STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-SOP-028

DOCUMENT TITLE:
Procedure for Manually Thawing Umbilical Cord Blood Units Frozen in Two Compartment Bags Using Dextran-Albumin Solution

DOCUMENT NOTES:
FACT # 6D.720

Document Information

Revision: 07	**Vault:** STCL-Processing-rel
Status: Release	**Document Type:** STCL

Date Information

Creation Date: 08 Oct 2021	**Release Date:** 18 Mar 2022
Effective Date: 18 Mar 2022	**Expiration Date:**

Control Information

Author: WATER002	**Owner:** WATER002
Previous Number: STCL-SOP-028 Rev 06	**Change Number:** STCL-CCR-522, STCL-CCR-5

STCL-SOP-028
PROCEDURE FOR MANUALLY THAWING UMBILICAL CORD BLOOD UNITS FROZEN IN TWO COMPARTMENT BAGS USING DEXTRAN-ALBUMIN SOLUTION

1 PURPOSE

1.1 To maximize viable cell recovery, cryopreserved cord blood units are rapidly thawed in a 37°C water bath. The slushy content is then slowly and gently diluted with a hypertonic solution containing 10% Dextran and 5% human albumin.

1.2 The hypertonic dextran/albumin solution buffers against the hypertonic intracellular environment created by DMSO and prevents influx of fluid into the cell. Albumin absorbs the intracellular Dimethyl Sulfoxide (DMSO) improving significantly the post thaw viability. Centrifugation helps in the removal of the solubilized DMSO, free hemoglobin and the majority of cell debris.

2 INTRODUCTION

2.1 Umbilical cord blood units are cryopreserved in a solution containing 10% Dimethyl Sulfoxide (DMSO) and 1% Dextran. Stem cells cryopreserved in DMSO have limited viability upon thawing, resulting in the potential for significant loss of cells available for transplantation.

2.2 DMSO, the cryoprotectant of choice, has cytotoxic effects when warmed to 37°C. Immediately upon thawing, intracellular DMSO creates a hypertonic intracellular environment which leads to sudden fluid shifts that compromise cell viability. In addition, DMSO causes adverse side effects in vivo after reinfusion, including blood pressure instability, fever, chills, and nausea. Lysis of red blood cells leads to accumulation of free hemoglobin that can be nephrotoxic when infused intravenously.

2.3 Mixing the thawed cells with a hypertonic solution, immediately upon thawing can ameliorate many of these problems. Typically, the hypertonic thawing solution contains 5% human albumin and 10% Dextran 40 in 0.9% sodium chloride solution. Dextran-Albumin thawing solution helps to restore the osmolarity of the blood cell suspension, promoting colloidal-osmotic intracellular equilibrium. Cell suspensions can then be washed to remove DMSO, free hemoglobin and other cellular products, thus allowing other procedures to be performed prior to reinfusion.

3 SCOPE AND RESPONSIBILITES

3.1 This procedure describes in detail the use of a hypertonic solution of Dextran and human albumin for thawing cryopreserved cord blood units.

3.2 The procedure covers all required steps for the application of the methodology. It starts with the initial preparations and continues from the time the selected unit is removed from the storage Dewar until the product is ready for patient infusion

3.3 Cord blood units are stored in double compartment cryopreservation bags, which enable working with the compartments independently if required. (**Figure 1**)

Figure 1

4 DEFINITIONS/ACRONYMS

4.1	DMSO	Dimcthyl sulfoxidc
4.2	BSC	Biological Safety Cabinet
4.3	CBB	Cord Blood Bank
4.4	NMDP	National Marrow Donor Program
4.5	mL	mililiter
4.6	C	celsius
4.7	USP	United States Pharmacopeia
4.8	ISBT	International Society of Blood Transfusion
4.9	LN2	liquid nitrogen
4.10	rpms	revolutions per minute
4.11	STCL	Stem Cell Laboratory
4.12	QC	quality control
4.13	DNA	deoxyribonucleic acid
4.14	DAT	dextran albumin thaw

5 MATERIALS

5.1 Albumin (human) USP 25% solution (0.25g/ml)

5.2 Dextran 40 in Sodium Chloride Injection, USP (10% Low Molecular Weight Dextran in 0.9% NaCl injection) (*ie Pfizer, BioLife Solutions, or equivalent*)

5.3 Trypan Blue vital stain at 0.4% solution, sterile filtered (or equivalent vital stain)

5.4 Aerobic & anaerobic culture bottles (BacT alert)

5.5 0.9% Sodium Chloride Solution (Saline)

5.6 Cell wash/infusion bag set (Pall Medical Cell Wash/Infusion Bag Set or equivalent)

5.7 Transfer Freezing Bag Set (Pall Medical Transfer Freezing Bag Set or equivalent)

5.8 150 mL transfer pack

5.9 300 mL transfer pack

5.10 Sterile disposable syringes: 3, 20, 30 & 60mL

5.11 16 gauge injection needles

5.12 Alcohol cleaned scissors or sterilized

5.13 5 mL sterile culture tubes (snap cap)

5.14 5mL polystyrene tubes

5.15 Cryogenic vials

5.16 Alcohol prep pads

5.17 ChloraPrep® SEPP (sterile) or equivalent disinfectant

5.18 Sterile (7x8 in.) zip-lock bags (or equivalent)

5.19 Hemostats (optional)

5.20 Gloves

5.21 Protective freezer gloves

5.22 Insul-ice mats *(if needed)*

5.23 250 mL Sorvall centrifuge insert

5.24 100 mL burette hemoset filter (or equivalent syringe filter), 150-260 microns

5.25 2 Gang, 3-Way Stopcocks with MLL or equivalent

5.26 Intralock Lipid compatible 3-way stopcock or equivalent

5.27 Clamp, Rubber Shod

5.28 60 inch extension set APV2.4 mL or equivalent

5.29 Neutral displacement needless connector (ICU Medical MicroCLAVE Endcap) or equivalent

5.30 parafilm

6 EQUIPMENT

6.1 Class II Laminar flow hood/Biological Safety Cabinet

6.2 Refrigerated centrifuge

6.3 Plasma extractor

6.4 Scale

6.5 Sterile tubing welder device

6.6 Tube heat sealer for PVC plastic

6.7 Automated cell counter

6.8 Optical microscope

6.9 Vortex mixer

6.10 Waterbath (4 liters or more at 37°C)

6.11 Thermogenesis canister opener

6.12 Refrigerator

6.13 Validated transport container

6.14 Tube Rack

6.15 Liquid Nitrogen (LN2) freezer

7 SAFETY

7.1 Wear all appropriate personal protective equipment when handling potentially infectious blood or body fluids to include, but not limited to, gloves, lab coats, goggles, face shields, sleeve covers, etc.

