



# Annotated Bibliography

## XXI. Cord Blood Banking



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### iv. PROCESSING, CRYOPRESERVATION AND INFUSION

#### 1. Does the volume reduction manipulation before cryopreservation influence cord blood cell recovery pretransplant?

Screnci M, Salvatori S, Carmini D, Arcese W. *Transfus Med.* 2007;17:208-9

The authors point out that cell recovery pre-transplant could be crucial especially for large-sized patients or when cell content is not exceeding the minimum recommended. They cite **two observations that have to be considered**:

1. **a median cell loss of 25% after thawing with the removal of DMSO** as reported by Laughlin et al (*NEJM* 2001;344:1815-1822)
2. **a better NC recovery when CBs are infused without manipulation** (Hahn et al – *Bone Marrow Transplant.* 2003;32:145-150; and Laroche et al *Transfusion* 2005;45:1909-1916)

To investigate whether the volume reduction process including RBC reduction before freezing can influence progenitor cell recovery after thawing, the authors analyzed results obtained in 78 CB units, 54% of which were unmanipulated and 46% of which were manipulated. Volume reduction was carried out by several methods: HES sedimentation; by differential centrifugation with automated expression of erythrocytes and plasma; and centrifugation with manual expression of plasma. Before infusion, all 78 samples were thawed and washed according to the procedure described by Rubinstein (1995). There was no difference in total NC counts or time of storage between the two groups of CB units (unmanipulated vs. manipulated) before thawing.

After thawing and washing, **NC recovery was better in the group of unmanipulated) CB than in the group of CB processed before storage (95.2 ± 14.7% recovery vs. 85 ± 15.4% recovery), and the difference was statistically significant (p=0.004)**. There was no difference in cell loss between the HES vs. non-HES volume reduction methods.

The authors conclude that their data indicate that cell recovery after thawing is significantly lower in CB units manipulated prior to storage. The authors state that in cases of CB units with a borderline cell dose, it could be reasonable to select the unmanipulated CB units. **Alternatively as suggested by Hahn et al and Laroche et al, CB units could be infused immediately after thawing without additional manipulation.**

#### 2. Analysis of hematopoietic cell transplants using plasma-depleted cord blood products that are not red blood cell reduced.

Chow R, Nademanee A, Rosenthal J, Karanes C, Jaing TH, Graham ML, Tsukahara E, Wang B, Gjertson D, Tan P, Forman S, Petz LD. *Biol Blood Marrow Transplant.* 2007;13:1346-57. **Abstract**

The authors have developed and evaluated the use of CB products from which plasma but not RBC have been removed in order to minimize cell loss. CB units were processed by centrifugation in the original collection bag at 1680 X g for 10 minutes at room temperature followed by variable amounts of plasma removal into an attached empty bag using a plasma expessor to reach a final CB volume of 60-84 mL before adding cryoprotectant solution. The plasma depleted (PD) products were then pre-cooled at 4°C for at least 30 minutes prior to cryoprotectant addition. The products were subjected to controlled rate freezing and, after attaining -90°C, were immediately transferred from the controlled rate freezer to liquid nitrogen tanks. There was <0.1% total nucleated cell (TNC) loss incurred by processing as measured by recovery in the discarded plasma fraction (n=27); however, due to sampling for various tests and archival purposes and clumping after processing, an average of 98% TNC recovery was observed in the cryopreserved units in the inventory.

The effect of CB processing techniques on post-processing cell dose among the NMDP banks was studied. Using information derived from the NMDP Cord Blood Bank Performance data, the percentage of high cell dose units among the 2 types of inventories (PD vs. RBC-reduced) in the NMDP were compared. At all 3 TNC count levels ( 125, 150, and 200 X 10<sup>7</sup>), the difference in proportions of inventories with high cell dose was significantly in favor of PD inventory (p<0.0001).

Analyses were carried on 118 infused products. Prior to infusion of the thawed CB, the product was washed in 67 instances whereas 50 products were infused without washing per the transplant center's decision (information unavailable for 1 unit). Although CB products that were washed had a higher median and mean TNC dose than unwashed products prior to cryopreservation, the post-thaw TNC dose as reported by the transplant centers was lower for washed products than unwashed products. Unwashed products engrafted faster for ANC500 (20 days vs 27 days) (log rank test; p<.02) and platelets (47 days vs 54 days (log rank test; p=.0003). The incidence of grade III-IV aGVHD and extensive cGVHD among all patients were 13% ± 4% and 17% ± 6%, respectively. Relapse rate for malignancies was 25% +/- 6% and 100-day treatment-related mortality (TRM) was 16% +/- 3%. With a median follow-up of 557 days, the 1-year overall survival and relapse-free survival are 65% +/- 5% and 51% +/- 6%, respectively.

Analysis of infusion-associated adverse reactions was divided into patients who received post-thaw washed products (W) and those who received CB that were not washed after thaw (NW). Events occurring during or after infusion include hypertension (6NW, 4W), hives (1NW, 1W), nausea/vomiting (2NW, 4W), and dyspnea (1NW, 1W). Hemoglobinuria (9NW, 1W) is an expected occurrence for PD CB because most RBCs lyse after thawing. Most symptoms attributed to DMSO administration were self-limiting or easily managed. One patient developed seizure and encephalopathy, although the relationship to infusion was uncertain.

