

## PACKING INFORMATION

***Cord Blood Bank (CBB) Instructions: Complete all information below. Then fax this sheet to the requesting Transplant Center AND include a copy with the shipped cord blood unit (CBU).***

Shipping to: \_\_\_\_\_ FAX: \_\_\_\_\_

This dry shipper contains a **frozen cryopreserved human placental umbilical cord blood unit (CBU)** for COBLT Recipient ID: \_\_\_\_\_

Contents of Dry Shipper:

COBLT CBU ID: \_\_\_\_\_

Accompanying Paperwork:  
Transplant Center Feedback Sheet  
Receipt Procedures  
Procedure for Thawing Cryopreserved Cord Blood Unit (CBU) for Transplantation  
Product Information  
COBLT Bar Code Labels

Name of CBB Supplying the CBU: \_\_\_\_\_

CBB Shipper Number: \_\_\_\_\_

Federal Express Tracking Number: \_\_\_\_\_

For questions, contact:

CBB Contact Person: \_\_\_\_\_

Phone #: \_\_\_\_\_ Fax #: \_\_\_\_\_

Alternate CBB Contact: \_\_\_\_\_

Phone #: \_\_\_\_\_ Fax #: \_\_\_\_\_

***Transplant Center Instructions: Upon receipt of this information, call the CBB contact person listed above for the combination of the dry shipper lock or if you have questions/concerns.***

**TRANSPLANT CENTER FEEDBACK SHEET**

CBU ID #: \_\_\_\_\_ Recipient ID #: \_\_\_\_\_

CBU Information:

Maternal Sample Test Results:

Volume (mL) - 25:

CMV IgM Antibody:

ABO Rh Type:

Anti-HBc:

HLA A:

Syphilis:

HLA B:

Anti-HCV:

HLA DRB1:

HbsAg:

CBU Collection Weight (gm):

Microbial Culture:

Total Viable NCC x 10<sup>8</sup>:

HIV-1/2:

CFU-GM x 10<sup>5</sup>:

HIV p-24 Ag:

CD 34+ x 10<sup>6</sup>:

HTLV-I/II:

CD 3+ x 10<sup>6</sup>:

Hemoglobinopathy Screen:

Infant gender:

.....  
*Complete the following information prior to packing the CBU in the dry shipper*

Charged Weight of Unpacked Dry Shipper: \_\_\_\_\_ lb.

.....  
*Complete the following information upon receipt of the CBU and fax to:  
Name and FAX Number of CBB and MCC - Computer Generated*

Transplant Center Code: \_\_\_\_\_ Time Zone (ET, CT, MT, PT)

Date and Time of Receipt: \_\_\_/\_\_\_/\_\_\_ :\_\_\_ (24 hour clock)

Confirm Label Checks: CBU ID and Recipient ID on Packing Information /  
Transplant Feedback Sheet / CBU Registration \_\_\_\_\_  
CBU ID on Unit / Transplant Feedback Sheet \_\_\_\_\_  
Transplant Feedback Sheet / Accompanying Labels \_\_\_\_\_

Weight of Unpacked Dry Shipper: \_\_\_\_\_ lb.

Condition of CBU at Receipt: 1 G Satisfactory 2 G Unsatisfactory, *specify*: \_\_\_\_\_

Condition of Dry Shipper: 1 G Satisfactory 2 G Unsatisfactory, *specify*: \_\_\_\_\_

Did shipper temperature monitoring device indicate temperature > -120°C? 1 G Yes 2 G No

Please specify conditioning regimen intended for Recipient ID \_\_\_\_\_

Information Completed By: \_\_\_\_\_ Study ID: \_\_\_\_\_ Date: \_\_\_/\_\_\_/\_\_\_

## RECEIPT PROCEDURES

### 1. Verifying and Storing the Cord Blood Unit (CBU)

- 1.1 Open the top of the dry shipper using the combination lock number supplied by the CBB contact person. Locate the Transplant Center Feedback Sheet included in the packing information. Verify that the COBLT CBU ID and the COBLT Recipient ID recorded in the packing information, on the Transplant Center Feedback Sheet, and on the COBLT Confirmation of Registration/CBU Release Request match.

If there is a discrepancy, DO NOT PROCEED. Immediately call the designated CBB contact person listed in the packing information.

