

Liquid storage, shipment, and cryopreservation of cord blood

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BACKGROUND: Cord blood banking requires methods for shipping and storage. This study examines the influence of shipping via overnight courier on postthaw viability of cord blood.

STUDY DESIGNS AND METHODS: Anticoagulated cord blood was divided with one sample diluted 1:1 using STM-sav (a storage solution) and the other undiluted. Units were shipped from Minneapolis to Memphis and returned, RBC-depleted, cryopreserved, stored for 14 days, and thawed. MNC counts, percent viable cells, quantity of CD34+ cells, and frequency of CFU-GM were measured. Temperature during shipment was continuously monitored.

RESULTS: Preliminary studies showed the packing and processing protocol influenced the temperatures experienced during shipping. Samples achieved temperatures below 10°C within 4 to 8 hours with a few units dropping near or below 1°C with cold ambient temperatures. The MNC recovery, CD34+45+ recovery, and frequency of CFU-GM for samples that were shipped were comparable to those observed using static liquid storage. The postthaw viable cell recovery was greatest for storage and shipping times of 24 hours and decreased when the storage and shipping times were longer.

CONCLUSION: Ambient conditions and the packing and processing protocol influence the temperature history of the sample. Samples stored beyond 24 hours in liquid storage and shipping exhibit a decreased postthaw recovery.

Collection and banking of cord blood (CB) for transplantation has grown considerably in recent years, with over 110,000 units stored worldwide.¹ Cord blood may be collected at any time (day or night) and may be shipped from a collection site to a central processing facility where it is processed (typically RBC depleted) and cryopreserved.

As with blood banking, CB collection is typically performed at multiple sites (typically hospitals) across the country and sent to a central processing facility where it is RBC depleted and cryopreserved. As such, the sample must be stored in liquid phase and shipped before cryopreservation. The influence of shipping conditions on blood cell function and survival during liquid storage has been of interest for over half a century.² Subsequent studies have examined the design and development of blood shipping containers³ and the influence of shipping conditions on biochemical markers.⁴ As international systems to coordinate hematopoietic progenitor cell (HPC) transplantation were developed, issues surrounding shipping of cryopreserved cells were examined.^{5,6}

In a recent series of articles, we have examined the influence of prefreeze processing on the viability of CB. Specifically, we examined the duration and conditions for optimal liquid storage of CB⁷ and the influence of liquid storage period on the postthaw recovery of CB.⁸ In these studies, we observed that CB was subjected to liquid storage for up to 72 hours with little loss in recovery. If the CB was frozen after liquid storage, liquid storage periods greater than 24 hours resulted in decreased cell recovery.

ABBREVIATION: CB = cord blood.

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The objective of the present study was to determine the influence of shipping during liquid storage on the postthaw viability of CB. An implicit objective was to determine shipping conditions that would permit us to maintain the desired thermal history for the sample. This information will be essential in developing and validating shipping protocols during liquid storage of HPCs.

STUDY DESIGNS AND METHODS

Umbilical CB collection

Human umbilical CB was aspirated with a 17-ga needle from volunteer donor cord veins using standard procedures (volume of CB range, 40-121 mL). All samples were collected with informed consent and with the approval of the local Institutional Review Board. Blood was collected into a blood bag (Baxter-Fenwal, Deerfield, NJ) containing 35-mL CPD (921 mg sodium citrate, 893 mg dextrose, 105 mg citric acid, and 78 mg monobasic sodium phosphate) while gently mixing.

Shipping protocol

Within 4 hours of the blood collection, the experiment was initiated. A blood sample of each CB unit was taken and analyzed for WBC counts, MNC counts, percentage and viability of CD34+ CD45+, TNC viability, and progenitor (CFU-GM) content. These assays were considered the 0 hour time point. The CB unit was divided equally, half serving as a control arm of the study. The remaining half of the unit was diluted with an equal volume of STM-Sav (STM-Sav: STM-Sav is composed of 1000 mL Lactated Ringers [Baxter, Deerfield, IL] solution with 50 mM Histidine (Ajinomoto, Paramus, NJ) and 1 percent HSA (Red Cross, Bern Switzerland) and was compounded at the Fairview-University Hospital Pharmacy.) Both resulting units were stored at room temperature before packaging for shipping. A schematic representation of the experimental protocol can be found in Fig. 1.