The authors point out that in centers that have opted not to wash the units after thawing, the additional 10%-30% cell loss due to wash is avoided. There were no clear benefits of performing post-thaw wash for PD CB.

The authors concluded that their results demonstrate that PD CBT is safe and effective, and that eliminating RBC reduction improves cell recovery during CB processing, resulting in a larger proportion of the inventory with high NC number.

### 3. Assessment of cord blood unit characteristics on the day of transplant: comparison with data issued by cord blood banks.

Wagner E, Duval M, Dalle JH, Morin H, Bizier S, Champagne J, Champagne MA. Transfusion. 2006;46:1190-8. [Abstract](#)

(Also see [Citation #12](#))

The goal of this study was to compare the graft characterization results obtained upon thawing and washing to those provided by CBBs at selection. With tests that assess CB graft characteristics known to impact engraftment, CB units were analyzed after thaw and before infusion. The results were compared to data provided by CBBs to determine the impact on engraftment and assess how CBB-supplied information can affect future CB unit selection.

All CBBs provided information on unit volume and TNC content; 96% of CBBs provided CD34+ cell content; 78% provided data on CFC content; and 43% provided results of cell viability testing. Most of the CBBs (83%) provided such information on measurements before cryopreservation.

**TNC content correlated rather well, both upon thawing ( $r^2= 0.085$ ) and after thawing and washing ( $r^2=0.799$ ). However, highly discrepant results were obtained for CD34+ cell enumeration, CFC content and cell viability.**

The authors suggest that 7-aminoactinomycin D staining is preferable to trypan blue dye exclusion for testing cell viability and that the latter method is of little value in assessing CB units.

In six patients, important differences may have accounted for the lack of neutrophil engraftment. The discrepancies included finding only 8% of the reported CD34+ content, and no growth or very low growth (1% of reported CFU-GM content) of hematopoietic progenitor cells.

Recent studies suggest that CB samples from the attached segment of the freezing bag or a separate cryovial can accurately predict the graft content. If validated on a larger scale, the assays should ideally be performed on a thawed sample as precryopreservation testing may not predict the quality of a CB unit.

*[Comment: How to evaluate the quality of an individual cord blood unit remains a problem in CB transplantation. Continued research on inter-laboratory standardization of methods is necessary. So far the results of such efforts have not been satisfactory. Results on a thawed sample would seem to be most representative of the infusion dose and may become the standard approach.]*

### 4. Multi-laboratory evaluation of procedures for reducing the volume of cord blood: influence on cell recoveries.

Takahashi T, Rebullia P, Armitage S, van Beckhoven J, Eichler H, Kekomaki R, Letowska M, Wahab F, Moroff G, For The Biomedical Excellence For Safer Transfusion Collaborative. Cytotherapy. 2006;8:254-64. [Abstract](#)

Various procedures can be used to isolate stem and progenitor cells from cord blood. This study evaluated the hydroxyethyl starch sedimentation (HES) with two centrifugation steps, and the top and bottom (T&B) isolation of buffy coat following a single centrifugation, and two filter systems for processing cord blood, one developed by Asahi Kasei Medical (filter A) and the second by Terumo (filter B). Each of seven laboratories was randomly assigned the evaluation of either the HES or T&B method and one of the filter methods (n=8 cord blood units, per laboratory, for each method). The composite results obtained by the seven laboratories were summarized.

Results of cell recovery of cells with various methods were as follows:

Method	Median TNC Recovery (%)	Median Mononuclear Cell Recovery (%)
HES	79	72
T&B	86	96
Filter A	58	79
Filter B	61	70
Group Traditional Methods	82	87
Filtration Methods	58	75

The authors concluded that filters that capture stem and progenitor cells may be an appropriate methodology for processing cord blood collected for banking.

*[Comment: Cell loss with each of the methods is significant. Cell dose has repeatedly been emphasized as critical to the success of cord blood transplantation. The cell losses reported in this study may significantly impact the appropriateness of a given unit for transplantation. Whether the value of removal of RBCs from the units compensates for the stem cell loss is being re-considered. This topic was discussed by Dr. Michael Creer at the Fourth Annual International Umbilical Cord Blood Transplantation Symposium. A CD with the audio and the slides of all speakers is available (See [Home Page](#) for details).]*

### 5. Multiple-laboratory comparison of in vitro assays utilized to characterize hematopoietic cells in cord blood.

Moroff G, Eichler H, Brand A, Kekomaki R, Kurtz J, Letowska M, Pamphilon D, Read EJ, Lecchi LP, Reems JA, Sacher R, Seetharaman S, Takahashi TA; Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Transfusion. 2006;46:507-15. [Abstract](#)

The authors conducted four exercises performed by multiple laboratories to assess assay variability on nucleated cell (NC), mononuclear cell (MNC) by hematology analyzers [HAs], and CD34+ cell (flow cytometry) measurements.

Intralaboratory reproducibility was highest for NC measurements and lowest for CD34+ cell measurements. Substantial variation was observed on measuring CD34+ cells.