- 1.2 Open the main storage compartment of the dry shipper and locate the plastic bag containing the CBU canister. Transfer the CBU from the dry shipper into the vapor phase of liquid nitrogen in a liquid nitrogen freezer at  $\leq -120^{\circ}\text{C}$ . Verify that the identification number on the CBU matches the CBU ID number on the Transplant Center Feedback Sheet. **THESE STEPS MUST BE COMPLETED AS QUICKLY AS POSSIBLE TO MINIMIZE THE TIME THE CANISTER CONTAINING THE CBU IS EXPOSED TO THE AMBIENT TEMPERATURE.**

If there is a discrepancy, proceed. After safely storing the CBU canister, immediately call the designated CBB contact person listed in the packing materials.

- 1.3 After the CBU is safely stored, inspect the condition of the dry shipper and locate the temperature monitoring device packed with the CBU canister. Contact the CBB immediately if there is any indication that the CBU was damaged or exposed to temperatures  $> -120^{\circ}\text{C}$ .
- 1.4 After all checks have been performed and any discrepancies resolved, notify the appropriate individuals at your institution that the CBU has arrived, and complete the receipt information on the Transplant Center Feedback Sheet. Fax the sheet to the CBB responsible person listed in the packing materials and to the MCC.

### 2. Returning the Dry Shipper (Contract Centers Only)

- 2.1 Make arrangements to return the dry shipper to the CBB immediately following storage of the CBU. Return the dry shipper via Federal Express using the enclosed return shipping label. Pack the Styrofoam packing, bubble paper, and the shipper lid. Lock the lid with the combination lock.
- 2.2 On the day the shipper is sent, inform the CBB designated contact person so that they can expect its arrival.

**Immediate return of the shipper is essential because it is needed for another patient's product. If there are any questions, please call the designated CBB contact person.**

## THAWING CRYOPRESERVED CORD BLOOD UNIT (CBU) CELLS FOR TRANSPLANTATION

### *Principle*

Cells cryopreserved in DMSO have limited viability upon thawing, resulting in significant losses of cells available for transplantation. DMSO, the cryopreservant used to maintain cell viability at ultra low temperatures, is toxic to cells when warmed to 37° C. Intracellular DMSO creates a hypertonic environment which leads to sudden fluid shifts and cell death upon warming. Lysis of red blood cells leads to accumulation of extracellular free hemoglobin which can be nephrotoxic if infused intravenously. In addition, DMSO causes adverse effects in vivo after reinfusion, including blood pressure instability, fever, chills, and nausea. These problems can be ameliorated by mixing the thawed cells with a hypertonic solution, Dextran 40 + 5% albumin, immediately upon thawing. Cells can then be washed and further manipulated to remove DMSO, free hemoglobin, and other cellular products, as well as to perform other procedures before infusion to the patient.

*NB: This procedure is designed to enable the technologist to sterilely thaw cryopreserved cord blood within a closed system while maximizing viable cell recovery. The final product can be resuspended in a variable amount of dextran/albumin solution, allowing for adjustment to a suitable volume for reinfusion into patients of varying sizes. The final product is stable for at least 6 hours and time should be taken to work carefully and calmly.*

### *Specimen*

Frozen CBU within a metal canister maintained in the vapor phase of liquid nitrogen in a liquid nitrogen freezer at -120° C. The cryo bag containing the CBU may be overwrapped with plastic and may have 1-2 sealed tubing segments attached.

### *Equipment*

Laminar Flow Hood	
Refrigerated Blood Bank Centrifuge	Sorvall
Plasma Expressor	Baxter 4R4414
Coupler	Baxter 4C2405
Transfer Packs with Spike (#2, 300 mL)	Baxter 4R2014
Sterile Docker	Haemonetics SCD 312
Balance	Sartorius or Mettler
Heat Sealer	Sebra
Hemocytometer with Coverslip	Hausser Scientific, Bright-Line 1492
Instrument to Count WBCs	Coulter
BacT/Alert 120	Organon Teknika
Glass Microscope Slides (2)	
Nunc Tube	
Sterile Gloves	
Protective Freezer Gloves	
COBLT Bar Code Labels	From CBB
CBU Thawing Form	

## ***Reagents***

Albumin 25% Human UPS (12.5 grams in 50 mL)	Baxter
Dextran 40 (10% Gentrin 40 in 0.9% NaCl, USP)	Baxter
Trypan Blue Vital Stain, 1% Solution	Gibco