8 PROCEDURE

8.1 Procedure Notes

8.1.1 Use aseptic technique in a biological safety cabinet for all processing steps, including all open-container processing and all spiking of blood bags.

8.1.2 Allow only sterile materials to come in contact with the cellular product.

8.1.3 Record the manufacturer, lot number and expiration date (if applicable) of all reagents and disposables.

8.1.4 Assemble all materials before thawing the cryopreserved product.

8.1.5 Treat the thawed cell suspension very gently. The cell membranes are fragile and the cells are lysed easily.

8.1.6 Dextran- albumin solution is to be added slowly so that the DMSO is gradually diluted, then removed.

8.1.7 The infusion time should be set up in advance with the transplant coordinator and the start time for this thawing procedure should be adjusted accordingly.

8.1.8 Verify that the water bath is full and the temperature is 37°C.

8.1.9 Verify that the refrigerated centrifuge is between 2-10°C.

8.2 Prepare Dextran 40/Albumin Solution (Thawing Solution)

8.2.1 Use sterile technique to connect an empty 300 mL transfer bag to a 500 mL bag of Dextran 40 solution.

8.2.2 Place the empty transfer bag on the scale and tare the scale.

8.2.3 Transfer 150 grams of pre-cooled Dextran 40 solution to the 300 mL transfer bag.

8.2.4 Heat seal tubing and detach Dextran 40 solution from the 300 mL transfer bag by cutting tubing at the sealed point leaving sufficient tubing length for sterile welding later.

8.2.5 Working in the biological safety cabinet, insert a sampling site coupler into one of the ports of the 300 mL transfer bag containing Dextran 40 solution.

8.2.6 Clean the rubber stopper of the human albumin bottle with alcohol wipes.

8.2.7 Draw up 30 mL of 25% human albumin.

8.2.8 Clean coupler port with alcohol and inject 30 mL of human albumin into the transfer bag containing Dextran 40 solution.

NOTE: The transfer bag now contains a total of approximately 180 mL of solution containing both Dextran-40 and human albumin and will be referred to as the Dextran40/Albumin bag in subsequent steps. The solution in this bag (with a final concentration of 8.3% Dextran-40/4.2% albumin) will be referred to as the thawing solution.

8.3 Assemble the Closed System

8.3.1 Clamp all tubing and place assigned ISBT 128 labels on the "cell wash/infusion bag set" (**Figure 2**).

(NOTE: The Cell Wash /Infusion bag will be referred to as Infusion Bag in subsequent steps).

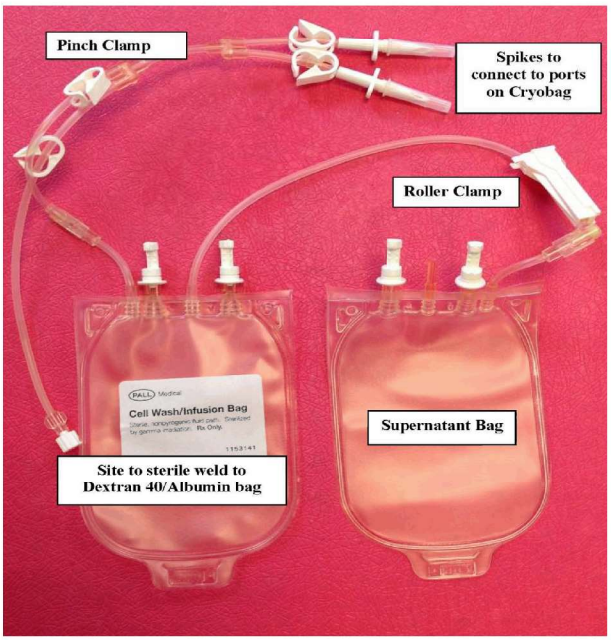
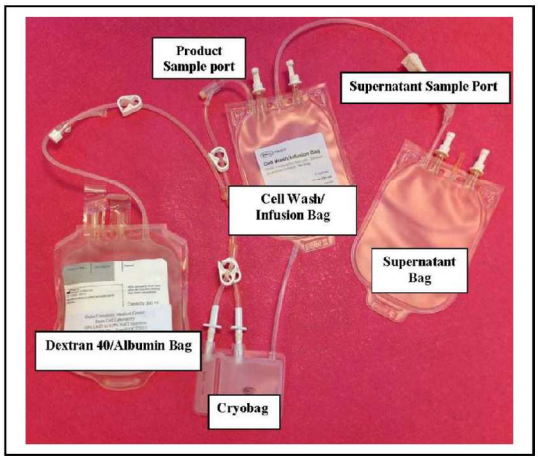
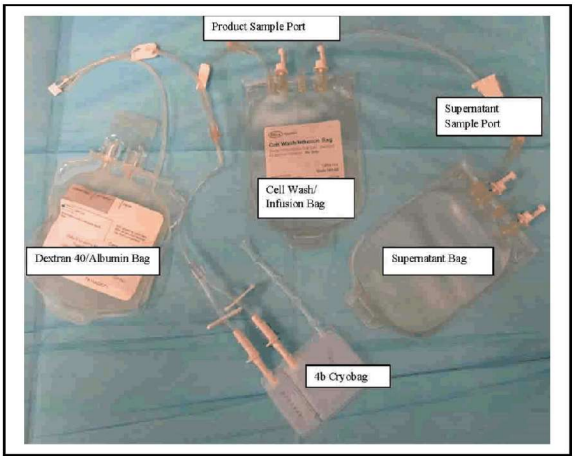