After separating the CB unit into two transfer bags, they were packaged for shipping. To simulate the test unit of CB being part of a shipment of several units, we packed the CB units being studied with several units of whole blood. One unit of normal human whole blood obtained for research use was aliquoted into four separate 300 mL transfer bags (Baxter). Three of the bags, containing 100 mL each, were refrigerated at 4°C. The fourth bag, also containing 100 mL, was allowed to acclimate to room temperature. The bags containing the CB (room temperature) were bundled with the whole blood (Fig. 2) with two rubber binders. A temperature probe programmed to take readings every minute was also placed in the center of the bundle. The probe (Model HiTEMP 102, ERTCO, West Patterson, NJ) uses a four-wire thermister to measure temperature. Temperature and time data are mea-

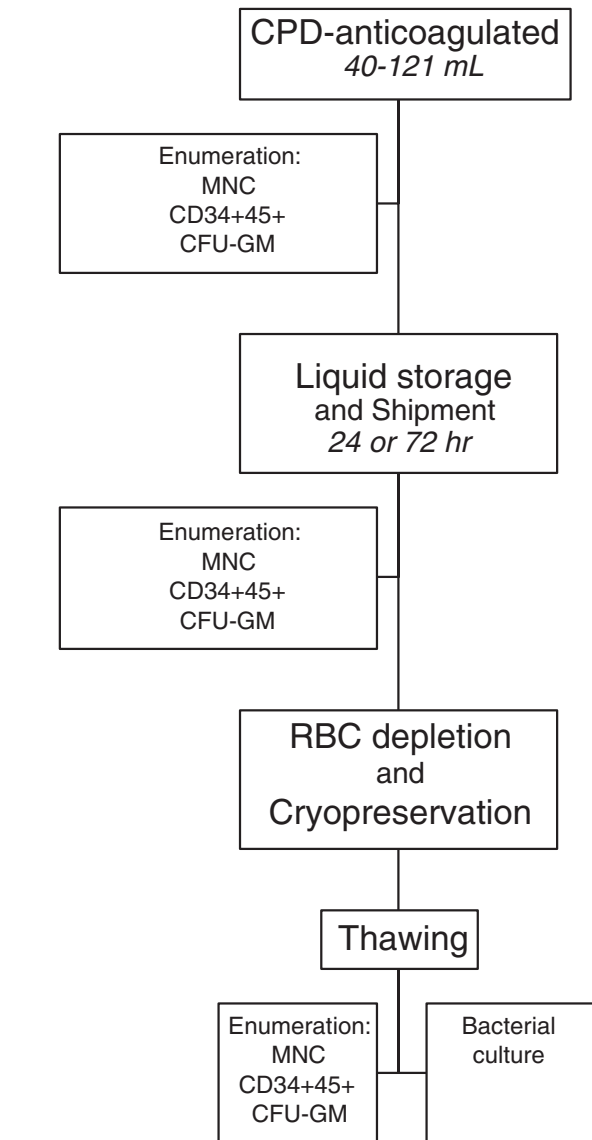


Fig. 1. Schematic representation of the CB-processing protocol.

sured at the specified reading interval and stored for later downloading. An absorbent pad was placed in the bottom of an E-54ARC insulated container. Bubble wrap was added to cover the bottom and the sides of the carton's interior. The blood and probe bundle was placed into a plastic liner, sealed, and then transferred to the carton. An additional absorbent pad and piece of bubble wrap covered the top of the liner. Seven pounds of ice was equally divided between two bags and placed on the top of the contents. The foam cover was replaced and the carton was sealed.

The carton containing the blood was shipped by a carrier (FedEx, Memphis, TN) priority overnight from Minneapolis to Memphis and returned. High and low temperatures of each city and tracking information were recorded.