## ***Supplies***

12 x 75 mm Tubes, Non-sterile	Falcon 2052/Fisher 149-596
12 x 75 mm Sterile Culture Tubes with Snap Caps	Labcon 3336-335-000/Port City, Inc.
Syringes: Sterile 1 cc, 20 cc	Becton-Dickinson: (1 cc) 309623, (20 cc) 309661
Injection Needles: Sterile 16 g, 19 g	Becton-Dickinson: (16 g) 305198, (19 g) 305187
Cell Wash/Infusion Bag Set	Pall Medical (791-03)
Hemoset (optional)	Abbott Labs #8948

## **Or**

Y-Type Blood/Solution Set with Large Standard Blood Filter (170-260 micron filter)	Baxter
Hemostats (optional)	
Insul - Ice Mat (optional)	POLYFOAM Packers #970362
Cup of Regular Ice	
Bucket of Dry Ice	
Small Plastic Zipper-locked Bags* *Gas sterilize in house	
Alcohol Prep Pads	Baxter Healthcare #40000-110
Iodine Swabsticks	Baxter Healthcare #40000-040

## ***Procedure***

1. Begin preparations.
  - a. Verify that the water bath temperature is 37° C.
  - b. Prepare and label QC materials: counting vials, glass slide(s) for Wright's stain, tubes for viability, nunc tube to refreeze cryo bag segment(s), and bacterial culture bottles. Nunc tubes should be labeled using one of the cryogenic ISBT-128 bar code labels supplied with the CBU. OPTIONAL: Tubes for immunophenotyping and sterile tubes for progenitor assays.
  - c. Assemble and bar code the necessary paperwork, completing as much as possible.

- d. Mark transfer and label packs at 150 cc, 50 cc, and 25 cc with a permanent marker using a template prepared in the laboratory.
- e. Place dry ice in bucket.
- f. Place regular ice in cup, then inside the hood for QC.
- g. Label the transplant bag and waste bag of the Cell Wash/Infusion Bag Set (Figure 1) and put 100, 125, 150, and 175 mL marks to the outsides of the transplant bag, using a template prepared in the laboratory, to aid in adding the correct volume of Dextran/albumin solution (Illustration 1). Additional marks for 50, 200, and 250 mL may also be added. Tare the scale and use the same tared scale to weigh the transplant bag and residual cells in Step 6d.

*NB: To obtain accurate weights, always position the transplant bag and attached tubing identically. To do this, obtain a plexiglass tray with pins which fit into the holes in the transplant bag. Also place a block next to the scale to rest the tubing attached to the transplant bag. Always rest the tubing on the block at the same distance from the bag. It is suggested that marking the tubing 15 cm from the top of the transplant bag will help in positioning the tubing.*

2. Prepare Dextran 40 + 5% albumin solution.

- a. With sterile technique, add 25 gm (100 mL from 2 bottles) of stock albumin (25% Human UPS, 12.5 gm/50 mL) to a UPS bag containing 500 mL of Dextran 40. Final volume is 600 mL Dextran/albumin solution with a final concentration of approximately 5% albumin (actually 4%).

**Or**

From a UPS bag containing 500 mL of Dextran 40, drain 250 mL Dextran 40 and sterilely add 12.5 gm (50 mL from one bottle) of stock albumin (25% Human UPS, 12.5 gm/50 mL) to the remaining 250 mL of Dextran 40. Final volume will be 300 mL of Dextran/albumin solution with a final concentration of approximately 5% albumin (actually 4%).

- b. Using sterile technique (sterile docking or spike), transfer 300 mL of Dextran/albumin solution into the labeled 300 mL sterile transfer pack. Heat seal the tubing and remove the transfer bag.
- c. If 600 mL of solution is prepared, using sterile technique (either sterile docking or spiking), transfer the remaining 300 mL of Dextran/albumin solution into a second marked 300 mL sterile transfer pack. Label bag with a 24 hour expiration date and time and save for another thaw procedure.