Figure 2
Cell Wash / Infusion Set

8.3.2 Sterile weld wash/infusion set to Dextran-40/Albumin bag (**Figure 3**).



or



8.3.3 Place the infusion bag on the scale (**Figure 4**).

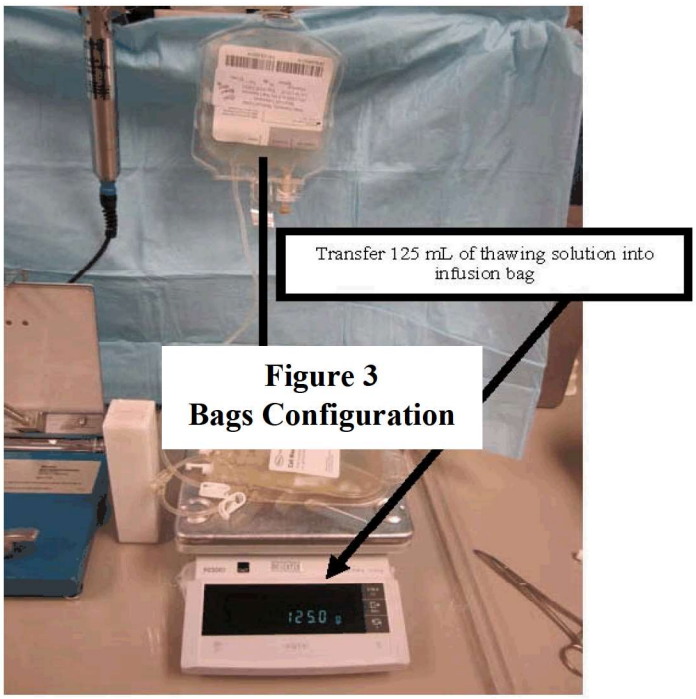


Figure 3
Bags Configuration

Figure 4

8.3.4 Tare scale and transfer 125 grams (125 mL) of thawing solution from the Dextran-40 / Albumin bag into the infusion bag (**Figure 4**).

8.3.5 Clamp off tubing between the Dextran-40/Albumin bag and the infusion bag, using pinch clamp provided with the set.

8.3.6 Wrap the infusion bag with an ice mat.

8.3.7 Place the Cell/Wash infusion set, joined to the Dextran-40 / Albumin bag containing thawing solution inside the hood (**Figure 5**).

8.3.8 Elevate (if desired) the Dextran-40/Albumin bag to facilitate flow (**Figure 5**).

Figure 5

8.4 Assemble materials and equipment

8.4.1 Prepare and label all site-specific forms, tubes, and bacterial culture bottles.

8.4.2 Assemble all materials needed in the biological safety cabinet before thawing. Leave one ice mat ready for the cryobag after thawing.

8.4.3 Place supplies, as needed for the procedure, inside the biological safety cabinet:

8.4.3.1 tube rack,

8.4.3.2 test tubes (2 mL cryogenic vials, 5 mL polystyrene tubes, 5 mL sterile culture tubes with snap caps),

8.4.3.3 syringes,

8.4.3.4 alcohol swabs and/or ChoraPrep® SEPP applicators,

8.4.3.5 disinfected scissors,

8.4.3.6 sampling site coupler,

8.4.3.7 vortex (as appropriate)

8.5 Cord blood Unit thawing

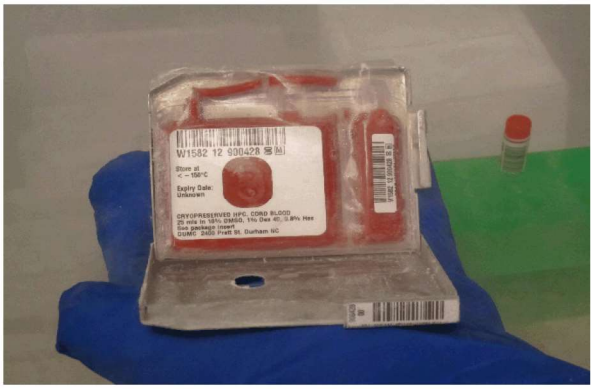
8.5.1 Working in the vapor phase of the LN2 tank, remove unit from metal cassette.

8.5.2 Confirm, with a second technologist or designee, the ISBT barcode on the cord blood unit. Document the label confirmation by both individuals.

8.5.3 Remove plastic overwrap, if present. (**Figure 6a**)

8.5.4 Visually inspect the cryobag for damage or cracks.

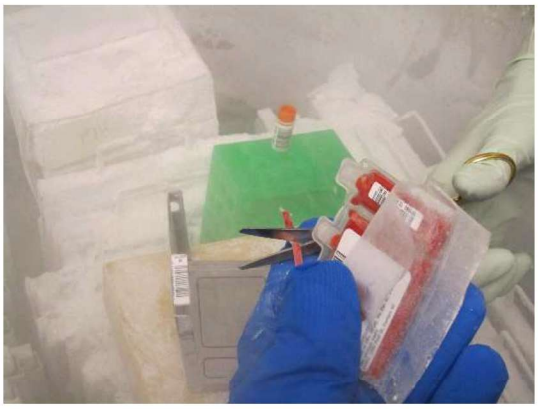
8.5.5 Cut the cryobag segments, if present. (**Figure 6b**).



MedSep bag

Figure 6a

BioSafe 4B bag



MedSep bag

Figure 6b

BioSafe 4B bag

8.5.6 Place the segments in a cryovial labeled with recipient and/or donor information, unit number, date, and product type.

8.5.7 Keep cryovial in vapor phase until assigning the storage location in designated LN2 freezer.

8.5.8 Spray the outside surface of the cryobag with alcohol and place the cryobag inside a sterilized zip-lock bag; remove the air and then seal the bag.

8.5.9 Confirm and document, with a second technologist or designee, that the label on the cryobag matches the label on the accompanying paperwork.

8.5.10 Thaw the unit in a 37°C water bath until the product reaches a slushy/liquid consistency. This generally takes approximately 1-3 minutes.

8.5.11 Treat the thawed cell suspension very gently. The cell membranes are fragile and the cells are lysed easily.

8.5.12 Dry the outside of the zip-lock bag containing the slushy cryobag and wipe with alcohol before placing in the biological safety cabinet.