**The authors concluded that it is important to recognize that the cell counts provided by processing laboratories to judge quality and suitability of a cord blood unit could be influenced by assay variability.** Currently NC counts obtained with a HA

is the preferred variable for judging quality and suitability. Although CD34+ cell counts may be a more important parameter for transplantation, the variation in assay results suggests that initially more standardized and interlaboratory validation of specific procedures is required. In addition, recent studies suggest that some of the CD34+ cells determined may be nonviable and that additional reagents may need to be utilized in the assays to exclude nonviable cells.

6. **Multiple-laboratory comparison of in vitro assays utilized to characterize hematopoietic cells in cord blood.** Creer MH. Transfusion. 2006;46:498-500.

This is an editorial by the medical director of a large American cord blood bank. He reviewed the article by [Moroff et al](#) and pointed out that the results of the study are, in general, very consistent with the results of an NMDP cord blood external proficiency study. Collectively, these two studies demonstrate that significant improvements in accuracy and precision of measurements of WBC subsets and CD34+ cell concentration in cord blood will be required before we can expect to be able to demonstrate a consistent correlation between these measurements and cord blood transplant outcome.

7. **Preservation of immunological and colony-forming capacities of long-term (15 years) cryopreserved cord blood cells.**

Kobylka P, Ivanyi P, Breur-Vriesendorp BS. Transplantation. 1998;65:1275-8. [Abstract](#)

Cryopreserved cord blood may be stored for decades before being used for allogeneic stem cell transplantation. The authors examined the recovery, viability, clonogenic capacity, and T-cell reactivity to HLA alloantigens of cord blood samples cryopreserved up to 15 years.

Progenitor cell recoveries were studied by (colony-forming unit-granulocyte-macrophage) clonogenic assays from 18 cord blood samples short-term frozen for 2-8 weeks and from 8 samples cryopreserved for 15 years. Proliferative and cytotoxic responses against HLA antigens of thawed cord blood mononuclear cells after short-term or long-term cryopreservation were tested in standard mixed lymphocyte cultures and cell-mediated lympholysis assays.

After thawing, the mononuclear cell recovery from long-term frozen cord blood low-density fractions averaged 80% (range, 64% to 92%). The data show that long-term frozen cord blood cells keep their clonogenic potential. No damaging effect was seen on the proliferative and cytotoxic capacities of long-term frozen cord blood T cells.

**The results support the possibility of long-term storage of progenitor cells from umbilical cord blood for future bone marrow reconstitution.**

8. **Prospective flow cytometric evaluation of nucleated red blood cells in cord blood units and relationship with nucleated and CD34(+) cell quantification.** Larghero J, Rea D, Brossard Y, Van Nifterik J, Delasse V, Robert I, Biscay N, Chantre E, Raffoux E, Socie G, Gluckman E, Benbunan M, Marolleau JP. Transfusion. 2006;46:403-6. [Abstract](#)

Nucleated red blood cells (NRBCs) are a physiological subset of CB population and high NRBC numbers can falsely elevate WBC counts requiring correction. The authors analyzed 826 CB units for total nucleated cells (TNCs), NRBCs, and CD34+ cells by flow cytometry. NRBCs were also counted conventionally by manual microscopy.

For 77.7% of the CB units, NRBC percentage was less than 10%. However, NRBC percentage was between 10 and 15% for 12% of the units and more than 15% for 10.3% of the units, with a maximum of 84%, thus demonstrating an important quantitative heterogeneity in CB.

The mean percentage of CD34+ cells was 0.27 percent (range, 0.01%-1.25%); when corrected for NRBC count, the mean percentage was 0.295% (p=0.0008 compared with the uncorrected percentage). The mean uncorrected TNC count was  $16.26 \times 10^8$  and the corrected count was  $14.8 \times 10^8$  (p< $10^{-4}$  compared to the uncorrected count).

The authors indicate that their findings could have implications for CB QC given that CD34+ and TNC numbers predict engraftment and transplant outcomes. Also, a high NRBC percentage in a cord blood unit could lead the cord blood bank to further investigate possible causes, such as sickle cell disease for which elevated numbers of circulating NRBC in the CB of neonates born to mothers with sickle cell trait has been described.

*[Comment: This study emphasizes the obvious fact that a high percentage of NRBCs in a cord blood unit can result in misleading figures for WBC and CD34+ cell counts if corrections are not performed. Although one study has suggested that the presence of NRBCs in CB units does not reduce their engraftment potential, this result is counter-intuitive and has not been confirmed.]*

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### iv. PROCESSING, CRYOPRESERVATION AND INFUSION

9. **The viability of cryopreserved PBPC depends on the DMSO concentration and the concentration of nucleated cells in the graft.** Liseth K, Bjorsvik S, Grottebo K, Bruserud O. *Cytotherapy*. 2005;7:328-33. [Abstract](#)

(Also see Citations #11, 14, 15, 20 and 21.)