3. Set up a closed system.
  - a. Sterile dock the 300 mL Dextran/albumin transfer bag to the Cell Wash/Infusion Bag Set (Illustration 2). Using the volume marks on the transplant bag, allow 125 mL of Dextran/albumin to flow into the transplant bag (see Step 1g).
  - b. Clamp off the tubing. Surround the bag with an ice mat to allow the solution to cool and place in hood (Illustration 3).
4. Thaw the cord blood and transfer to transplant bag.
  - a. Remove the cryopreserved cord blood from the freezer carefully. Two technicians will perform label checks as per institution SOP.
  - b. Working in vapor phase of liquid nitrogen, open the cassette, remove cryo bag plastic overwrap if present, and quickly separate segment(s) from the cryo bag. Place segment(s) in labeled nunc tube and put nunc tube in vapor phase of liquid nitrogen immediately to prevent thawing of the cord blood in the segment(s). Nunc tubes should be placed in a permanent storage location at a later time.
  - c. Remove the frozen CBU from the cassette. Wipe down outside of cryo bag quickly and carefully place into a sterile zipper-locked bag. Thaw the CBU in the zipper-locked bag in the water bath until the product reaches a slushy/liquid consistency. Remove the zipper-locked bag containing the CBU from water bath, dry outside of zipper-locked bag, and place under the hood.
  - d. Under the hood, remove CBU cryo bag from zipper-locked bag and, if necessary, dry the outside of the cryo bag. Clean the cryo bag port covers with iodine solution, cut the covers over the ports, and clean the cut and inner surfaces again with alcohol.

*NB: The ports on the cryo bag are covered with a plastic seal. In addition, there is an internal seal that the spikes will break as they enter the bag.*

Use sterile gauze to dry the ports. Spike into the cleaned ports with the spike lines attached to the Cell Wash/Infusion Set. Envelope the cryo bag with an ice mat. **Open the connection to the Dextran/albumin bag, but do not begin mixing the cells with the dextran/albumin until you read through Steps e and f below.**

- e. Hold the cold pack-wrapped cryo bag in one hand and the cold pack-wrapped transplant bag in the other hand, lower the cryo bag, and raise the transplant bag to allow the Dextran/albumin solution to run into the cryo bag (Illustration 4). (Approximately 25 mL of Dextran/albumin solution should flow into the bag by gravity over 1-2 minutes.) Adjust the cold pack to allow the cryo bag to expand to accommodate this additional volume (Illustration 5). Massage the cryo bag by hand to thoroughly mix the 50 mL of cells plus Dextran/albumin solution.

- f. Continue to **gently mix** the cells in the cryo bag with the Dextran/albumin solution by alternatively raising the cryo bag (with ice mat) relative to the transplant bag (with ice mat) and then the transplant bag relative to the cryo bag. Allow gravity to facilitate mixing of the cells and remaining Dextran/albumin solution. Gradually and completely mix cells with Dextran/albumin over a minimum of 4-5 additional minutes. After complete mixing, lower the ice pack-wrapped transplant bag and raise the ice packed-wrapped cryo bag to allow the cells to run into the transplant bag (Illustration 6). A small amount of residual fluid and cells will remain in the cryo bag and tubing. Clamp off the lines between the cryo bag and transplant bag in preparation for the rinsing procedure.
  
- g. To rinse the cryo bag, unclamp the tubing between the Dextran/albumin bag and the cryo bag. Allow approximately 25 mL of Dextran/albumin solution to run into the cryo bag. Close the clamp on the tubing between the Dextran/albumin bag and the cryo bag. Swirl the solution around the cryo bag to mix any remaining cells with the Dextran/albumin solution. Open the tubing between the cryo bag and the transplant bag and allow the rinse solution with cells to run from the cryo bag into the transplant bag (Illustrations 7a-c). Repeat this process a second time. The total volume in the transplant bag should now be approximately 200 mL. [125 mL Dextran/albumin + 25 mL cells in DMSO + 25 mL (rinse 1) + 25 mL (rinse 2)]. CAUTION: Do not add more than 250 mL to the bag. Overfilled bags may break when centrifuged.

*Note: To enhance flow during rinsing, add air from the transplant bag to the cryo bag. Sometimes the liquid in the 5 mL section of the cryo bag will not drain out of the bag. If this happens, close the clamp between the 20 mL section of the cryo bag and transplant bag. The fluid will then run out of the 5 mL compartment. CAUTION, do not add more than 250 mL to the bag. Overfilled bags may break when centrifuged. Allow a few mLs of well-mixed Dextran/Albumin + cells to enter back into the cryo bag. Drain back into Transplant bag.*

5. Centrifuge the cord blood to pellet the cells.
  - a. Place the Cell Wash/Infusion Bag Set and Dextran/albumin transfer bag into a centrifuge bucket. It is suggested that the bags be placed in a sterile zipper-locked bag prior to placement in the centrifuge bucket. The assembly must be supported and cushioned and all bags must be standing upright with ports facing up (Illustration 8). Bags can be cushioned by placing two 250 mL bags filled with water or saline placed on either side of the transplant bag. Weigh and tare the balance. Balance with a second bucket.
  
