STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Procedure or Thawing Bone Marrow and Peripheral Stem Cells Using Dextran-Albumin Solution

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STCL-SOP-048
PROCEDURE FOR THAWING BONE MARROW AND PERIPHERAL STEM CELLS USING DEXTRAN-ALBUMIN SOLUTION

1 PURPOSE

1.1 To maximize viable cell recovery, cryopreserved stem cells are thawed rapidly in a 37°C water bath, and then slowly diluted with a hypertonic solution containing 10% Dextran and 5% human albumin. The removal of the supernatant containing most of the Dimethyl Sulfoxide (DMSO) and cellular debris potentially decreases the occurrence of side effect reactions.

2 INTRODUCTION

2.1 Harvested stem cells are cryopreserved in a solution containing a final concentration of 10% (DMSO). Bone marrow and peripheral stem cells cryopreserved in DMSO have limited viability upon thawing; this results in the potential for significant loss of cells available for transplantation.

2.2 DMSO, the cryoprotectant of choice, may cause adverse side effects after reinfusion, including blood pressure instability, fever, chills and nausea.

2.3 Thawing solution containing dextran-albumin helps to restore the osmolarity of the freshly thawed cells, promoting colloidal-osmotic intracellular equilibrium. Cell suspensions can be washed to remove DMSO, free hemoglobin, and other cellular debris.

2.4 This procedure covers the thawing methodology using a hypertonic solution of 10% dextran, 5% human albumin in 0.9 % normal saline from the time the cryopreservation bag(s) is (are) removed from the freezer until the final product is ready for infusion to the recipient.

3 SCOPE AND RESPONSIBILITIES

3.1 The Medical Directors, Laboratory Manager, and laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met

4 DEFINITIONS/ACRONYMS

4.1	DMSO	Dimethyl Sulfoxide
4.2	USP	United States Pharmacopeia
4.3	ISBT	International Society of Blood Transfusion
4.4	LN2	Liquid nitrogen
4.5	QC	Quality Control
4.6	SOP	Standard Operating Procedure
4.7	G	gauge
4.8	mL	milliliter

4.9	°	degrees
4.10	C	Celsius
4.11	g	grams
4.12	RPM	Revolutions per minute
4.13	RFLP	Restriction fragment length polymorphism
4.14	NaCl	Sodium Chloride

5 MATERIALS

5.1 Specimen

5.1.1 Red blood cell and/or volume reduced bone marrow or peripheral stem cells that have been frozen in a controlled rate freezer. Frozen products are cryopreserved in a liquid or vapor phase of liquid nitrogen at a temperature of -150°C or colder.

5.2 Reagents

5.2.1 Human albumin (human) USP 25% solution

5.2.2 Dextran 40 (10% Gentran 40 and 0.9% NaCl) *(ie. Pfizer, BioLife Solutions)*

5.2.3 0.9% NaCl Solution (Normal Saline)

5.2.4 Trypan Blue at 0.4% solution

5.3 Supplies

5.3.1 Aerobic & anaerobic culture bottles

5.3.2 150 mL transfer pack

5.3.3 300 mL transfer pack

5.3.4 Sterile disposable syringes

5.3.5 Disinfected scissors, if applicable

5.3.6 5 mL sterile culture tubes (snap cap)

5.3.7 5 mL polystyrene tubes

5.3.8 16 G needles (*or equivalent*)

5.3.9 Alcohol prep pads

5.3.10 ChloraPrep® SEPP® applicator (*or equivalent*)

5.3.11 Sterile (7 x 8 inch) Ziploc bags

5.3.12 Hemostats

5.3.13 Regular and protective freezer gloves

5.3.14 Insul-ice mats (if needed)

5.3.15 Sampling site couplers

5.3.16 4-way stop cock

5.3.17 Plasma transfer tubing spike

5.3.18 Sorvall centrifuge insert

6 EQUIPMENT

6.1 Class II Laminar flow hood / Biological Safety Cabinet

6.2 Refrigerated centrifuge

6.3 Plasma extractor

6.4 Balance

6.5 Sterile Welding device

6.6 Tube heat sealer for PVC plastic

6.7 Automated hematology instrument

6.8 Optical microscope

6.9 Vortex mixer

6.10 37° C Water bath

7 SAFETY

7.1 Wear appropriate personal protective equipment when handling any/all potentially hazardous blood and body fluids to include, but not limited to, gloves, goggles, lab coats, sleeve covers, disposable gowns, disposable aprons, etc.