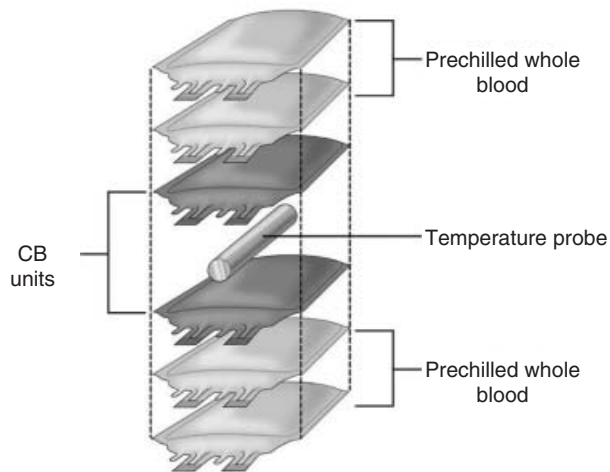


Fig. 2. Schematic representation of packing procedure for CB and chilled blood units. A total of six units were packed. Control CB unit and unit diluted with STM-sav were packed around a temperature probe. Surrounding those units were four prechilled whole-blood units of approximately 100 mL each.

Cryopreservation

After shipping and return to our laboratory, the CB samples were processed for cryopreservation. The total liquid storage time was approximately 24 hours (average, 21 ± 1 hr) for the 24-hour studies and approximately 48 hours (44 ± 2 hr) for the 48-hour studies. Each unit was transferred to a separate 150-mL transfer bag (Terumo, Tokyo, Japan). A volume of 6-percent hetastarch (Baxter, Deerfield, IL) solution equal to 20 percent of the CB volume was added to each bag. After mixing, the resulting solutions were centrifuged at $90 \times g$ for 6 minutes (Sorvall, RC-3 series, Kendro Laboratory Products, Newtown, CT) to remove RBCs. Plasma and 15 mL of RBCs were transferred to a new transfer bag. After mixing, product was centrifuged at $450 \times g$ for 10 minutes, and the supernatant was removed down to the RBC layer. The final volume of the unit was reduced to about 15 mL. A sample of the RBC-depleted product was assayed for WBC counts, MNC counts, percentage, and viability of CD34+ CD45+, TNC viability, and progenitor (CFU-GM). The remaining processed CB was transferred to 50-mL cryocyte bags (Baxter, Chicago, IL).

The cryopreservation medium containing 55 percent DMSO wt/vol and 5 percent Dextran 40 wt/vol (Protide, St. Paul, MN) was added to the RBC-depleted CB to a final concentration of approximately 10 percent DMSO. The sample was transferred to a controlled-rate freezer (Planer, Kryo 10, series III, Middlesex, UK) that was pre-cooled to 0°C . Then the chamber temperature of the controlled-rate freezer was cooled at 1°C per minute to -12°C , cooled at 20°C per minute to -60°C followed by warming of the sample at 15°C per minute to -18°C , cooled at 1°C per minute to -60°C , and finally, 3°C per minute to

-100°C . This protocol resulted in a 1°C per minute cooling rate for a probe inserted in a sample cassette containing the cryopreservation solution. After completion of the freezing protocol, the units were removed from the controlled-rate freezer and stored in liquid nitrogen.

Thawing protocol and postthaw viability assessment

After a minimum of 14 days of storage in liquid nitrogen, units were thawed in a 37°C water bath (National, Portland, OR) for analysis. Before further processing, samples were drawn from both units for assessing viability. A minimum of 15 mL each of Dextran 40 (Abbott Laboratories, North Chicago, IL) and 5 percent HSA (Baxter Healthcare, Glendale, CA) were slowly added to each unit. The resulting solutions containing the thawed cells were centrifuged at $250 \times g$ for 10 minutes in a precooled centrifuge (Sorval, RC series, Kendro Laboratory Products, Newtown, CT). After removal of the supernatant, cells were re-diluted for analysis. As with previous studies, WBC counts, MNC counts, percentage and viability of CD34+/CD45+, TNC viability, and progenitor (CFU-GM) assays were performed. In addition, samples were also taken for bacterial culture to determine if contamination of the sample was present.

Assays

Manual MNC counts were performed with a hemocytometer (Hauser Scientific, Horsham, PA). Cells were stained with acridine orange/PI⁹ and examined using a fluorescent microscope (Zeiss Axioskop, Zeiss, Jena, Germany or Leitz Laborlux S microscope, Leica, Wetzlar, Germany). All cells fluorescing green were scored as viable. The MNC recovery was calculated by dividing the MNC counts at a given time point (24, 48 hours or after thaw) by the Time 0 hour MNC count. The percent viability was determined by dividing the total number of green cells by the sum of the green and the orange cells and multiplying by 100.