  - b. Pellet the cells at 880 G for 20 minutes at 4° C via centrifugation. On their Sorvall model, personnel at Duke University spin at 1800 rpm to achieve 880 G . Each center should validate its centrifuge prior to thawing a COBLT CBU.

6. Express supernatant and prepare cells for transplantation.
  - a. After centrifugation, gently remove the Cell Wash Infusion Bag Set and Dextran/albumin bag, taking care not to disrupt the cell pellet at the bottom of the transplant bag.
  - b. Hang the transplant bag on the plasma expessor without disturbing the pellet at the bottom of the bag.
  - c. Express the majority of the supernatant from the transplant bag into the waste bag. Continue to apply pressure until the cells in the pellet at the bottom of the transplant bag start to move or when the net remaining cell solution reaches a volume of approximately 25 mL.

*Note: Clear the tubing between the transplant bag and the waste bag by adding air from the waste bag.*

- d. After completion of expression of the supernatant, move the transplant bag to the tared scale (see Step 1g). The transplant bag and tubing must be placed in the same position used to tare the scale.
- e. Carefully open the tubing between the Dextran/albumin bag and transplant bag. Resuspend cells in 50 mL (or desired volume between 30-150 mL) of fresh Dextran/albumin. Record the weight from the tared scale of the washed and resuspended CBU on the CBU Thawing Form. Heat seal and remove the waste and Dextran/albumin bags.

*Note: If patient weight is  $\leq 20$  kg, use approximately 30 mL. If patient weight is  $\leq 40$  kg, use approximately 40 mL.*

- f. Using sterile technique, remove a 0.5 mL sample of the cells for QC. Obtain a cell count and viability on the final product. Record values on the CBU Thawing Form.

Calculate viable cell recovery using the following formula:

$$\frac{\text{Total viable nucleated cells in washed and resuspended CBU}}{\text{Total viable nucleated cells of cryo-preserved CBU}}$$

*Note: To standardize viability reporting, viability should be performed by Trypan Blue dye exclusion within 5 minutes of taking the QC sample. See Procedure Notes: Viability counts using Trypan Blue.*

If  $< 75\%$  of total viable nucleated cells are recovered (calculated above), re-spin supernatant in waste bag to recover additional cells, as detailed in Procedure Note 1.

*OPTIONAL: Calculate the amounts needed for progenitor cell assays and immunophenotyping.*

- g. If the final cord blood product contains clumps, filter using a burette filter (100 mL burette hemosep, Abbott Labs #8948) or Fenwal Y-type filter set (blood component filter set). Do not use any filter small enough to deplete leukocytes (e.g. < 60 microns). A pore size 60 - 270 microns is recommended. Do not irradiate product.
- h. Label the transplant bag with information per institutional SOP.
- i. Remove 15 mL from the waste bag and add to sterility testing tubes.

### ***Procedure Note***

1. To attempt to recover additional cells from the waste bag, follow the procedure below.
  - a. Heat seal the tubing between the transplant bag and the waste bag proximal to the transplant bag. The transplant bag can be transported to the transplant unit for infusion into the patient.
  - b. Using sterile technique, attach the tubing on the waste bag to a new transfer bag. Pellet the residual cells in the waste bag. Centrifuge the bag at 880 G at 4 degrees centigrade x 15 minutes. Remove the bag from the centrifuge carefully without disrupting the cell pellet.
  - c. Express the supernatant into the transfer bag, leaving a residual volume of approximately 10-20 mL.
  - d. Resuspend the cell pellet in the remaining supernatant and remove an aliquot of 0.1 mL. Perform cell count and viability.
  - e. If  $\geq 1 \times 10^6$  cells/kg of patient body weight are recovered from the waste bag and if patient received a cell dose of  $1-5 \times 10^7$  cells/kg in the initial transplant bag, infuse these additional cells. These cells may be added to the original transplant bag or may be given as a boost later on Day 0. Transport residual cells to the patient's room for reinfusion. If  $< 1 \times 10^6$  but  $> 1 \times 10^5$  cells/kg are recovered, cells should be cryopreserved in 10% DMSO for future testing or other uses. These cells can be stored in a nunc vial or small cryo bag at the discretion of the transplant center lab.

### ***Quality Control***

Cell count

Viability

Smear for Wright's stain

Progenitor assay (Optional) \* Do not remove more than 0.5% of total cells.

CD34+ Procount (Optional) \* Do not remove more than 0.5% of total cells.