7.2 Personnel should handle ONLY **one cellular product** at any given time in an effort to minimize cross-contamination.

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

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

8.1.6 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

8.2 Procedure Steps

8.2.1 Record the supplies, reagents, and equipment, used during this procedure, on the designated worksheet.

8.2.2 Assemble all materials needed to perform this procedure BEFORE thawing the cryopreserved product.

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

8.2.4 Place the following supplies inside the laminar flow hood: tube rack, sterile snap-cap tubes, test tubes, syringes, alcohol swabs, disinfected scissors (*if applicable*), ice mats, sampling site coupler, and bacterial culture bottles.

8.2.5 Assemble the double stopcock, transfer tubing lines, a 300 mL transfer bag, and a 16 G needle as shown on ***Figure 1***

8.2.6 Using a hemostat, **clamp off** the tubing line attached to the 300 mL transfer bag.

NOTE: **This tubing line will be used later in the procedure**

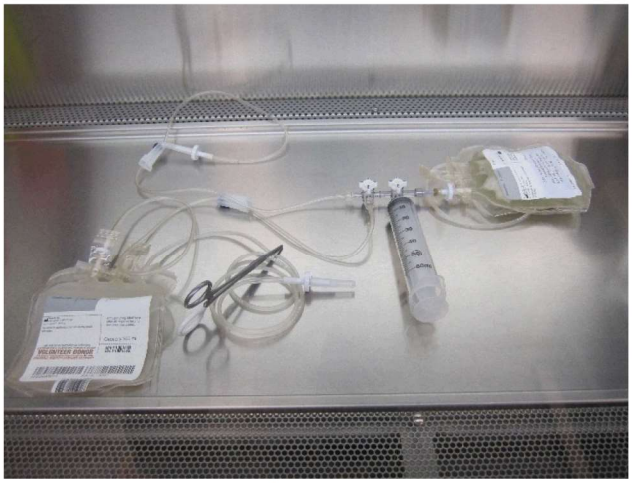

Figure 1

8.3 Preparation of Dextran-Albumin thawing solution *(unless 250 mL pre-filled bag is available from the refrigerator*)

NOTE: (Human albumin at final concentration of 4.2% in 10% Dextran/saline solution.)

8.3.1 Spike a 300 mL transfer bag into a 500 mL bag of Dextran *(that has been removed from the refrigerator)*.

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

8.3.3 Transfer 250 g of Dextran solution to the transfer bag.

8.3.4 Heat seal tubing and detach Dextran bag by cutting tubing at the sealed point.

8.3.5 Working in the laminar flow hood, spike a sampling site coupler into one of the ports of the 300 mL transfer bag containing 250 g of Dextran.

8.3.6 Clean the rubber stopper of a 25% human albumin solution bottle with alcohol prep pad.

8.3.7 Draw up 50 mL of albumin using a 60 mL syringe.

8.3.8 Clean the coupler with an alcohol prep pad (*or equivalent*).

8.3.9 Inject the albumin solution into the Dextran bag.

8.3.10 Mix the contents and place Dextran-Albumin solution in the refrigerator.

NOTE: If thawing only one bag of cells for infusion to the patient, Dextran-Albumin solution can be prepared using 150 g of Dextran and 30 mL of Albumin.

8.4 Preparation and thawing

8.4.1 Spike the Dextran-Albumin bag (pre-cooled) to the 16 G needle in one end of the double stop cock setting

8.4.2 Working in vapor phase of LN2 tank, remove the unit from the metal cassette as shown in ***Figure 2.***

Figure 2

8.4.3 Confirm, with a second technologist or designee, the recipient's name (*and donor's name, if applicable*), medical history number, and ISBT barcode on the bag of cells being prepared for infusion. Both individuals must initial *STCL-FORM-043 Thawing and Infusion Worksheet* indicating that this step was completed.

