cord blood stem/progenitor cell measures.](image)

![Fig. (2). Effect of cryopreservation delays on cord blood stem/progenitor cell outcomes.](image)
the time of cryopreservation. This finding agreed with analyses of CD34+ cell recovery after freezing and thawing of the incubated samples. That is, samples which had been incubated on ice for varying periods of time were frozen, stored in liquid nitrogen (for at least 5 days) and then later thawed for analysis. The incubated and thawed CB samples demonstrated no loss of stem/progenitor cells (compared to immediately frozen samples) as shown by measurement of the CD34 phenotypic marker. In fact, CD34+ cell recovery increased slightly for several time points (up to 114% of control), most likely due to loss of nucleated red cells and mature white blood cells (i.e., PMNs) after thawing. However, analysis of CFU-GM (biological) content produced a somewhat different result. Although a delay in cryopreservation of up to 6 hours had minimal effects on stem/progenitor cell activity (95% of control, immediately frozen cells), further delays did result in losses of 10-15% in terms of functional activity, bordering on statistical significance.

**Storage and Banking Variables**

As shown in Fig. (3), after initial collection and processing, all ten (10) CB samples displayed approximately 100% viability. It was not until after the fourth round of freezing/thawing that cell viability decreased to approximately 50% of baseline values (p<0.05). That is, although there was a statistically significant difference from baseline values after the third round of freezing and thawing (mean of 75% versus 99%; p<0.05), the biological significance was thought to be marginal. Subsequent rounds of freezing and thawing resulted in much greater losses of viability (means of 31% viability after the fifth cycle and 10% viability after the sixth cycle). Maintenance of sample viability for three freezing/thawing cycles is likely to be attributed to utilization of the

![Fig. (3). Cord Blood Viability after Repeated Cycles of Freezing/Thawing.](image)

Ten independent cord blood samples were collected, processed, frozen and thawed as described in Methods. Samples were assessed for viability initially before freezing and after each round of thawing. Data are shown as the percent viable cells 30min after each thawing and washing. Numbers at the top of each bar represent percent viability for the 10 samples at that cycle. Standard errors of the means are shown as bars above each column. A (*) next to the cycle number on the x-axis indicates significantly different from fresh, unfrozen samples at p<0.05. The viable MNC counts for the 10 CB samples ranged from 250-600x10^6 cells.

![Fig. (4). Cord Blood CD34+ Cell Recovery after Repeated Cycles of Freezing/Thawing.](image)

Ten independent cord blood samples were collected, processed, frozen and thawed as described in Methods. Samples were assessed for the percentages of CD34+ cells initially and after each round of thawing. Data are shown as the percent of control as compared to fresh, unfrozen samples. All data were acquired 30min after thawing and washing. Numbers at the top of each bar represent percent of control for the 10 samples at that cycle. Standard errors of the means are shown as bars above each column. A (*) next to the cycle number on the x-axis indicates significantly different from fresh, unfrozen samples at p<0.05. Initial baseline sample values were 1.0+/-.0.5% CD34+ cells.
mononuclear cell fraction of each CB sample that resulted from the initial processing methodology which removed the more fragile neutrophil (PMN) population.

Subsequent analyses were performed to assess the stem and progenitor cell content of the samples after such multiple cycles of freezing and thawing. Measurements of CD34 content and CFU-GM assays were used as surrogates for stem/progenitor cell activity. As shown in Fig. (4), repeated rounds of freezing and thawing had neither a statistical nor a biologically significant effect on viable CD34+ cell content (whether measured as a percentage or as absolute cell counts for the initial 3 rounds of freezing/thawing). In fact, the percent CD34 content was found to apparently increase during the rounds of freezing and thawing, probably due to lysis of confounding nucleated red blood cells as well as the loss of mature white blood cells (i.e., PMNs). Thus, it did not appear that repeated rounds of freezing/thawing induced any significant loss of CD34+ cells present in the cord blood samples (until such time as significant viable cell losses were observed).