### ***Procedure Notes***

#### Viability Counts using Trypan Blue

1. Add 10 $\lambda$  of post-thaw cells and 10 $\lambda$  of trypan blue, 1% solution, to a sterile tube.
2. Mix thoroughly and incubate for 5 minutes.
3. Remove 10 $\lambda$  and place under a coverslip on a hemacytometer.
4. Allow to settle.
5. Count 200 cells, scoring live versus dead cells.

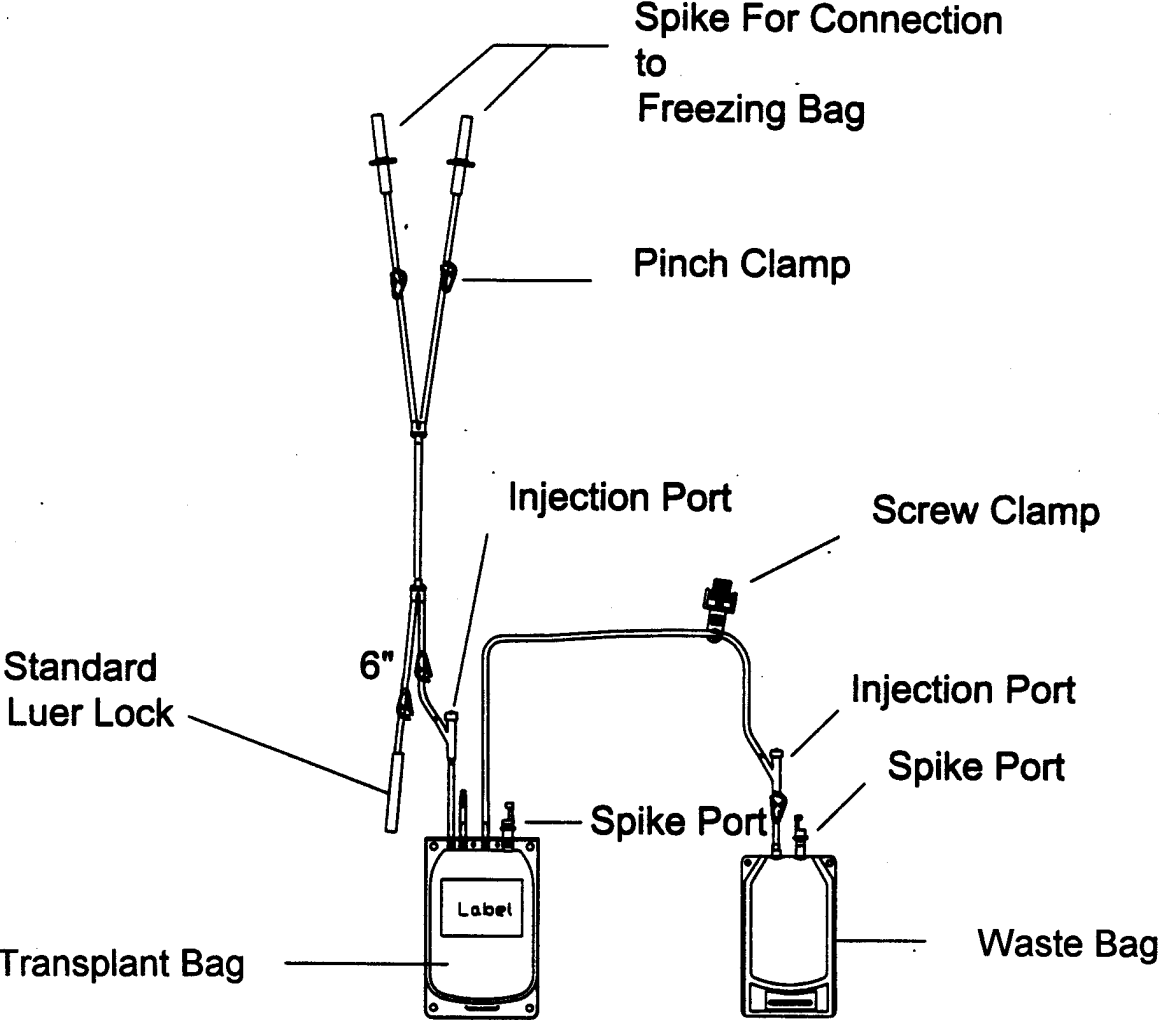
NOTE: The live cells are NOT blue, the dead cells are blue. Results are expressed as percent of cells that exclude the dye (i.e., are alive).