8.6 Cord blood dilution

8.6.1 Remove the cryobag from zip-lock bag.

8.6.2 Clean the outside of the port covers with ChloroPrep® SEPP.

8.6.3 Cut both port covers with sterile or disinfected scissors.

8.6.4 Clean cut surfaces with ChloroPrep® SEPP applicators followed by alcohol prep as shown in **Figure 6c**.

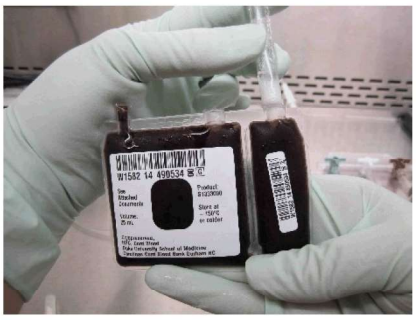


Figure 6c

8.6.5 Allow the cut surfaces to air dry.

8.6.6 Insert the spikes of the infusion set in the dry and disinfected ports (one at the time).

8.6.7 Wrap the cryobag with an ice mat.

8.6.8 Unclamp tubing between infusion and cryobag.

8.6.9 Transfer cold thawing solution (**Figure 7a**) from the infusion bag into the cryobag slowly over approximately 1 to 2 minutes until both compartments of the cryobag bulge (as shown in **Figure 7b**).

(**NOTE**: It is important to add the thawing solution slowly over 1-2 minutes so that the DMSO in the product is gradually diluted).

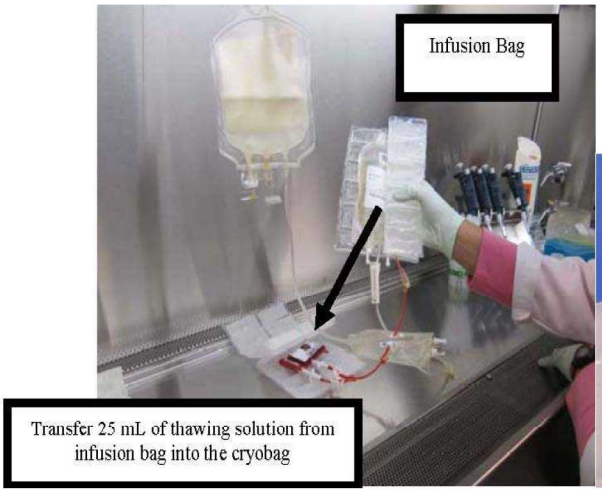


Figure 7a

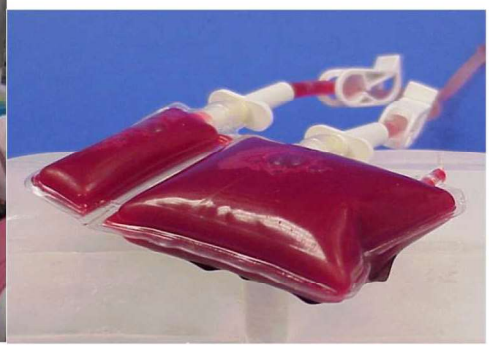


Figure 7b

8.6.10 Gently mix by hand the incoming thawing solution and slushy product during transfer.

8.6.11 Gently rock the cryobag for 1-3 minutes by hand for complete homogenization of its contents.

8.6.12 Elevate the cryobag to gradually transfer the diluted cell suspension from the cryobag into the infusion bag which is wrapped in an ice mat to keep the product cool.

(**NOTE**: At this point in the procedure, the infusion bag contains 50 mLs of material from the cryobag (25 mL product + 25 mL thawing solution) mixed with the remaining 100 mL of thawing solution in the infusion bag for a total of approximately 150 mL.

8.6.13 Mix fluids during transfer by moving bags up and down as shown in **Figure 8**.

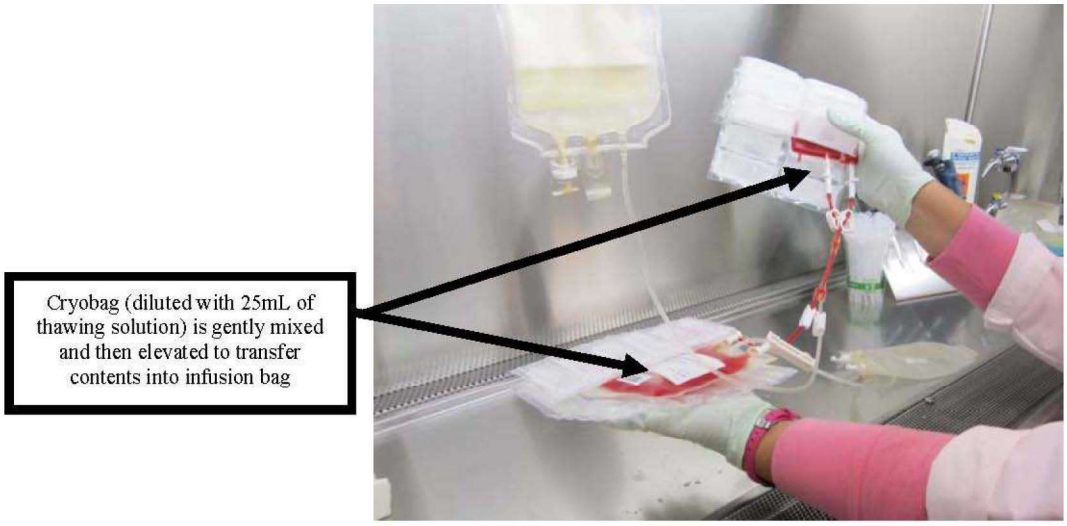


Figure 8

8.6.14 Leave the remaining residual fluid and cells in the cryobag at this time.

8.6.15 Gently rock the infusion bag by hand for 1-2 minutes to allow complete mixing.

8.6.16 Clamp the lines between the cryobag and the infusion bag in preparation for the rinsing process.

8.7 First Rinse of the Cryobag

8.7.1 Unclamp tubing between the Dextran-40 /Albumin bag and the cryobag.

8.7.2 Add approximately 25mL of thawing solution from the Dextran-40/Albumin bag to both compartments of the cryobag.

(**NOTE**: A total of 150 mL of thawing solution has been used to mix with the product at the completion of this step).