DMSO is widely used as a cryoprotectant for PBPC. It is desirable to reduce the amount of DMSO without jeopardizing the quality of the stem cell product. The present study was undertaken to investigate whether recovery and survival of CD34<sup>+</sup> cells would be significantly altered when **peripheral blood progenitor cells (PBPC) used for autologous transplantations were cryopreserved with four different DMSO concentrations.**

Apheresis samples of PBPC from 20 consecutive patients were mixed in parallel with 2%, 4%, 5% and 10% DMSO, frozen with identical cell concentrations at a controlled rate, and stored in liquid nitrogen for 6-8 weeks. PBPC samples from 11 consecutive patients were also cryopreserved with two different cell concentrations (150 and 300 x 10<sup>6</sup>) nucleated cells/mL) to investigate the effect of increasing the cell concentrations while decreasing the DMSO concentration. The flow cytometric absolute count method, based on ISHAGE guidelines, was used to measure the absolute count of total and viable CD34<sup>+</sup> cells in the post-thaw samples.

Results indicated that PBPC cryopreserved at 150 x 10<sup>6</sup> cells/mL with 2% DMSO yielded significantly inferior CD34<sup>+</sup> cell recovery (P < 0.001) and survival (P < 0.001) compared with cryopreservation with 4% and 5% DMSO. This was also observed when comparing higher cell concentrations. However, a reduced cell survival (P = 0.02) was observed when the nucleated cell concentration was increased from 150 to 300 x 10<sup>6</sup> cells/mL in samples cryopreserved with 5% DMSO.

The authors concluded that 5% DMSO may be the optimal dose for cryopreserving PBPC as long as the cells have not been concentrated at much more than 200 x 10<sup>6</sup> nucleated cells/mL.

*[Although this study was done using PBPC, the results should be of interest to cord blood bankers.]*

10. **Analysis and cryopreservation of hematopoietic stem and progenitor cells from umbilical cord blood.** Meyer T, Hofmann B, Zaisserer J, Jacobs V, Fuchs B, Rapp S, Weinauer F, Burkhart J. *Cytotherapy*. 2006;8:265-76. [Abstract](#)

(Also see Citations #3, 6, 7, 9, 20 and 21 )

The objective of this study was to optimize cryopreservation conditions for CD34<sup>+</sup> HSC/HPC from UCB. Experimental variations were concentration of the cryoprotectant, the protein additive and cell concentration. In addition, protocols involving slow, serial addition and removal of DMSO were compared with standard protocols (fast addition and removal of DMSO) in order to avoid osmotic stress for the cryopreserved cells. Viability and recoveries of MNC, CD34<sup>+</sup> cells and total colony-forming units (CFU) were calculated as read-outs.

The optimal conditions for cryopreservation of CD34<sup>+</sup> HPC in MNC preparations were 10% DMSO and 2% human albumin at high cell concentrations (5x10<sup>7</sup> MNC/mL) with fast addition and removal of DMSO. After cryopreservation using a computer-controlled freezer, high viabilities (89%) and recoveries for CD34<sup>+</sup> cells (89%) as well as for CFU (88%) were observed.

The authors emphasized that fast addition of DMSO is essential for improved cryopreservation and post-thaw quality assessment results, whereas the speed of DMSO removal after thawing has little influence on the recoveries of CD34<sup>+</sup> cells and CFU.

11. **Cryopreservation of Hematopoietic Cells.** Rowley SD. In: Blume KG, Forman SJ, Appelbaum FR (eds) *Thomas' Hematopoietic Cell Transplantation*. 3rd edition. Malden (MA), Blackwell Publishing, 2004, pp 599-612.

Although this comprehensive review is not related exclusively to cryopreservation of cord blood (CB) units, it contains much information that is of critical importance for CB banking and transplantation.

The author reviews the scientific basis for cryopreservation including the physics of cooling and warming of cell products. The only **"assay" for the cryosurvival of hematopoietic cells** is engraftment after transplantation. Progenitor cell assays such as culture for CFU-GM after thawing can be predictive of engraftment, but the validity of these assays must be determined for each group of patients and the assay used. The different cooling properties of vials compared to bags diminishes the reliability of these small aliquots for determining cryosurvival. If an attempt is made to use small aliquots for clinical decisions, a system of cooling, thawing, washing and culture that correlates with engraftment kinetics must first be developed.

**Cooling rates** for hematopoietic cells are reviewed. Different cells have different optimal cooling rates, and the optimal cooling rate is also dependent on the type and concentration of cryoprotectant used. Cooling at slow rates limits intracellular ice nucleation, and mechanical disruption of the cell from ice recrystallization during warming is less likely. Rapid warming is appropriate.

Some laboratories perceive storage in the **liquid phase of liquid nitrogen** to be safer because of temperature gradients in **vapor-phase** refrigerators and because of the larger quantity of nitrogen present. However, **liquid nitrogen can serve as a reservoir for viruses**. This problem was dramatically illustrated by the transmission of hepatitis B infection to at least three patients whose cells were immersed in the same liquid nitrogen refrigerator as those of the index case.

The **duration of storage may be indefinite** if adequate temperatures are maintained and appropriate cryopreservation techniques are used. DMSO and HES need not be removed before infusion if consideration is given to the potential toxicities of these agents. Most centers performing autologous or allogeneic marrow hematopoietic cell transplantation infuse the cells within a few minutes after thawing and without any post-thaw processing.