### ***References***

Pablo Rubinstein, M.D., Director of Placental Blood Bank, New York Blood Center, New York (personal training and communications).

Rubinstein P, Dobrila L, Rosenfield RE, Adamson JW, Migliaccio G, Migliaccio AR, Taylor PE, Stevens CE. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci USA* 92:10119-10122, October 1995.

Cell Wash/Infusion Bag Set



Insert here:

Illustration for COBLT CBU Thawing SOP - Page 1 of 3

(Found in m:\cor\wp>manuals\sop\bld-appf.fig1)

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Illustration for COBLT CBU Thawing SOP - Page 2 of 3

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Illustration for COBLT CBU Thawing SOP - Page 3 of 3

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## PROCEDURE FOR INFUSING THAWED CBU

### *Principle*

Unrelated cord blood, banked for public use, can substitute for bone marrow as the source of reconstituting stem cells after marrow ablative therapy used to treat patients with cancer, bone marrow and immunodeficiency diseases, and selective genetic diseases. After selection for transplantation, the designated unit is shipped from the bank to the transplant center in a dry shipper before the patient begins cytoreductive therapy. At the transplant center, the unit is stored in the vapor phase of liquid nitrogen until the day of transplant, when it is thawed and washed in dextran/albumin, a process which increases cell viability and removes approximately 90% of the DMSO cryoprotectant. The washed cells are resuspended in dextran/albumin and transported to the patient's bedside in a labeled transplant bag for infusion.

The unit is infused into the patient's blood via the central venous catheter over 2-30 minutes. Every effort should be made to be sure that all the cells in the transplant bag and IV tubing are delivered to the patient, maintaining a closed system during the infusion procedure.

### *Materials*

Labeled transplant bag  
Y-infusion set  
IV extension tubing  
500 mL bag of Normal Saline  
Blood filter, 170-260 microns

### *Procedure*

1. Verify that the patient is stable and has received scheduled premeds for transplant.
2. Verify that the transplant bag label matches patient identifiers via institutional procedures.
3. Examine the transplant bag to be sure that cells are in solution and that large clumps are not present in the bag. If clumps are present, prepare to use blood filter in infusion set-up.
4. Close all clamps on the tubing on the Y-infusion set and blood filter.
5. Spike one arm of the Y-infusion set into the bag of normal saline.
6. Connect extension tubing to the distal arm of the Y-infusion set.
7. Prime all tubing with normal saline.
8. Spike the other arm of the Y-infusion set into the transplant bag.
9. Connect the distal end of the extension tubing to the patient's central venous catheter.

10. Open the clamps between the transplant bag, the extension tubing, and the patient's central line and allow cells to infuse into the patient's blood.
11. Rinse residual cells in the transplant bag and tubing. After all cells have dripped out of the transplant bag, close the clamps on the extension tubing and Y-set connected to the transplant bag. Open the clamps between the normal saline bag and transplant bag and allow approximately 25 cc of saline to run into the transplant bag. Close the clamps between the saline bag and transplant bag and open the clamps between the transplant bag and patient and infuse this saline to the patient. Repeat rinse x 1.
12. Monitor the patient's vital signs before, during, and after infusion per institutional practices.

## PRODUCT INFORMATION

### General Information

Bone marrow transplantation (BMT) from human leukocyte antigen (HLA)-identical sibling donors has been successfully utilized in the treatment of high-risk or recurrent hematological malignancies, bone marrow failure syndromes and selected hereditary immunodeficiency states and metabolic disorders. In an attempt to increase the availability of suitable donors and reduce the morbidity and mortality associated with allogeneic bone marrow transplantation, clinical investigators worldwide have evaluated placental and umbilical cord blood as an alternate source of hematopoietic stem and progenitor cells for transplantation (1 - 21).

As of June 2000, umbilical cord blood from sibling and unrelated donors has been used to reconstitute hematopoiesis in approximately 1200 patients with malignant and non-malignant disorders treated with myeloablative therapy. Reports from individual institutions and the International Cord Blood Transplant Registry (ICBTR) suggest that umbilical cord blood contains sufficient numbers of hematopoietic stem and progenitor cells for both early and late engraftment at least in recipients weighing less than 40 kilograms. The purpose of the Cord Blood Transplantation (COBLT) Study is to accurately describe 180-Day survival and other events after cord blood transplantation.