To assess the fraction of cells that were CD34+45+, the RBCs present in the sample were lysed and the remaining MNCs were stained with anti-CD45-FITC and anti-CD34-PE (Hle-1 and HPCA-2 Becton-Dickinson Immunocytometry Systems, San Jose, CA). The cells were stained with Via-Probe (BD Pharmingen, San Jose, CA) to assay viability. Utilizing a flow cytometer (FACSCalibur, Becton-Dickinson), specimens were then evaluated using International Society of Hematotherapy and Graft Engineering (ISHAGE) gating.¹⁰ The number of CD34+45+ cells was calculated by multiplying the total number of MNCs times the percent of CD34+45+ cells times the percentage of viable CD34+ cells. The fraction of dead cells was determined based on the fraction of cells that incorporated the viability dye. The recovery of CD34+45+ cells was calculated by dividing the number of viable CD34+45+ cells at

a given time point by the number of viable CD34+45+ cells at time 0 hour.

The proliferative capability of the cells was determined using a colony formation assay. The RBCs were removed from the sample by lysis. The cells were then diluted and added to methylcellulose (Methocult GF H4434, Stemcell Technologies, Vancouver, BC, Canada)¹¹ in a final concentration of 30,000 MNC per mL. Progenitor assays were performed using methylcellulose. One mL of cells per methylcellulose was plated in duplicate into 22-mm diameter non-tissue culture treated, hydrated 12-well plates (Becton Dickinson, Franklin Lakes, NJ). Plates were incubated at 37°C and 5 percent CO₂ for 14 days. CFU-GM results are the mean number of colonies per 10⁵ nucleated cells represented by the formula

$$\text{CFU-GM colonies counted}/\# \text{ cells plated} \times 100,000$$

Statistical analysis

The percentage of viable cells, percentage of viable CD34+45+ cells, CFU-GM per 10⁵ cells and viability were determined as a function of the experimental conditions (time period for liquid storage and storage in STM-Sav compared to control). The frequency of CFU-GM, CD34+45+ recovery, and MNC recovery were analyzed using the student's t test. Due to non-normal distribution of CD34+45+ counts, a log transformation of the data was performed before analysis using the t test.

RESULTS

Thermal conditions during shipping

The first phase of this investigation involves the development and validation of the shipping protocol. Specifically, we were interested in the thermal conditions for the blood units during the shipping process. In preliminary studies, the CB/whole blood units were packed using the protocol described in Materials and Methods. The blood units were not prechilled before packing. The temperature as a function of time for one experiment is given in Fig. 3. There is a gradual decline in the temperature as a function of time, but the probe does not meet the desired temperature range (1-10°C) at any time during the shipping protocol. These studies were performed in warm weather conditions.

Based on this experience, we decided to prechill the whole-blood units shipped. These units were placed in a standard refrigerator for at least 4 hours to precool the units. The units were then packed as described previously

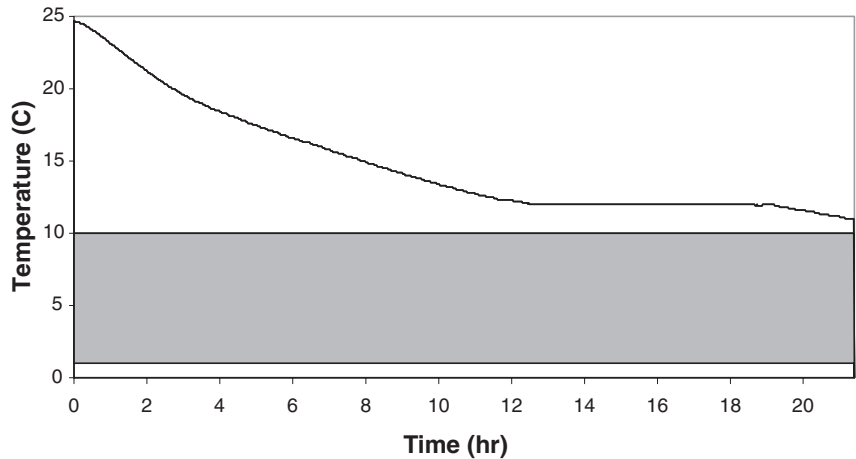


Fig. 3. Temperature as a function of time for CB unit shipped from Minneapolis to Memphis and return. Shaded area indicates the desired range of temperatures (1-10°C). CB units were packed with unchilled whole-blood units.