A high incidence of generally mild, **infusion-related morbidity** has been reported with the infusion of either marrow of peripheral blood and this is generally attributed to the effects of DMSO compounded by the presence of lysed blood cells. More significant adverse reactions include the rare anaphylactic reaction and profound hypotension. Skin flushing, dyspnea, abdominal cramping, nausea and diarrhea have been attributed to DMSO-induced histamine release. These complaints resolve over a few hours and are treated symptomatically. DMSO may also cause increased blood pressure and bradycardia, which may be maximal about 1-3 hours after completion of the hematopoietic cell infusion. Cardiac rhythm abnormalities generally resolve spontaneously within 24 hours of infusion. CNS complications are rare and generally related to the amount of DMSO infused. Encephalopathy has been reported and may promptly resolve following plasmapheresis.

The authors summarize by stating that current cryopreservation techniques are satisfactory for the treatment of many patients, but there has been no comprehensive study of hematopoietic cell cryobiology to quantify the cell losses resulting from cell freezing. There is considerable, but generally minor, toxicity from the currently used cryoprotectants.

**12. Cell loss and recovery in umbilical cord blood processing: a comparison of postthaw and postwash samples.** Laroche V, McKenna DH, Moroff G, Schierman T, Kadidlo D, McCullough J. *Transfusion* 2005; 45:1909-1916. [Abstract](#)

This study examines the effect of the wash step as well as that of postthaw storage on various quality control variables of UCB units. Ten units were thawed and washed. Samples were removed from each unit at six time points: prefreeze, immediately postthaw, immediately postwash, and 1, 2 and 5 hours postwash. On each sample, total nucleated cell (TNC) count, CD34+ cell enumeration, colony-forming unit (CFU)-granulocyte-macrophage, and viability assays were performed.

Results indicated that TNC counts decreased postthaw and at subsequent time points; the postthaw TNC recovery was 89% compared to 82% postwash. TNC recovery decreased to 90% of postwash (82% of postthaw) values and 83% of postwash (76% of postthaw) values at 2 and 5 hours postwash, respectively. CD34+ cell loss postthaw was not significant. Viability decreased postthaw and plateaued over time. CFUs were significantly lower postthaw, recovering postwash.

Some results are particularly noteworthy. The mean TNC count decreased significantly at each step of processing. Of the 18% total loss of TNCs associated with standard postcryopreservation processing, the washing step accounted for 7 percent. The recovery of CD34+ cells was 97% postthaw and a remarkable 148.9% postwash (95% Confidence Interval (CI), 112.8-185%) (This was not statistically significant.) The majority of the increase in CD34+ cells occurred after washing. CFUs decreased to 17% of their prefreeze level but increased postwash returning to 64% of prefreeze values.

The authors conclude that thawing and washing result in a substantial loss of cells, with TNC loss approaching 20% when compared with prefreeze counts; the wash step was responsible for nearly half of the cell loss. Elapse of time postwash resulted in further loss of nucleated cells but no detectable significant changes in CD34+cell content and viability and/or CFU.

The authors comment that the rationale behind RBC depletion has been to reduce the total volume of the product being frozen to maximize storing capacity. However, a number of publications presenting data on post-RBC depletion processing have shown total cell losses falling in the range of 20-30%. Washing postthaw has been justified by the in vitro inhibitory effect of DMSO on progenitor cell viability and clonogenicity, better control over the thawing conditions, and reduction in infusional toxicity related to DMSO.

The authors indicate that their data suggest that cell loss associated with thawing and washing significantly impacts the number of UCB units suitable for transplantation in patients weighing 40 kg or more. Patients weighing between 40 and 60 kg would seem to gain more if the washing step was removed.

The authors suggest that their results support the need for an assessment or reevaluation of the rationale for washing in cord blood processing. The potential benefits of removing debris and DMSO should be weighed against the impact of cell loss in the infused product.

**13. Cryopreserved human haematopoietic stem cells retain engraftment potential after extended (5-14 years) cryostorage.** Spurr EE, Wiggins NE, Marsden KA, Lowenthal RM, Ragg SJ. *Cryobiology*. 2002;44:210-7. [Abstract](#)

The effect of long-term cryostorage (5-14 years) on the viability and functional capacity of haematopoietic stem cells (HSCs) was investigated in 40 bone marrow and peripheral blood harvests using standard in vitro methods, the colony forming unit-granulocyte/macrophage (CFU-GM) assay and a single platform viable CD34(+) cell absolute count by flow cytometry. Forty percent of harvests had CD34(+) HSC counts of at least  $0.7 \times 10^6$ /kg bodyweight and 85% had CFU-GM counts of at least  $1.0 \times 10^5$ /kg bodyweight. These values represent minimum requirements for safe transplantation at the authors' institution. Based on these results, it appears that HSC collections can remain adequate for safe transplantation after up to 14 years of cryostorage. However, as deterioration of HSC quality and viability may occur, some precautions may be warranted, namely harvesting higher than normal numbers of HSCs in collections intended for long-term storage and repeating in vitro assays on harvests after long-term storage prior to transplantation.