8.7.3 Clamp tubing between the Dextran-40/Albumin bag and the cryobag after transfer.

8.7.4 Apply gentle pressure and massage the cryobag to dislodge all remaining cells.

8.7.5 Swirl the thawing solution around the inside of the cryobag to resuspend and harvest all remaining cells.

8.7.6 Open clamp between cryobag and the infusion bag.

8.7.7 Elevate cryobag to allow the thawing solution and cells to flow into the infusion bag.

8.7.8 Gently mix the fluids during transfer by rocking the infusion bag by hand.

8.7.9 Compress and roll the cryobag to remove all the remaining cell suspension.

8.7.10 Drain all of the solution from the cryobag into the infusion bag.

8.7.11 Clamp off the line from the infusion bag in preparation for the second rinsing.

8.8 Second Rinse of the Cryobag

8.8.1 Again, unclamp the tubing between the Dextran-40/Albumin bag and the empty cryobag.

8.8.2 Add approximately 25mL of thawing solution from the Dextran-40/Albumin bag to both compartments of the empty cryobag.

(**NOTE**: A total of 175 mL of thawing solution has been used to mix with the product at the completion of this step).

8.8.3 Repeat steps 8.7.1 to 8.7.10 of the first rinse of the cryobag procedure above.

8.8.4 Drain any remaining thawing solution from the Dextran-40/Albumin bag into the infusion bag.

(**NOTE**: At this point in the procedure, the product and thawing solutions are mixed together in the infusion bag for a total volume of approximately 200 to 205 mL).

8.8.5 Heat seal tubing between the infusion bag and the cryobag then detach the cryobag by cutting at the sealed point.

8.8.6 Remove the unit identifying label from the cryobag, place it on the paperwork, and discard empty cryobag.

8.8.7 Heat seal tubing between the infusion bag and the Dextran-40/Albumin bag then detach the infusion bag by cutting at the sealed point.

(NOTE: The volume of the diluted product should be close to 200mL. A volume higher than 225mL may cause bag breakage).

8.8.8 If the product is going to be administered in the diluted state (ie. without further washing):

8.8.8.1 Label the infusion bag with patient and donor information per internal labeling procedure.

8.8.8.2 Attach a tie tag containing the recipient and donor demographic information to the infusion bag.

8.8.8.3 Take a 0.9 mL sample for quality control testing (see **section 8.13**).

8.8.8.4 Remove an additional 2.0 mL sample for sterility testing.

8.8.8.5 Transport diluted product to the site of administration to the recipient in a validated transport container.

8.8.8.6 Infuse product within one hour of thawing.

8.8.8.7 Infuse product according to institutional nursing administration procedures. **Do NOT use leukoreduction filters**.

8.9 Cord Blood Unit Wash

8.9.1 Centrifugation of thawed/dilute product

8.9.1.1 At this point the infusion bag remains attached, by tubing, to the supernatant bag.

8.9.1.2 Check to ensure that all clamps are closed.

8.9.1.3 Place the infusion bag inside a sterile zip lock bag.

8.9.1.4 Place the infusion bag in 250 mL centrifuge adapter (see **Figure 9**).

8.9.1.5 Arrange the insert and the supernatant bag inside the centrifuge bucket with the supernatant bag placed between the adapter and the inner wall of the centrifuge bucket (see **Figure 10**).

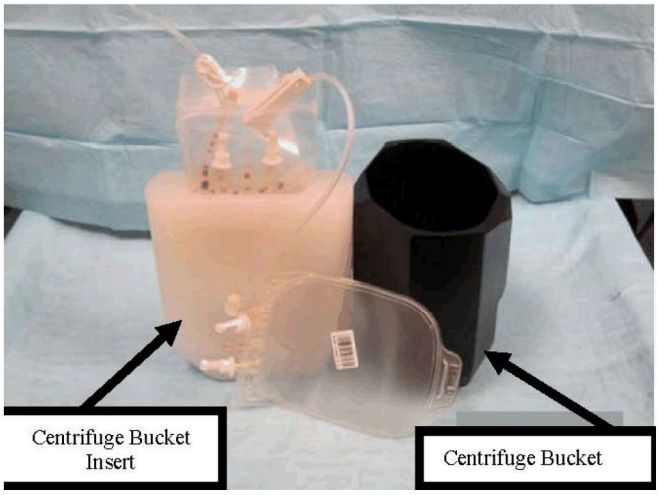


Figure 9

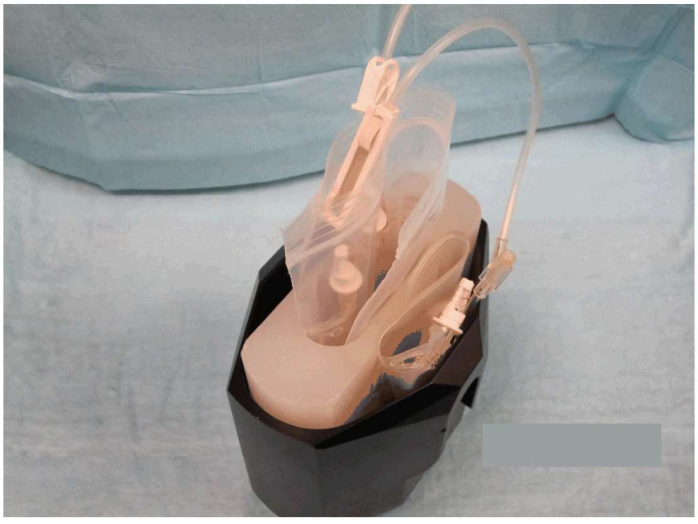


Figure 10

8.9.2 Tape tubing inside the bucket as shown in **Figure 11**.

Figure 11

8.9.3 Balance the centrifuge buckets before starting the centrifuge.

8.9.4 Pellet the cells via centrifugation at 1800 rpms for 20 minutes at 2-8°C.

8.10 Removal of the Supernatant:

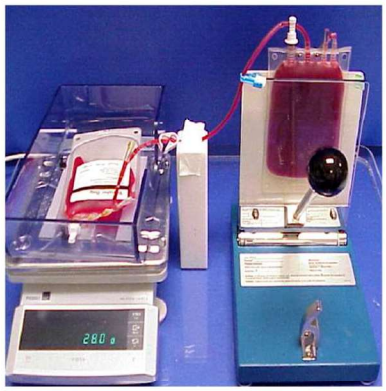


Figure 12

8.10.1 Remove the infusion bag attached to the supernatant bag from the centrifuge bucket.

8.10.2 Place the centrifuged infusion bag in the plasma expressor as shown in **Figure 12**.

8.10.3 Place the transfer bag on the scale as shown in **Figure 12.**

8.10.4 Tare the scale.

8.10.5 Open the tubing clamp between the two bags.

8.10.6 Transfer most of the supernatant to the supernatant bag after leaving a volume of product appropriate for the recipient's weight as determined by the transplant team (see JA1 Thawing Job Aide for reference), while taking into account the volume of product being recovered in **step 8.11** below.