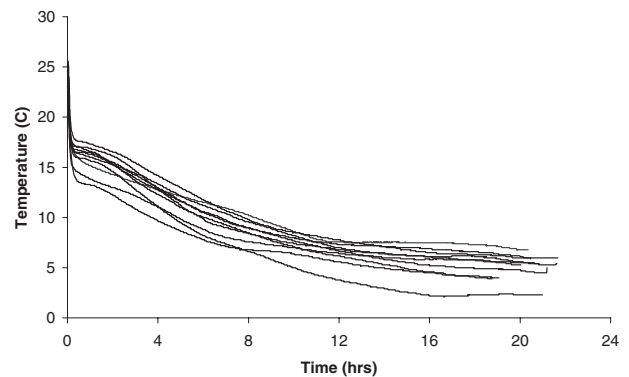


Fig. 4. Temperature as a function of time for CB unit shipped from Minneapolis to Memphis and return. The temperature traces for 10 different units are each represented. Two CB units were packed with four chilled whole-blood units.

and shipped. The temperature as a function of time for the temperature probe packed in the units was given in Fig. 4. For 10 different shipping experiments, the thermal histories were recorded and graphed. There were three distinct patterns in the thermal histories measured. From 0 to 15 minutes, the cooling rate was -0.55°C per minute (95% CI, -0.62 to $-0.49^{\circ}\text{C}/\text{min}$). From 16 minutes to 12 hours, the cooling rate was 0.014°C per minute (95% CI, -0.016 to $-0.012^{\circ}\text{C}/\text{min}$). For times greater than 12 hours, the cooling rate is -0.003°C per minute (95% CI, -0.004 to $-0.002^{\circ}\text{C}/\text{min}$). All three cooling phases are statistically different from each other ($p < 0.01$).

Using this shipping protocol, a total of 13 units were studied (10 units with a 24-hour and 3 units with a 48-hour storage/shipping protocol). All units achieved temperatures below 10°C at between 4 and 8 hours after the start of the shipping protocol. The probe for only one of the

studies monitored dropped below 1°C (Table 1). This drop was observed during very cold weather conditions in Minneapolis. The shipping studies were performed over a range of outdoor temperatures (Table 1). During warmer weather conditions, the temperature of the units as measured by the probe did result in the range of desired temperatures (1-10°C). For cold weather conditions, our results suggest that different methods of packing the units or maintaining thermal conditions may be required to reduce the potential for dropping below the lower temperature limit (1°C).

TABLE 1. Minimum (MIN) and maximum (MAX) air temperature during shipment of CB units for Minneapolis and Memphis and minimum CB temperature during shipment

ID	Date	Minneapolis		Memphis		CB MIN (C)
		MAX (C)	MIN (C)	MAX (C)	MIN (C)	
SH4	09/19/2002	23	11	26	18	6.8
SH5	10/07/2002	15	6	22	9	6.0
SH7	10/16/2002	7	-3	18	8	5.3
SH8	10/24/2002	8	0	22	13	5.9
SH9	10/29/2002	7	-5	20	7	4.5
SH10	11/18/2002	7	-1	19	4	5.4
SH11	11/26/2002	-3	-12	4	-4	5.3
SH12	12/02/2002	-3	-16	13	1	2.1
SH14	01/02/2003	-1	-10	8	-3	4.0
SH15	02/12/2003	-4	-22	17	2	3.9
SH16	02/26/2003	1	-16	4	-2	3.6
SH17	02/27/2003	1	-9	9	2	3.9
SH18	03/05/2003	3	-21	17	5	0.2

MNC Recovery

The average initial MNC count for umbilical CB before storage was $2.7 \pm 0.6 \times 10^6$ cells per mL. Due to changes in the volume and cell concentration during the protocol, we calculated the recovery of MNCs at different time points in the protocol. The recovery is defined as the total number of MNCs at a given time point divided by the total number of MNCs at time 0 hour. During liquid storage and shipment, the MNC recovery for samples stored in STM-Sav was 166 ± 64 percent and 145 ± 22 percent at 24 and 48 hours, respectively. Similarly, the MNC recovery for control samples was 161 ± 68 and 137 ± 20 percent at 24 and 48 hours liquid storage and shipment, respectively (Fig. 5). No significant difference in the MNC recovery during liquid storage and shipment for control and STM-Sav samples were observed at both 24 and 48 hours ($p > 0.60$).