8.4.4 Place the frozen unit inside a sterilized Ziploc bag.

8.4.5 Thaw the unit in the 37°C water bath until the product reaches a slushy, liquid consistency

8.4.6 Take the product and place inside the biological safety cabinet (hood).

8.4.7 Remove the product from inside the protective Ziploc bag.

8.4.8 Remove the port cover from one of the available ports.

8.4.9 Clean the port with a ChloraPrep® SEPP® applicator.

8.4.10 Spike the available transfer line into the disinfected (cleaned) port.

8.4.11 Fill a 60 mL syringe with dextran-albumin solution.

8.4.12 Slowly add the solution to the thawed product bag as seen in ***Figure 3***.

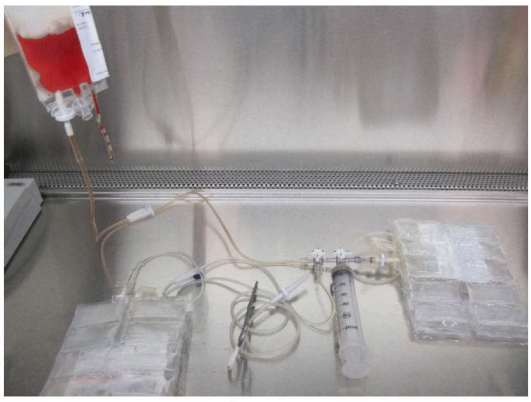

Figure 3

8.4.13 Mix the contents during transfer.

8.4.14 Using the 60 mL syringe and the stopcock, transfer the dilute product to the 300 mL transfer bag (Infusion Bag) labeled with an ISBT barcode and record the volume.

NOTE: **Do not exceed 250 mL/bag (*in a 300 mL transfer bag*) or the cells will not fit into the centrifuge insert.**

8.4.15 Mix the contents well during transfer.

8.4.16 Continue to draw up dextran-albumin solution and add it to the cryobag in an effort to rinse out any remaining cells left in the bag.

8.4.17 Transfer washed cell suspension(s) into the infusion bag and record the total volume

8.4.18 After transfer is complete, close off the transfer lines with the roller clamps.

8.4.19 Carefully remove the spike from the transfer line of the infusion bag port. Place a sampling site coupler into that open port (*see pic below*)

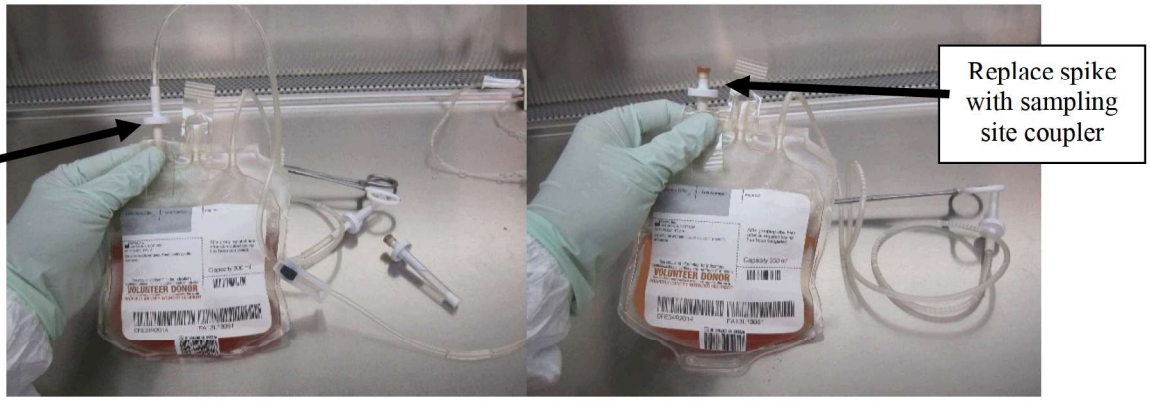

Figure 4

8.5 Centrifugation of the thawed/diluted product

8.5.1 Sterile dock a 300 mL transfer bag to the infusion bag tubing line using the directions on the sterile tubing welder.