(**NOTE**: If cells are being prepared for transplant in a SYRINGE, the final infusion volume must not exceed 60mL).

8.10.7 Be sure that the cell pellet is not disrupted during transfer of the supernatant.

8.10.8 Empty the tubing between the bags by transferring the air from the supernatant bag to the infusion bag.

8.10.9 Clamp the tubing to the infusion bag and remove the supernatant bag from the scale.

8.10.10 Place the infusion bag on the scale.

8.10.11 Record the displayed weight on appropriate worksheet.

8.10.12 Heat seal the tubing connecting the infusion bag and the supernatant bag and detach the supernatant bag.

8.10.13 Gently mix the cellular contents of the infusion bag. Keep the infusion bag in an ice mat when completing subsequent steps.

(**NOTE**: The cell suspension after the completion of this step will be referred to as ***cell suspension #1***).

8.10.14 Remove a 0.2mL sample of cell suspension # 1 for cell count and viability testing using sterile technique in the BSC.

8.10.15 Perform the cell count.

8.11 Recovery of remaining cells

8.11.1 Place supernatant bag inside a sterile zip lock bag and then inside the centrifuge insert.

8.11.2 Please the centrifuge insert and the bags in the centrifuge bucket.

8.11.3 Balance the buckets.

8.11.4 Pellet the cells at 1800 rpms for 20 minutes at 2-10°C.

8.11.5 Close roller clamp (if applicable) on the supernatant bag before sterile welding.

8.11.6 Sterile weld the supernatant bag to a transfer/freezing bag set.

8.11.7 Place the supernatant bag containing the pelleted cells, which is now attached to the transfer bag, on the plasma expressor.

8.11.8 Open the roller clamp (if applicable) between the supernatant bag and the transfer bag.

8.11.9 Express the supernatant from the supernatant bag into the transfer bag without disrupting the pellet leaving a volume appropriate to add to the infusion bag that will not exceed the final volume requested by the transplant team (refer to Thawing Job Aide).

8.11.10 Gently mix the cellular content of the supernatant bag after expression of supernatant and then withdraw the contents into a 20 or 30 mL syringe using the supernatant sample port.

(**NOTE**: The cell suspension at the completion of this step will be referred to as ***cell suspension # 2*** *if 3rd spin is necessary*).

8.11.11 Measure the volume in the syringe.

8.11.12 Transfer approximately 0.2 mL of sample of cell suspension # 2 from the syringe into a 5 mL test tube for a cell count using sterile technique in the BSC. Mix sample adequately before testing.

8.11.13 Perform the cell count.

8.11.14 Inject cell suspension # 2 into the product sample port on the tubing attached to the infusion bag containing cell suspension # 1 to combine to two cell suspensions. Refer to **Figure 3**.

8.11.15 Determine the combined volume and/or weight of the final product.

8.11.16 Proceed to the next section for transplantation preparation steps.

8.12 Preparation of cells for transplantation:

8.12.1 BAG METHOD

8.12.1.1 Remove 0.9mL aliquot for QC testing from the product sample port (see **Figure 3**) on the tubing attached to the infusion bag using sterile technique in the BSC. Mix sample adequately before testing. This QC sample is used to perform viability, total nucleated cell count, and other testing, as recommended. See **section 8.13** of the procedure for quality control testing.

8.12.1.2 Calculate the viable cell recovery the following formula:

$$\frac{\text{Total Viable nucleated UCB cells in the infusion-ready product}}{\text{Total viable nucleated cells of cryopreserved unit (provided on CBB/ NMDP documents)}}$$

8.12.1.3 Record all values on the designated laboratory thawing form.

8.12.1.4 Label the infusion bag with recipient and donor information per institutional labeling procedures.

8.12.1.5 Attach a tie tag containing the recipient and donor demographic information to the infusion bag.

8.12.1.6 Prepare a saline wash bag by transferring 20-100 mL of 0.9% sodium chloride solution into a 150 mL transfer bag with extension tubing. This saline solution will be used to wash the infusion bag after the product has been infused into the recipient.

8.12.1.7 Sterile weld the infusion bag to the saline wash bag (**see Figure 13**).

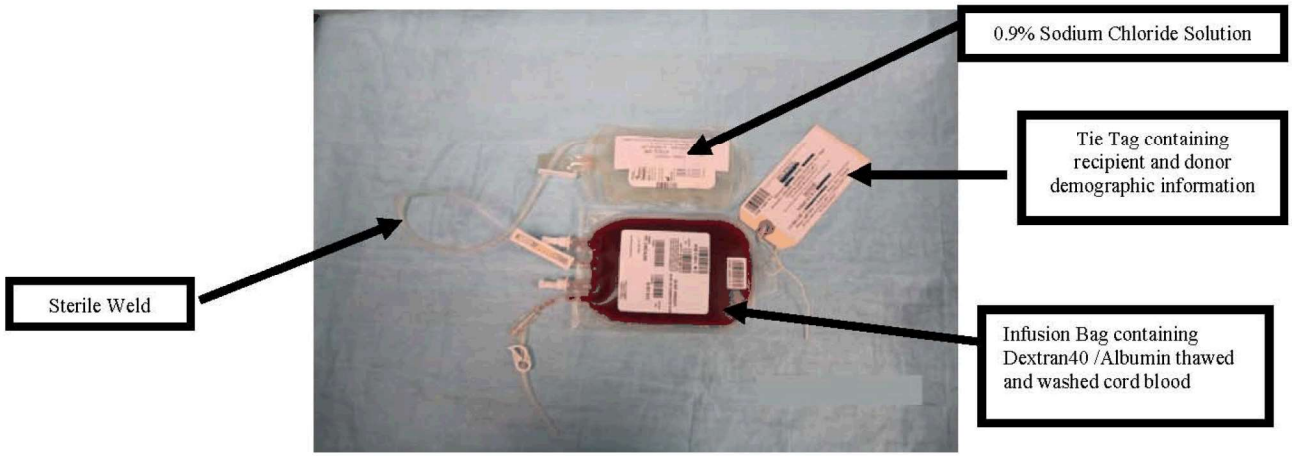


Figure 13

8.12.1.8 Clamp the tubing between the infusion bag and the saline wash bag using the clamp on the tubing connected to the infusion bag.