### Drug Description

Umbilical cord blood is collected from the delivered placenta by insertion of a sterile transfusion set needle into the umbilical vein. Gravity causes the blood to drain into the collection bag, which is part of a sterile closed system. The anticoagulant citrate-phosphate-dextrose (CPD) is included in the bag. Collection volumes range from 40-300 mL. Informed consent is obtained from every mother and samples of the mother's blood are screened for CMV, Hepatitis B, Hepatitis C, HIV-1/2, HTLV-I/II, and syphilis. A sample of the cord blood unit is tested for microbial contamination and HLA type. Results from newborn sickle cell disease screening are obtained. A maternal medical history is obtained, and a six month follow-up of the baby is requested. A nucleated cell count, a CD34+ cell count, and a colony forming unit assay are performed on cells from the unit. Units are cryopreserved using a solution of 10% dimethyl sulfoxide (DMSO) and 1% dextran. Cryopreserved CBUs are permanently stored in liquid phase of liquid nitrogen. Small aliquots for additional testing and unit identification are also frozen. The collection bag, cryobag, test samples, and data forms are labeled with a study bar code. When selected for transplant, the unit is shipped to the transplant center using an express carrier. It is thawed in the laboratory at 37°C, washed with 10% dextran 40 and 5% human albumin to remove cryoprotectant, resuspended in fresh 10% dextran 40 and 5% human albumin, and infused into a patient who has received an appropriate conditioning regimen.

### Pharmacological/Toxicological Effects

In early studies, there were reports of reactions to the cryoprotectant used for freezing the cord blood unit. A change in the thawing procedure (22) has reduced or eliminated that problem and improved viability of the infused cells. Reported graft versus host disease, even in patients who received two and three HLA antigen mismatched cord blood units, appears to have been less than would have been expected with marrow from an unrelated donor (16).

## **Pharmacokinetics**

The median time to engraftment (ANC >500 on the first of three days) for patients who receive cord blood stem cell transplants has been reported to vary from 17 to 26 days (17). Hematopoietic recovery may have been related to the use of growth factors in this study. Others have suggested it may also be related to cell dose per kg of patient weight, and perhaps to other unidentified factors. Platelet engraftment is significantly delayed in recipients of cord blood compared to other types of stem cell transplants (median time to platelets >50,000 = 67 days in the Wagner study and 82 days in the Kurtzberg study).

## **Safety and Effectiveness**

Nearly 375 unrelated donor UCB transplants have been performed at Duke University and University of Minnesota. In July 2000 (21), a detailed analysis of their combined data sets was performed to determine the potential influence of various factors (e.g., graft cell dose and donor/recipient HLA disparity) on rate of hematopoietic recovery and probabilities of engraftment, acute GVHD, chronic GVHD, non relapse mortality, relapse and overall survival. In comparison to prior reports on unrelated donor UCB transplantation, the present study benefits from standardized HLA typing with high resolution typing of HLA-DR, greater homogeneity in supportive care treatments between two centers, and the ability to internally verify data accuracy.

The results from the analysis demonstrated that cryopreserved UCB from HLA 0-3 antigen mismatched unrelated donors contains sufficient numbers of transplantable hematopoietic stem and progenitor cells for most small patients. The data presented indicated that the probabilities of grade III-IV acute GVHD and extensive chronic GVHD are low. Please see the reference list for complete citation and additional studies.

## **Risks and Toxicities**

Recipients of cord blood transplants, like recipients of allogeneic marrow transplants, incur risks from pre-transplant conditioning and graft versus host disease (GVHD) prophylaxis which must be weighed against the risk of malignancy or other disease for which they are receiving a transplant. Compared to other forms of transplantation, the following risks may be increased in recipients of cord blood.

1. Failure to engraft or secondary graft failure can occur. It appears that both white cell and platelet engraftment are slower compared to other sources of stem cells. Graft failure is of special concern in larger patients. Additional stem cells will not be available from the same donor to treat graft failure.
2. Relapse of the underlying disease may occur, especially in patients with advanced disease at the time of transplant. Because of the naive nature of the cord blood cells, relapse may be an increased problem in this kind of transplant. Additional stem cells will not be available from the same donor to treat graft failure.
3. GVHD may be increased compared to marrow transplants, especially in patients with two or three HLA antigen disparity.

4. Infections can be life threatening in the transplant patient population, especially patients receiving immunosuppressive therapy for GVHD.
5. Unknown toxicity from residual cryoprotectant or other agents in the cord blood infusion is a theoretical possibility.

Despite these potential risks and toxicities, the published data to date suggest that cord blood transplants are an acceptable alternative to other forms of stem cell transplantation and prompted this study.

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