8.5.2 Place product into a sterile Ziploc bag.

8.5.3 Place cell suspension bag inside the appropriate centrifuge insert.

8.5.4 Arrange the insert and the empty bag inside the centrifuge cup (***Figure 4***).

8.5.5 Once centrifuge cups are balanced, place them in the centrifuge.

8.5.6 Pellet the cells by centrifugation at 890G (1800 rpm) for 20 minutes at 2-8° C.

8.5.7 Complete the necessary paperwork.

8.6 Expression of supernatant and addition of fresh thawing solution

8.6.1 Place centrifuged infusion bag on the plasma extractor (***Figure 5***).

Figure 5

8.6.2 Place the transfer bag on the scale. Tare the scale (***Figure 5***).

8.6.3 Open the clamp between the two bags.

8.6.4 Without disturbing the cell pellet, allow the wash/supernatant to flow out until the cells from the pellet start to move or the desired volume is reached

8.6.5 Use the weight of the supernatant to estimate the volume remaining in the infusion bag.

8.7 Preparation of cells for transplant / reinfusion

8.7.1 Heat seal the transfer line between the two transfer bags

8.7.2 Detach bags and save the supernatant for sterility tests

8.7.3 Weigh the product bag and subtract the tare weight of the empty bag

8.7.4 Working inside the hood, mix the contents and remove a 0.7 mL aliquot for QC tests (***remove 0.9 mL if RFLP is ordered***):

NOTE: *If product is going to the inpatient unit, if the volume and cell count permit, filter the product into 60 mL syringe and add stopcock and extension tubing; add parafilm to the end of the tubing to prevent leaking* ***(Figure 6)***

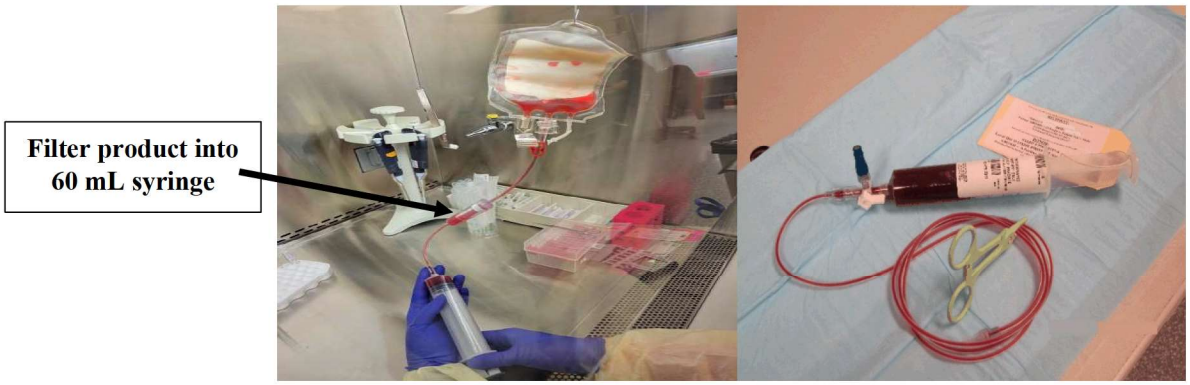

Figure 6

8.7.5 Label the infusion bag with product, recipient, and donor information

8.7.6 If the product volume is too large to send in a 60 mL syringe, sterile dock a 100 mL saline bag to the infusion bag as shown in ***Figure 7***.

