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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 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 then 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. It covers all required steps from the time cryopreservation bags are removed from the freezer until they are ready for infusion.

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 volume reduced bone marrow or peripheral stem cells frozen in a controlled rate freezer. Frozen products are cryopreserved in a liquid phase of liquid nitrogen at the temperature of approximately -196°C.

5.2 Reagents
5.2.1 Human albumin (human) USP 25% solution
5.2.2 Dextran 40 (10% Gentran 40 and 0.9% NaCl)
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 CloroxPrep® SEPP® applicator
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
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 blood bank 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.

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](image_url)

8.3 Preparation of Dextran-Albumin thawing solution

**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.

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.
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 made using 150 g of Dextran and 30 mL of Albumin.

8.4 Preparation and thawing

8.4.1 Spike the Dextran-Albumin bag to the 16 G needle in one end of the double stop cock setting.
8.4.2 Wrap Dextran-Albumin bag with ice mat.
8.4.3 Working in vapor phase of LN2 tank, remove the unit from the metal cassette as shown in **Figure 2**.

![Figure 2](image)

8.4.4 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 to be transplanted. Both individuals must initial *STCL-FORM-043 Thawing and Infusion Worksheet* indicating that this step was completed.
8.4.5 Place the frozen unit inside a sterilized Ziploc bag.
8.4.6 Thaw the unit in the 37°C water bath until the product reaches a slushy, liquid consistency.
8.4.7 Take the product and place inside the biological safety cabinet (hood).
8.4.8 Remove the product from inside the protective Ziploc bag.
8.4.9 Remove the port cover from one of the available ports.
8.4.10 Clean the port with a ChloraPrep® SEPP® applicator.
8.4.11 Clean the port with an alcohol prep pad.
8.4.12 Spike the available transfer line into the disinfected (cleaned) port.
8.4.13 Fill a 60 mL syringe with dextran-albumin solution.
8.4.14 Slowly add the solution to the thawed product bag as seen in Figure 3.

![Figure 3](image)

8.4.15 Mix the contents during transfer.
8.4.16 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.

8.4.17 Mix the contents well during transfer.
8.4.18 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.
8.4.19 Transfer washed cell suspension(s) to the infusion bag and record the total volume.
8.4.20 After transfer is complete, close off the transfer lines with the roller clamps.
8.4.21 Carefully remove the spike from the transfer line of the infusion bag port. Place a sampling site coupler into that open port.

![Remove spike of the transfer line](image)

![Replace spike with sampling site coupler](image)
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 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).

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).

8.7.5 Per SOP, label the infusion bag with product, recipient and donor information.

8.7.6 Sterile dock a 100 mL saline bag to the infusion bag as shown in Figure 6.

![Figure 6](image_url)

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 Saline solution will be used to rinse the remaining cells left in the infusion bag.

8.7.9 Confirm, with a second technologist, or designee, the patient’s name, medical history number, and ISBT barcode on the bag of cells to be transplanted 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-056 Cellular Therapy Infusion Request Form
9.3 STCL-FORM-046 Processing Lot Numbers-Bone Marrow or PSC DA Thaw

10 REFERENCES

11 REVISION HISTORY

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<td>05</td>
<td>B. Waters-Pick</td>
<td>Formatted and checked grammar of entire document</td>
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<td></td>
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<td>Section 2 – moved section 2.4 from 3.1.</td>
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<td>Sections 5 &amp; 6 – removed manufacturer references for reagents, supplies, and equipment</td>
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<td>Section 8.2.6 – Added new picture for Figure 1.</td>
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<td>Section 8.4.21 – Added two new pictures to this step.</td>
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<td>Section 8.5 – Added new picture for Figure 4.</td>
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## STCL-SOP-048 Procedure For Thawing Bone Marrow And Peripheral Stem Cells Using Dextran-Albumin Solution

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