8.12.1.9 Complete any other appropriate laboratory forms.

8.12.1.10 Confirm and document, with a second technologist or designee, the demand 128 label, ISBT barcode affixed on, and the tie tag attached to the infusion bag for accuracy.

8.12.1.11 Transport product to the site of administration to the recipient in a validated transport container.

8.12.1.12 Infuse the product within four (4) hours of thawing.

8.12.1.13 Infuse product according to institutional nursing administration procedures. **Do NOT use leukoreduction filters**.

8.12.2 SYRINGE Method for pediatric recipient or recipients with fluid restrictions.

8.12.2.1 Remove filter set from its packaging

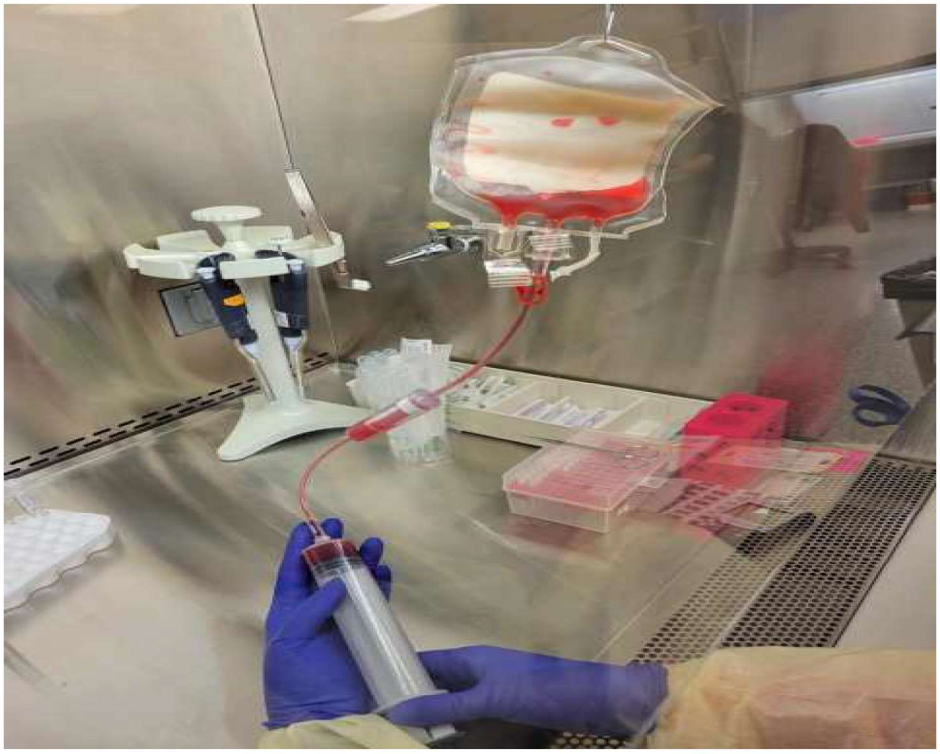


Figure 14

8.12.2.2 Insert the spike from the filter set into one of the ports of the infusion bag.

8.12.2.3 Mix and pull the cellular product into the 60 mL syringe so the entire volume can be accurately measured (see **Figure 14**).

8.12.2.4 Post filtering product, remove 0.5 mL of product to be used for QC testing. This QC sample is used to perform viability and a total nucleated cell count (refer to **section 8.13** of the procedure for quality control testing).

8.12.2.5 Detach the 60 mL syringe containing the cellular product and attach it to the lipid-compatible 3-way stopcock, the neutral displacement needless connector, and the 60 inch 2.4 mL extension set tubing. See **Figure 15**.

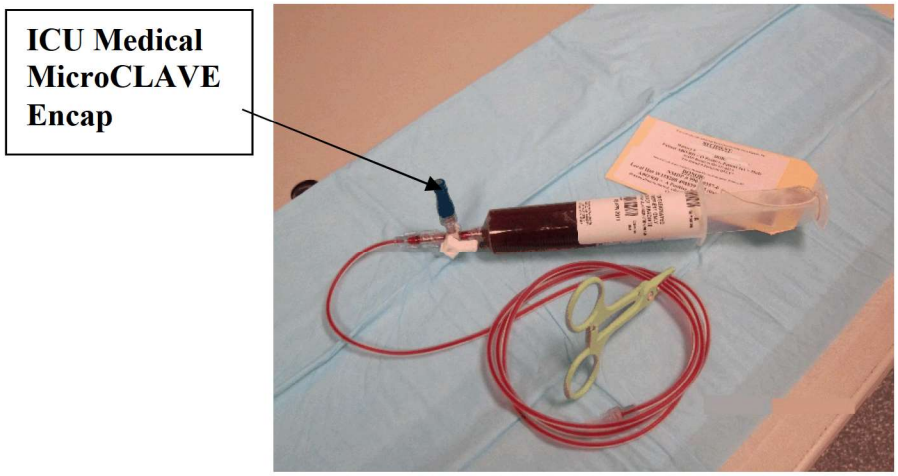


Figure 15

8.12.2.6 Prime the extension set tubing with cellular product. Be sure to place the stopcock in the OFF position after the tubing has been primed.

8.12.2.7 Attach rubber shod clamp to the tubing to ensure there is no leakage.

8.12.2.8 Place parafilm on the tubing cap at the end of the extension tubing to prevent it from becoming detached.

(NOTE: If product is being distributed to the Children's Health Center (CHC), a second stopcock is attached to the tubing cap).

8.12.2.9 Viable cell recovery values are calculated using the following formula:

$$\frac{\text{Total Viable nucleated UCB cells in the infusion-ready product}}{\text{Total viable nucleated cells of cryopreserved unit (provided on CBB/ NMDP documents)}}$$

8.12.2.10 Record all values on the designated laboratory thawing form. If TNC recovery is <70%, notify medical director and/or designee.