As expected, cryopreservation reduced the MNC recovery. The postthaw MNC recovery was 66 ± 33 and 74 ± 4 percent for control samples that experienced a 24- or 48-hour liquid storage and shipping phase, respectively. For samples stored in STM-Sav for 24 or 48 hours before cryopreservation, the postthaw MNC recovery was 69 ± 30 and 80 ± 21 percent, respectively (Fig. 5). No significant difference in the postthaw MNC recovery was observed following processing, cryopreservation, and thawing of control and STM-sav samples for the time periods studied ($p > 0.56$).

Viable CD34+ 45+ cell recovery

The average initial concentration of CD34+45+ cells was $11 \pm 6 \times 10^3$ cells per mL. There was no significant decline in CD34+45+ cells observed during the liquid storage and shipping period studied ($p > 0.42$) (Fig. 6). During liquid storage and shipment, CD34+45+ cell recovery for samples stored in STM-sav was 203 ± 112 percent and 249 ± 91 percent at 24 and 48 hours, respectively. Similarly, the CD34+45+ cell recovery for control samples was 166 ± 84

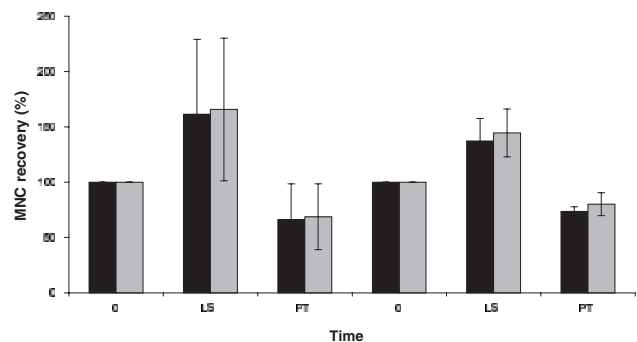


Fig. 5. MNC recovery percentage during liquid storage and shipment (LS) and postthaw (PT) for CB stored in STM-sav and undiluted control CB for either 24 or 48 hours before cryopreservation. Error bars correspond to the SD of the mean. ■, control; ■, STM-Sav.

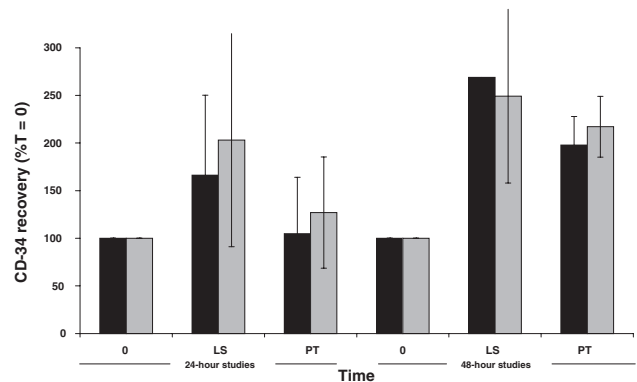


Fig. 6. CD34+CD45+ recovery percentage during liquid storage and shipment (LS) and postthaw (PT) for CB stored in STM-sav and undiluted CB stored for either 24 or 48 hours before cryopreservation. Error bars correspond to the SD of the mean. ■, control; ■, STM-Sav.

and 269 ± 137 percent at 24 and 48 hours of liquid storage and shipment, respectively.

For control samples that experienced 24- or 48-hour liquid storage and shipping periods, the postthaw CD34+ recovery was 105 ± 59 and 198 ± 30 percent, respectively. For samples stored in STM-Sav for 24 or 48 hours before cryopreservation, the postthaw CD34+ recovery was 127 ± 58 and 217 ± 32 percent, respectively. The differences in postthaw recovery for CB stored in STM-Sav and control samples were not statistically different and did not vary with time in liquid storage before cryopreservation ($p > 0.41$).

Progenitor assays

The frequency of CFU-GM per 10^5 cells was 105 ± 24 for fresh CB samples studied. During liquid storage and shipment, the frequency of CFU-GM for samples stored in STM-Sav was 109 ± 26 and 99 ± 17 percent at 24 and 48 hours, respectively. Similarly, the frequency of CFU-GM per 10^5 cells for control samples was 84 ± 25 and 98 ± 17 percent at 24- and 48-hour liquid storage and shipment, respectively (Fig. 7).