However, a functional assessment of the effects of repeated freezing/thawing on cord blood stem/progenitor cells revealed a quite different outcome. Assessment of CFU-GM activity in the samples (as shown in Fig. 5) demonstrated that only two rounds of freezing/thawing were required to produce a 50% loss of CFU-GM activity (p<0.05 as compared to fresh, unfrozen CB). Even one round of freezing/thawing was found to induce borderline effects with respect to statistical significance (mean of 74% versus 100%), albeit biologically equivalent in terms of transplant utility (i.e., there were 460 CFU-GM per 1x10^6 MNC present in the initial samples on average versus 340 CFU-GM per 1x10^6 cells after thawing on average). These findings were reminiscent of those observed for cell viability after freezing/thawing. Thus, CFU-GM activity appeared to be the most sensitive indicator of the deleterious effects of repeated freezing/thawing.

DISCUSSION

The collection, shipping and banking of umbilical cord blood has become a routine process that occurs hundreds of times a day in the United States. Most cord blood banking facilities are of modest size, generally processing a dozen such samples or less each day. For many of these facilities in order to increase the numbers and diversity of their inventory the samples are collected at a variety of distant sites, batched and shipped by air for later processing. Thus, shipped samples may be exposed to a variety of environmental hazards during this process, as well as experience delays in reaching the final destination. One of the earliest concerns that arose during the development of the cord blood banking field was the potential effects of exposure to ionizing radiation on the stem and progenitor cells contained in the samples. Exposure could occur during passage through airport security (i.e., from x-ray machine inspection of baggage) or possibly during shipment on planes flying at high altitudes (i.e., from cosmic radiation at high altitudes).

Cord blood banking offers the advantage of a stem cell sample that can be economically collected in advance of its use, and kept in frozen storage for a prolonged period of time. The disadvantage is that one generally does not know at the time of collection for what use it will be put. Therefore, how (i.e., in which condition) to bank the sample is often a matter of economy than utility. That is, it is twice as expensive to bank a frozen sample in two containers as in a single container (e.g., bag). It costs 50% more to bank a CB sample in three containers as two containers, and so on, due to space considerations (i.e., the number of dewars required). If it were possible to store a CB sample in one or two containers, and then later thaw the sample for use (and possible expansion) before subsequent re-freezing, this approach would alleviate many of the concerns and expenses facing cord blood stem cell banking. One would not need to know all possible future uses for the sample, and rare samples could potentially be expanded at a future point in time when needed without loss of the entire sample.

**Fig. (5). Recovery of Cord Blood CFU-GM Activity after Repeated Cycles of Freezing/Thawing.** Ten independent cord blood samples were collected, processed, frozen and thawed as described in Methods. Samples were assessed for total CFU-GM initially before freezing and after each round of thawing. Data are shown as the percent of control 30min after thawing and washing as compared to unfrozen samples. Numbers at the top of each bar represent percent of control for the 10 samples at that cycle. Standard errors of the means are shown as bars above each column. A (*) above the bars indicates significantly different from fresh, unfrozen samples at p<0.05. Baseline CFU-GM values were 460+/-.50 CFU-GM per 1x10^6 MNC.
In the current study we have endeavored to address the above questions. To do so, we have examined cell viability (a gross assessment), CD34+ cell content (a phenotypic assessment) and CFU-GM content (a biological assessment and surrogate indicator of stem/progenitor cell function) of the CB samples after various manipulations. Both of the latter assessments are correlated with transplant utility of the sample [27-29].