**14. Variation in dimethyl sulfoxide use in stem cell transplantation: a survey of EBMT centres.** Windrum P, Morris TC, Drake MB, Niederwieser D, Ruutu T; EBMT Chronic Leukaemia Working Party Complications Subcommittee. *Bone Marrow Transplant*. 2005;36:601-3. [Abstract](#)

The cryoprotectant dimethyl sulfoxide (DMSO) is known to have toxic side effects, yet guidelines for its use in stem cell transplantation do not exist. To assess current practice in the use of DMSO and the incidence of DMSO-related complications, a single page questionnaire was mailed to 444 EBMT centers involved in **autologous transplantation**. The responses from 97 centers showed a wide variation in practice between transplant units regarding the concentration of DMSO used, daily DMSO dose restriction and the use of cell washing. There was an upper limit in the amount of DMSO given per day by 57 centers as follows: 80 g (n=5), 60 g (n=9), 40 g (n=12), 20 g (n=2) and others (n=29). Among "others", the most frequently cited limit was 1 g/kg. Five centers washed cells before return while some did so "sometimes".

The overall incidence of DMSO toxicity was approximately one in 70 transplants and most cases were cardiovascular and respiratory in nature. Other effects such as CNS, renal, hepatic and anaphylaxis are less common. Only once case of mortality attributed to DMSO was cited, and that was in a patient with cardiac amyloidosis and end-stage renal failure. There was a trend to reduced complication rates in centers using lower concentrations of DMSO or washing cells prior to return.

A large-scale prospective study of the strategies for reduction in exposure to DMSO and reduction in toxic effects is required before guidelines in the use of DMSO in stem cell cryopreservation can be promulgated.

15. **Effect of dimethyl sulfoxide on post-thaw viability assessment of CD45+ and CD34+ cells of umbilical cord blood and mobilized peripheral blood.** Yang H, Zhao H, Acker JP, Liu JZ, Akabutu J, McGann LE. Cryobiology. 2005;51:165-75.

**Abstract**

**This study was designed to evaluate the pre- and post-cryopreservation effect of Me<sub>2</sub>SO and Me<sub>2</sub>SO removal on the enumeration of CD45+ and CD34+ cells by flow cytometry.** While a logical predictor of hematologic recovery would be the establishment of a minimum post-cryopreservation dose of viable CD34+ cells, the lack of a validated or standardized test for post-thaw enumeration of CD34+ cells has hindered this development.

In most studies, Me<sub>2</sub>SO is removed prior to the enumeration of CD34+ cells from post-thaw samples, although some investigators perform the enumeration of CD34+ cells in the presence of different concentrations of Me<sub>2</sub>SO. In practice, thawed mobilized peripheral hematopoietic progenitor cells containing Me<sub>2</sub>SO are infused into patients, whereas thawed cord blood hematopoietic cells are [usually] washed to remove Me<sub>2</sub>SO before infusion. Some studies suggest that the toxicity of Me<sub>2</sub>SO to progenitor cells warrants its removal. However, **Me<sub>2</sub>SO toxicity to human progenitor cells has not been found at a concentration of 10% (v/v) at either 4 or 37°C for incubation of up to one hour.**

Cells from leukapheresis products from multiple myeloma patients and umbilical cord blood cells were suspended in 1, 2, 5, or 10% Me<sub>2</sub>SO for 20 min at 22°C. Cells suspended in Me<sub>2</sub>SO were then immediately assessed or assessed following removal of Me<sub>2</sub>SO. In other samples, cells were suspended in 10% Me<sub>2</sub>SO, cooled slowly to -60 degrees C, stored at -150 degrees C for 48 h, then thawed. The thawed cells in 10% Me<sub>2</sub>SO were diluted to 1, 2, 5, or 10% Me<sub>2</sub>SO, held for 20 min at 22 degrees C and then immediately assessed or assessed after the removal of Me<sub>2</sub>SO. CD34+ cell viability was determined using a single platform flow cytometric absolute CD34+ cell count technique incorporating 7-AAD.

The results indicated that after cryopreservation neither recovery of CD34+ cells nor viability of CD45+ and CD34+ cells from both post-thaw progenitor cells mobilized from peripheral blood and umbilical cord blood were a function of the concentration of Me<sub>2</sub>SO. Without cryopreservation, when 10% Me<sub>2</sub>SO is present, recovery and viability of mobilized peripheral blood CD34+ cells but not CD45+ cells were significantly decreased. Removing Me<sub>2</sub>SO by centrifugation significantly decreased the viability and recovery of CD34+ cells in both mobilized peripheral blood and umbilical cord blood before and after cryopreservation.

**The authors concluded that their results indicate that to reflect the actual number of CD45+ cells and CD34+ cells infused into a patient, removal of Me<sub>2</sub>SO for assessment of CD34+ cell viability should only be performed if the HPC are infused after washing to remove Me<sub>2</sub>SO.**

16. **Cord Blood Transplantation Study (COBLT): cord blood bank standard operating procedures.** Fraser JK, Cairo MS, Wagner EL, McCurdy PR, Baxter-Lowe LA, Carter SL et al. J Hematother 1998; 7:521-561. **Abstract**

The Cord Blood Transplantation Study (COBLT) Cord Blood Bank Standard Operating Procedures were published in 1998. The medical coordinating center publishes updates on the EMMES website ([www.EMMES.com](http://www.EMMES.com)).