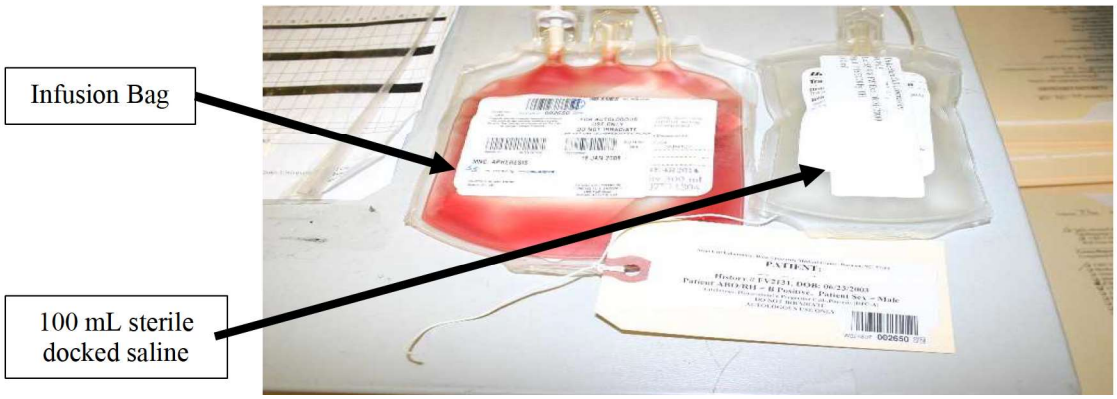

Figure 7

8.7.7 Place a slide, pinch, or roller clamp on the tubing of the product bag. Close the clamp and create a sterile dock between the clamp and the saline bag.

8.7.8 The infusion bags will be rinsed with saline solution to capture any cells left in the bag

8.7.9 Confirm, with a second technologist, or designee, the patient's name, medical history number, and ISBT barcode on the infusion bag of cells prior to sending it to the transplant unit. Both individuals must initial the *Thawing and Infusion Worksheet* indicating that this step was completed

8.7.10 Send the product to the transplant unit in a validated transport container (cooler).

8.8 Quality Control Tests

8.8.1 Cell Counts on infusion-ready product.

8.8.2 Viability test by trypan blue exclusion dye on infusion-ready product.

8.8.3 Progenitor cell assay on infusion-ready product.

8.8.4 CD34 positive cells on infusion-ready product.

8.8.5 RFLP test on infusion-ready product, *if ordered by a physician*.

8.8.6 Aerobic and anaerobic cultures (sterility) testing on washed supernatant.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-FORM-043 *Thawing and Infusion Worksheet*

9.2 STCL-FORM-046 *Processing Lot Numbers-Bone Marrow or PSC DA Thaw*

9.3 STCL-FORM-056 *Cellular Therapy Infusion Request Form*

10 REFERENCES

10.1 Kernan NA, Scroeder ML, Ciavarella D et al. Umbilical cord blood infusion in a patient for correction of Wiskott-Aldrich syndrome. Blood Cells 1994; 20: 242-4.

10.2 Neudorf SML, Blatt J, Corey S et al, Craft failure after an umbilical cord blood transplant in a 11 Allogeneic Umbilical Cord Blood Banking and Transplantation.

10.3 Rubenstein, P, L Dobrila, RE Rosenfield, JQ Adamson, G Migliaccio, AR Migliaccio, PE taylor, and CE Stevens. 1995. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. PNAS 92:10119-10122.

10.4 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

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
06	B. Waters-Pick	• Added sources for Dextran-40 in 0.9% sodium chloride in Section 5.2.2 • Section 5.3.14 – Added Insul-ice mats *"(if needed)"* • Added wording in italic to Section 8.3 "Preparation of Dextran-Albumin thawing solution *(unless 250 mL pre-filled bag is available from the refrigerator)*" • Added wording in italic to Section 8.3.1 "Spike a 300 mL transfer bag into a 500 mL bag of Dextran *(that has been removed from the refrigerator)*" • Modified Section 8.4.1 and removed original 8.4.2 "Wrap dextran albumin bag in ice mat" • Removed 8.4.11 "Clean the port with an alcohol prep pad" (step 8.4.10 states "clean the port with a ChloraPrep® SEPP® applicator) so 8.4.11 is a duplicate statement that can be removed • Added NOTE to Section 8.7.4 **NOTE: *If product is going to the inpatient unit, if the volume and cell count permit, filter the product into 60 mL syringe and add stopcock and extension tubing; add parafilm to the end of the tubing to prevent leaking*** • Added 2nd picture at the end of Section 8.7.4 on the LEFT side to show filter used and added ***Figure 6*** and added text box and arrow • Section 8.7.5 removed "Per SOP" resulting in "Label the infusion bag with product, recipient, and donor information" • Changed wording in Section 8.7.6 to include "*If the product volume is too large to send in a 60 mL syringe*", sterile dock a 100 mL saline bag to the infusion bag as shown in ***Figure 7*** and added text boxes and arrows. This allows for the use of syringes or bags depending on the volume available for infusion.

Signature Manifest

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