In terms of variables that could affect the collection and transport of CB collections we observed that exposure to doses of ionizing radiation ranging from 0-500 rads (the approximate equivalent of 0-500 rems or 0-5Sv) had no observable effects on sample viability that could be immediately assessed. However, there were significant losses of both CD34+ cells and CFU-GM activity even at the lowest doses tested (50 rads). Therefore, was it possible that shipped samples might encounter such exposure doses during transit and therefore be damaged? Previous studies have reported that aircraft are exposed to 0.5-4 mrad/hour of flight, or to 3-25 mrad on a typical 6h cross-country shipment [30, 31]. Further, analysis of airport x-ray machines has shown that such devices expose samples to 0.1 rads per inspection [32]. Thus, the exposure levels that are most likely encountered during CB sample shipment are at doses 100-fold or lower than the lowest ionizing radiation doses found to impact CB stem/progenitor cell activity in the current study, and should not be of any concern. However, in this study the lowest radiation dose at which no effect(s) were observed still remains to be determined, and is difficult to estimate. Anecdotally, however, Cord Blood Registry (a large family cord blood bank) routinely receives thousands of such samples monthly and has used such samples for transplant with no adverse effects (private communication, H. Brown, Cord Blood Registry).

There are also processing variables that might affect CB sample utility. In order to minimize expenses some institutions elect to hold (i.e., batch) unprocessed samples, either at the point of shipment or at the facility itself, and then process and freeze all samples at once. There have been multiple publications that have examined this particular aspect of sample batching before processing. However, the reports have been mixed with regard to holding unprocessed cord blood samples. Campos et al. [33] reported that unprocessed cord blood samples could be held for 24 h at 25C with minimal loss of progenitor cells, but that holding the sample at 4C before processing resulted in significant losses. In contrast, a different investigator [34] reported that 4C incubation was better than room temperature for unprocessed samples for up to 2 days post-collection, as assessed by viability and CFU-GM measures. Finally, Shlebak et al. [35] reported that neither 4C nor 25C holding of unprocessed samples was feasible after 9h post-collection. Some of the discrepancies may have resulted from the anti-coagulant used to collect the samples (heparin vs. CPD) as well as the processing method post-incubation (buffy coat vs. density gradients). Most regulatory guidelines specify that collections be processed with 32-48h of harvesting, but do not specify at what temperature the samples should be kept, as long as the process is validated.

However, other facilities process samples as the collections arrive, but then hold the processed samples on ice until later batch freezing. In contrast to the above situation, there have not been any studies that have analyzed holding samples for cryopreservation after processing. It was our belief that most of the sample variability described in the literature could be eliminated by processing the sample, as well as by removing the majority of non-MNC prior to 9C incubation. We observed that neither cell viability nor CD34+ cell recovery was affected by incubation of the processed CB cells on ice for up to 24h. However, there was a potentially biologically significant decrease in CFU-GM activity after 12h of such incubation. As such biological assessment of stem/progenitor cell function may be more relevant to end use in transplant (as compared to phenotypic assessments; [28, 29]) the data would seem to indicate that processed CB samples cannot be held on ice indefinitely. However, the ability to hold samples for up to 12h should allow most CB banks to receive and process samples throughout the day, while performing a single cryopreservation run. It should be noted that in vitro CFU assessments are surrogate indicators for biological function, and other assays such as reconstitution of NOD-SCID mice need to be examined for confirmation of these findings or to determine if any differences might exist.

Finally, we evaluated variables pertaining to how processed CB units should be cryopreserved for storage. That is, was it feasible to freeze CB collections in one (or two) containers and then rely upon later expansion of such thawed units before re-freezing? Unfortunately, the effects of repeated freezing and thawing on cord blood stem cell samples are not clear. Investigators have postulated that stem and progenitor cells are the most resistant cells to these potentially deleterious effects [36]. In fact, there are several studies to support this assumption. For example, Timeus et al. found that two rounds of freezing/thawing had minimal effects on cord blood viability, CD34+ cell numbers and CFU activity [21]. Other investigators have reported contrary results demonstrating that even one single round of thawing was sufficient to induce losses of cell viability ranging from 40-70%, and losses of CFU activity of up to 33% [22, 23]. A portion of these conflicting results is probably due to the type of samples examined. Some investigators analyzed cord blood, while others examined PBSC. Discrepancies did not appear to be solely due to methodological differences (i.e., Trypan blue versus 7AAD dye exclusion for viability determinations, or total CFU content versus individual colony types measured) as relative changes remained intact, and consistent, within an investigator’s laboratory. However, many CB samples are both RBC depleted and reduced in PMN content which may explain some of the differential findings as compared to studies performed with unseparated PBSC.