[<<PREVIOUS PAGE](#) [Page 1](#) | [2](#) | [3](#) [NEXT PAGE >>](#)

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Page Updated  
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### iv. PROCESSING, CRYOPRESERVATION AND INFUSION Page 3

17. **A simple and reliable procedure for cord blood banking, processing, and freezing: St Louis and Ohio Cord Blood Bank experiences.** Alonso JM, III, Regan DM, Johnson CE, Oliver DA, Fegan R, Lasky LC et al. *Cytotherapy* 2001; 3:429-433.

**Abstract**

This report provides details of a modification of the hetastarch sedimentation and volume reduction approach of Rubinstein et al (Rubinstein et al PNAS USA 1995;92:10119-22). Cord blood is mixed with a 1:5 v/v ratio of hetastarch. The product is incubated for 45 minutes in an inverted position in a refrigerated centrifuge and then is spun for 5' at 50g. RBC concentrate is drained from the bottom. The volume drained is calculated to remove 80% of RBCs. The cord blood unit is then resuspended and spun for 13 min at 420 g. Plasma is expressed from the top. A final product volume of 27 mL (range 16-58 mL) was obtained from an original 50-200 mL. The average yield of TNC pre and post-processing was 90% for the first 4,055 units banked. Pre- and post-processing CFU and CD34 yields were tested in a cohort and were similarly conserved. With a processing time of 3 hours for a single cord, this process is time efficient and lends itself well to processing several units at the same time. The technique has been exported to other laboratories with similar yields.

18. **Predictive utility of the attached segment in the quality control of a cord blood graft.** Rodriguez L, Garcia J, Querol S. *Biol Blood Marrow Transplant.* 2005;11:247-51. **Abstract**

A reliable method to assess the cell content and viability of a graft before transplantation is crucial. Although it has been shown that the number of total nucleated cells (TNCs), CD34+ cells, and colony-forming cells (CFCs) per kilogram body weight of the recipient in the graft are good predictors of patient survival, all of these variables are normally defined before cryopreservation. However, the quality of a cord blood graft might be affected during the freezing process, storage, and transportation to the transplant unit, as well as during the thawing process.

To validate the use of a segment attached to the umbilical cord blood (UCB) unit as a quality-control tool for the final product, UCB units (n = 20) stored in liquid nitrogen were analyzed. The UCB units and their attached segments were thawed, and the number and viability of total nucleated cells, mononucleated cells, CD45+ cells, and CD34+ cells were determined, as were colony-forming cell counts. There was no significant difference between UCB units and segments for any of the parameters assessed. Additionally, the linear correlation coefficient ( $R^2$ ) in these paired samples was 0.85 and 0.78 for CD34+ cells and colony-forming cells, respectively.

On the basis of these data, the authors concluded that the cell sample in the tube segment physically linked to the transplant UCB bag predicts the total cell content and functionality of the unit and may serve as a source for final quality control of the UCB unit before transplantation.

19. **Use of nonvolume-reduced (unmanipulated after thawing) umbilical cord blood stem cells for allogeneic transplantation results in safe engraftment.** Hahn T, Bunworasate U, George MC, Bir AS, Chinratanalab W, Alam AR et al. *Bone Marrow Transplant* 2003; 32:145-150. **Abstract**

Twenty-six 26 patients underwent transplantation with unmanipulated (n = 18) or volume-reduced (n = 8) cord blood units. Engraftment was similar in the two groups, and similar to reported series and to the authors' series using volume-reduced units for cord blood transplantation. The results of this small study indicated that there was no serious toxicity from cord blood infusion using unmanipulated cord blood units. The authors concluded that unmanipulated cord blood units may be infused safely with adequate engraftment and survival.

20. **Cryopreservation of umbilical cord blood: 1. Osmotically inactive volume, hydraulic conductivity and permeability of CD34+ cells to dimethyl sulphoxide.** Hunt CJ, Armitage SE, Pegg DE. *Cryobiology* 2003;46:61-75. **Abstract**

21. **Cryopreservation of umbilical cord blood: 2. Tolerance of CD34+ cells to multimolar dimethyl sulphoxide and the effect of cooling rate on recovery after freezing and thawing.** Hunt CJ, Armitage SE, Pegg DE. *Cryobiology* 2003;46:76-87. **Abstract**

Cryopreservation protocols for umbilical cord blood (UCB) have been based on methods established for bone marrow and peripheral blood stem cells. The a priori assumption that these methods are optimal for progenitor cells from UCB has not been investigated systematically. Studies of UCB stem cells have largely been directed to establishing that such methods provide an adequate level of post-thaw survival rather than attempting to derive optimal cryopreservation protocols from first principles. The authors established addition and elution protocols that prevent osmotic damage and they used these to investigate the effect of multimolar concentrations of Me<sub>2</sub>SO on membrane integrity and functional recovery. They determined that the optimal recovery of CD34+ cells requires serial addition of Me<sub>2</sub>SO, slow cooling at rates between 1°C and 2.5°C/min and serial elution of the cryoprotectant after thawing.