8.12.2.11 Label the syringe with recipient and donor information per institutional labeling procedures.

8.12.2.12 Attach a tie tag containing the recipient and donor demographic information to the syringe.

8.12.2.13 Complete any other appropriate laboratory forms.

8.12.2.14 Confirm and document, with a second technologist or designee, the demand 128 label, ISBT barcode affixed on, and the tie tag attached to the syringe for accuracy.

8.12.2.15 Transport product to the site of administration to the recipient in a validated transport container.

8.12.2.16 Infuse the product within four (4) hours of thawing.

8.12.2.17 Infuse product according to institutional nursing administration procedures. **Do NOT use leukoreduction filters.**

8.13 Quality Control Tests

8.13.1 Cell Counts: performed on final product to be infused and cells for freezing from the supernatant bag.

8.13.2 Viability test: performed on cell suspension #1 and on the final product. If viability is <70%, notify medical director and/or designee.

8.13.3 Colony assay for CFU-GM, GEMM, and BFU-E: performed on final product to be infused.

8.13.4 Viable CD34+ determination by flow analysis, along with CD 3/4/8 (if allogenic product) performed on the final product.

8.13.5 Sterility Test: performed on 10mL of supernatant from the final wash (5 mL for aerobic and 5 mL for anaerobic culture bottles).

8.13.6 RFLP or equivalent DNA-based identity test: performed on approximately 1 x 10e6 cells (0.2mL) from the final product, upon request.

8.14 Emergency Product Recovery in the Event of a Container Failure

8.14.1 If the bag is compromised at any point during this procedure, move further handling into biological safety cabinet.

8.14.2 It is the transplant physician's (or designee's) responsibility to determine whether the product will be used or discarded if the container is compromised at any step of the procedure.

8.14.3 If the transplant physician (or designee) decides that the product should be used, recovery of the product may be attempted.

8.14.4 Take pictures, when applicable, of any container failures.

8.14.5 Complete Non-Conforming Product form when applicable.

8.15 Products returned from issue

8.15.1 Products returned to the STCL due to clumping, bag punctures, etc. should be documented using the HPC Return from Issue Form. Capture all pertinent information on the form and contact medical director and/or designee to get instructions regarding salvage or disposal.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-FORM-056 Cellular Therapy Infusion Request Form

9.2 STCL-FORM-043 Thawing and Infusion Worksheet

9.3 STCL-FORM-050 Processing Lot Numbers - CBU Processing

9.4 STCL-SOP-028 JA1 Thawing Job Aid

9.5 STCL-SOP-028 JA2 Dextran Albumin Thawing Vials and Small Cryobags

9.6 FLOW-GEN-012 FRM1 STCL Flow Cytometry Worksheet

9.7 STCL-PROC-022 FRM1 STCL Clinical HPCA Worksheet

9.8 STCL-PROC-025 Cryopreservation of Back-up Cells from Original UCB Reinfusion

9.9 STCL-QA-007 FRM1 Non-Conforming Product form

9.10 STCL-DIST-001 FRM1 HPC Return from Issue Form

10 REFERENCES

10.1 Kurtzberg J, Graham M, Casey J et al. The use of umbilical cord blood in a mismatched related and unrelated hematopoietic stem cell transplantation. Blood Cells 1994; 20: 275-84.

10.2 Kurtzberg J, Laughin, Graham ML et al. Placental blood as source of hematopoietic stem cells for transplantation in unrelated recipients. N. Engl. J. Med. 1996;335: 157-66

10.3 Wagner JE, Rosental J, Sweetman R, et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: Analysis of engraftment and acute graft-versus-host disease. Blood 1996; 88:795-802.

10.4 Rubinstein P, Carrier C, Scaradavou A, et al. Outcomes among 562 recipients of placental blood transplants from unrelated donors. N. Engl. J. Med. 1998; 1565-77.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
7	B. Waters-Pick	• Section 5.2 - Added sources of Dextran-40 in 0.9% NaCl • Section 5.22 – Add Insul-ice mats *"(if needed)"* • Section 8.2.12.1 – Removed reference to "100 mL burett hemoset and 170-260 microns" • Section 8.4.3.7 – Removed "ice mats" • Section 8.11.9 – Removed NOTE • Section 8.11.10 – Added "*if 3rd spin is necessary*" • Section 8.12.2.2 – Removed "*close the roller clamp on the filter tubing set*" • Section 8.12.2.3 – Removed "*a 3 way stopcock to the end of the 100 mL burette hemoset filter line*" • Section 8.12.2.4 – Removed "*attach 1 3 mL syringe for QC testing and 60 mL syringe to the 3 way stopcock*" • Section 8.12.2.5 - Removed *NOTE* after Figure 14 • Section 8.12.2.7 – Removed "*Adjust the stopcock and use the 3 mL syringe to remove 0.9 mL sample" and replaced with "Post filtering product, remove 0.5 mL*" • Section 8.12.2.8 – Added *NOTE*

		• Section 8.13.1 – Removed "*diluted product (optional), cells suspension #1 & #2 (final product) from sentence*
		• Section 8.24 – Added 100 mL burette hemoset filter (or equivalent *"syringe filter)*", 150-260 microns
		• Made corrections in Section 9 to the document listed
		  ○ Section 9.3 - Corrected title to STCL-FORM-050
		  ○ Section 9.4 – Corrected document # and name
		  ○ Section 9.5 – Added "0" to STCL-028 document name
		  ○ Section 9.6 – Added # to document
		  ○ Section 9.7 – Added # to document

Signature Manifest

Document Number: STCL-SOP-028 **Revision:** 07

Title: Procedure for Manually Thawing Umbilical Cord Blood Units Frozen in Two Compartment Bags Using Dextran-Albumin Solution

Effective Date: 18 Mar 2022

All dates and times are in Eastern Time.

STCL-SOP-028 Procedure for Manually Thawing UBCs Frozen

Author

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		01 Mar 2022, 06:33:56 PM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		01 Mar 2022, 06:34:11 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		02 Mar 2022, 11:05:44 AM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
Isabel Storch De Gracia (IMS19)		04 Mar 2022, 04:28:04 PM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandra Mulligan (MULLI026)		07 Mar 2022, 12:23:00 PM	Approved