There was no difference in the postthaw frequency of CFU-GM for either time point (24 or 48 hours) or control versus STM-Sav ($p > 0.52$).

For control samples held for 24 or 48 hours of liquid storage and shipment, the postthaw frequency of CFU-GM per 10^5 cells is 84 ± 25 and 98 ± 17 , respectively. For samples stored in STM-Sav for 24 or 48 hours before cryopreservation, the postthaw frequency of CFU-GM per 10^5

cells is 94 ± 31 and 91 ± 2 , respectively. The postthaw frequency of CFU-GM per 10^5 cells was no different for cells diluted in STM-Sav versus control samples ($p > 0.41$) for 24- and 48-hour liquid storage and shipping protocols ($p > 0.33$).

The microbial testing of all the samples for the study was negative, indicating that no samples had become contaminated during processing.

DISCUSSION

In this investigation, we developed and implemented a standard shipping protocol. Our initial studies indicated that the initial temperature of the product to be shipped had a strong influence on the thermal history of the product during liquid storage and shipment. Specifically, we observed that the desired temperature range ($1-10^\circ\text{C}$) would not be achieved during the shipping period (24 hour) unless the four whole-blood units were pre-chilled. This suggests that if several CB units are packed together, they may not reach the desired temperature ($<10^\circ\text{C}$) if the units that have been collected recently and are not precooled before shipping.

After developing our standard protocol, the thermal history of the samples reached the desired temperature range within a 4- to 8-hour time period from the beginning of the shipping protocol. All 10 samples analyzed exhibited a consistent range of cooling rates after initiation of the shipping protocol. These thermal histories were obtained under realistic conditions: different ambi-

ent temperatures with the motion and vibrations associated with shipment. Previous studies have centered on characterizing shipping temperatures under controlled laboratory conditions.^{2,3,12} For this study, we only examined the influence of shipping on viability for a consistent number of units ($n = 6$). The actual thermal history of the units being shipped may be influenced by the total volume shipped, the initial temperature of the units shipped, and the ambient temperatures. Further studies should be performed to validate these results for small volumes (one unit) or large shipments (>20 units).

Another implicit variable in this study is the volume of the individual CB units. We were typically able to obtain only small-volume units not suitable for banking. The small sample volume may have influenced the pH of the sample. Specifically, the units were collected in CPD, which influenced the initial pH of the solution.⁷ Previous investigators

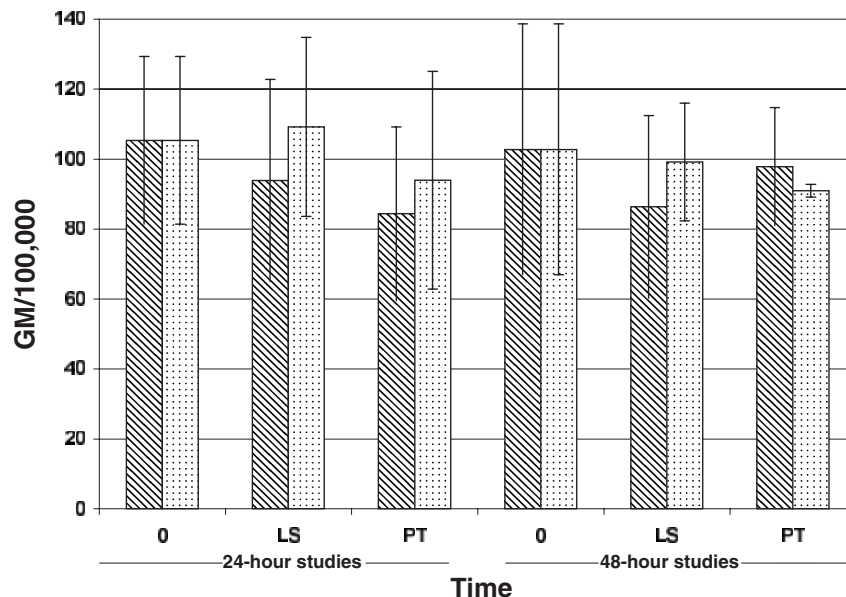


Fig. 7. Frequency of CFU-GM per 10^5 cells during liquid storage and shipment (LS) and postthaw (PT) for CB stored in STM-Sav and undiluted control CB stored for either 24 or 48 hours before cryopreservation. Error bars correspond to the SD of the mean.