To clarify this issue we examined RBC- and PMN-depleted CB samples to determine the maximum number of times it was possible to freeze and thaw a single CB sample before biologically significant effects were observed. We have defined biologically significant (as opposed to statistically significant) as changes that approximate a 50% reduction from baseline values. We have chosen this value as one that is reminiscent of values observed when thawing bags of bone marrow and PBSC for use in transplant (personal observations), and as ones that should provide sufficient numbers of stem and progenitor cells for use in transplant,
stem cell expansion and/or regenerative medicine. We found that repeated rounds of freezing and thawing of a single cord blood sample had minimal effects on either cell viability or CD34+ cell content for up to 4 such cycles. It is worth noting at this point that many transplant centers utilize cell viability and CD34 content (i.e., numbers) as the sole indices of a sample’s suitability prior to transplant (generally because such assessments are inexpensive and expedient). Significantly, biological assessment of the effects of repeated freezing/thawing cycles produced a different result. That is, as few as 2 cycles of freezing and thawing was sufficient to reduce CFU-GM activity by 50% as compared to initial values. Although recent clinical studies with CB [27] have implied that viable MNC numbers alone are sufficient to predict cord blood engraftment and success in transplant, such samples are only thawed once. Subsequent cycles of freezing/thawing would be expected to provide a different outcome. In fact, many investigators believe biological assessments of stem cell grafts (e.g., CFU assays) are more sensitive and accurate predictors of stem and progenitor cell activity [28, 29]. Therefore, measures of CFU activity may be a more sensitive indicator of graft utility. This finding may be especially critical for cord blood grafts wherein the number of stem/progenitor cells may be limiting.

In conclusion, the current studies have demonstrated that CB stem/progenitor cells are inherently resistant to the effects of very low dose ionizing radiation that might be encountered from the time of collection until processing as assessed by phenotypic and biological assays. Such cells are also unaffected by prolonged incubation at 9C after processing. Therefore, two of the concerns that would routinely impact cord blood banking have been addressed. Exposure of the CB sample to ionizing radiation during shipment or to the banking facility should not be a major concern as the exposure doses that would normally be encountered are 100-1000-fold lower than the doses found to significantly affect the samples in our studies. Second, although there are mixed reports in the literature as to whether one can hold CB samples prior to processing, it seems apparent from our results that RBC and PMN depletion during processing allows for extended post-processing incubation at 9C before final cryopreservation. This finding should permit the economization of banking operations of moderate to small cord blood facilities unable to afford multiple liquid nitrogen runs per day involving only 1-2 samples per run. Finally, it seems that cord blood samples are able to undergo only limited rounds of freezing and thawing while maintaining stem/progenitor cell activity. Therefore, cord blood samples should be cryopreserved in multiple aliquots (as many as feasible based on processed cell numbers) if samples are ever to be used for expansion, tissue engineering or gene therapy in the future.

The overall recommendations that can be derived from these studies are that shipment of off-site CB collections by standard air transport is a safe and efficient method by which to quickly obtain CB units; that CB units should be processed upon arrival and then frozen no later than 6-12 hours after processing (as long as the units are held on ice to avoid loss of biological activity); and that CB samples should be cryopreserved in multiple aliquots (whether in vials or compartmentalized bags) to avoid repeated cycles of freezing and thawing which rapidly and negatively impacts sample utility.

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CONFLICTS OF INTEREST

Dr. Harris is a consultant to Cord Blood Registry. No other author has any conflict to declare.

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