22. **Assessment of cell viability and apoptosis in human umbilical cord blood following storage.** Xiao M, Dooley DC. *J Hematother Stem Cell Res.* 2003 Feb;12:115-22. **Abstract**

The authors attempted to identify a simple and rapid technique for assessing the quality and recovery of umbilical cord blood

(UCB) cells following laboratory manipulation. They determined that UCB held for 72 hours showed higher levels of cell deterioration than were present in UCB <48 hours old. Staining with 7-AAD was more sensitive to cellular damage than was uptake of Trypan Blue, and correlated with retention of hematopoietic function (progenitor assays). The authors concluded that 7-AAD staining of UCB mononuclear cells provides a rapid and simple technique for assessing the viability, recovery, and hematopoietic functionality of stored UCB.

**23. Patient care during infusion of hematopoietic progenitor cells.** Sauer-Heilborn A, Kadidlo D, McCullough J. *Transfusion*. 2004;44:907-16.

The authors provide a detailed review of the factors which should be taken into consideration and the adverse reactions that may occur in relation to the process of hematopoietic progenitor cell (HPC) infusion. Most published data refer to autologous or allogeneic BMT or PBPC infusions, and adverse reactions associated with cord blood infusions are less frequent and appear to be less severe. The thawed HPC product contains granulocyte debris, RBC stroma, and free hemoglobin which may cause side effects when infused. Washing of the cells is an option but must be weighed against the loss of viable cells. In the largest study reported, severe reactions occurred in only 0.4 percent of 1412 patients. The most frequent symptoms were nausea, vomiting, hypertension, hypotension and bradycardia. Some reports describe more side effects with a higher dose of DMSO. Hemoglobinuria has been reported in a high percentage of patients in relation to the RBC content of the transfused product. Side effects are usually mild to moderate; however, life-threatening cardiologic, neurologic, pulmonary, anaphylactic, and renal events have been reported. Patients receiving autologous BM containing an average of 1.55 ml of RBCs per kg experienced significantly more arrhythmias than patients receiving PBPC with a median RBC content of 0.23 ml per kg. There are reports of severe bradycardia and even two case reports of a cardiac arrest immediately after the infusion of autologous BM. To minimize the time of contact, there is an incentive to infuse the HPCs as rapidly as possible. However, in the study with the lowest rate of bradycardia and heart blocks, the authors suggested that that might be due to a slow infusion rate of 5.5 to 6 ml per minute. One study used an infusion rate of 20 to 50 ml per minute and found cardiac arrhythmias and hypertension in a high percentage of the patients (82 and 41%, respectively). Patients should be carefully monitored during and after the infusion for 6-24 hours.

**24. Microbial contamination of hematopoietic progenitor cell grafts—incidence, clinical outcome, and cost-effectiveness: an analysis of 735 grafts.** Kamble R, Pant S, Selby GB, Kharfan-Dabaja MA, Sethi S, Kratochvil K, Kohrt N, Ozer H. *Transfusion*. 2005;45:874-8. [Abstract](#)

The authors point out that the current guidelines of AABB recommend bacterial and fungal microbial surveillance of HPC grafts. Microbial cultures are therefore obtained immediately after HPC collection and also after HPC processing. However, the clinical significance of microbial contamination on HPC engraftment and transplant outcome is unclear. It is the general experience of the transplant community that positive cultures are not associated with adverse events. Unfortunately, there are few published data available to support this impression.

In this study, a retrospective analysis of 735 consecutive marrow and peripheral blood progenitor cell harvests between 1998 and 2003 was performed. Analysis included incidence, clinical outcome, and cost outcomes of positive blood cultures and antibiotic therapy.

Thirty-three of 735 (4.5%) harvests were contaminated. The incidence of microbial contamination varied with the source of the graft: 4 of 26 (15%) were cord blood, 8 of 177 (4.5%) were marrow, and 21 of 532 (3.9%) were peripheral blood. Coagulase-negative *Staphylococcus* (n=22) and *Propionibacterium acnes* (n=8) were most frequently isolated. Potentially pathogenic organisms were isolated in 6 of 735 (0.81%) grafts. The estimated total cost of surveillance was approximately \$81,585. The cost of vancomycin therapy in 4 patients who received prophylactic antibiotic therapy was approximately \$10,000.

No adverse sequelae followed infusion of contaminated grafts.

The authors concluded that clinical sequelae following infusion of microbially contaminated progenitor cells is extremely rare. Prophylactic empiric antibiotics may be unnecessary. Routine microbial surveillance of progenitor cell grafts is a low-yield procedure.

*(Present requirements for cord blood units by accrediting agencies (FACT/NETCORD and AABB) require that, if positive cultures are obtained, antibiotic sensitivities must be provided to the transplant center. Some investigators (See v. Quality Issues, Citation #1) recommend that cord blood units with a positive culture should not be placed into useable inventory and available for transplant. The data presented in this article suggest that transplant physicians should continue to have the option to accept or refuse units with positive cultures.)*

[<<PREVIOUS PAGE](#) [Page 1](#) | [2](#) | [3](#)

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