▨, control; ▤, STM-Sav.

have observed that shifts in pH can influence colony-forming potential and differentiation potential.^{13,14} In previous studies, we observed that the shifts in pH did not influence colony-forming efficiency or viability by any of the measures observed^{7,15} for short time periods (24 hour). Further studies are, however, needed to quantify what if any effect shifts in pH have at longer time periods of storage (48 hours, 72 hours). Sample volume may also influence outcome. This investigation does not suggest a specific mechanism for the decline in viability observed with liquid storage (lack of oxygen, nutrients, toxicity of products of metabolism, etc.). It is our hypothesis, however, that the concentration of cells has more of an influence over viability than total sample volume. This hypothesis has been supported by this and our previous studies in which the use of a storage solution (hence, a dilution of cell concentration) enhances recovery and extends liquid storage times.^{7,15}

The recovery of MNCs observed in this investigation was consistent with those observed in our previous study⁸ in which the postthaw MNC recovery for CB after 24 hours in liquid storage was 64 ± 10 percent for samples stored in STM-Sav. For this investigation, the postthaw MNC recovery was 68 ± 30 percent for samples stored in STM-Sav for 24 hours during liquid storage and shipment. These results indicate that the process of shipping did not result in a decrease in MNC recovery. The postthaw MNC recoveries observed in this investigation were also consistent with those observed in other studies where there was a period of liquid storage before cryopreservation of CB.^{16,17}

In our previous study, the recovery of CD34+45+ cells stored for 24 hours in STM-Sav before cryopreservation was 140 ± 39 percent.⁸ For this investigation, the postthaw CD34+45+ cell recovery was 203 ± 112 percent. As with the MNC recovery, there is no significant difference in the CD34+45+ recovery resulting from shipping. The large variance in the CD34+45+ cell recovery is concerning. We have observed previously that the variance in the CD34+45+ cell enumeration appears to increase with time in liquid storage.⁷ The results of this investigation are consistent with that observation. There appears to be an increase in the postthaw CD34+45+ cell recovery for cells stored and shipped for 48 hours. The small number of samples ($n = 3$) combined with the variance associated with CD34+45+ cell enumeration makes it difficult to conclude that there is an increase in CD34+45+ cell recovery for 48-hour liquid storage and shipment.

For cells cryopreserved after 24 hours in storage, the frequency of CFU-GM per 10^5 cells varied little with storage condition. The frequency of CFU-GM per 10^5 cells for CB stored in STM-sav for 24 hours before cryopreservation was 100 ± 28 .⁷ For cells that experienced 24 hours of liquid storage and shipment, the frequency of CFU-GM per 10^5 cells was 94 ± 31 . As with previous measures, the shipping process does not seem to adversely affect the

frequency of CFU-GM per 10^5 cells. Beaujean et al.¹⁸ observed similar results for the frequency of CFU-GM per 10^5 cells after liquid storage.

The membrane integrity of frozen and thawed cells was determined and the postthaw recovery determined. Unlike MNC recovery, CD34+45+ cell recovery and frequency of CFU-GM per 10^5 cells, the postthaw viable cell recovery was less for cells during liquid storage and shipment of 48 hours than that observed at 24 hours. The decline in viable cell recovery is consistent with our previous study and suggests further that liquid storage for more than 24 hours followed by cryopreservation may result in reduced postthaw recovery.¹⁵

This study suggests that the storage and shipping conditions examined do not result in a significant decrease in cell recovery compared to liquid storage without shipment for the measures studied. This outcome is in contrast with our previous studies in which a significant difference was observed during liquid storage for samples diluted with a storage solution and those not.⁷ One likely explanation is that the process of RBC depletion and cryopreservation introduced significant variance to the process. Thus, quantifying the influence on viability of an early step in the process (liquid storage) may be difficult. Specifically, it is not clear that using a storage solution during the liquid storage phase provides a quantifiable benefit to postthaw viability for 24 hours in liquid storage. The use of a storage solution appears to provide a benefit for longer periods of storage (72 hour).¹⁵

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