STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Cryopreservation of Back Up Cells from Original UCB Reinfusion

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STCL-PROC-025
CRYOPRESERVATION OF BACK UP CELLS FROM ORIGINAL UCB REINFUSION

1 PURPOSE

1.1 At the time of transplant the patient receives the majority of the stem cells present in the cryopreserved cord blood unit. The remaining fraction of the cells left in the supernatant are re-frozen and cryopreserved for possible post-transplant procedures.

2 INTRODUCTION

2.1 Maintenance of the transplantable hematopoietic cells found in umbilical cord blood is achieved by storage in liquid nitrogen (temperature <-150°C or colder). This protocol details the steps involved in preparing the remaining cord blood cells after thaw and reinfusion for re-storage, with minimal loss of cell viability.

3 SCOPE AND RESPONSIBILITIES

3.1 This procedure covers all required steps to perform the assay. Beginning at the time the transfer/freezing bag set is sterilely connected to the transfer/infusion set. Remaining cells can be refrozen in an enclosed system.

4 DEFINITIONS/ACRONYMS

4.1	w/w	weight/weight
4.2	v/w	volume/weight
4.3	ml	milliliter
4.4	C	Celsius
4.5	Na	sodium
4.6	Co	company

5 MATERIALS

5.1 Specimen

5.1.1 Cord blood cells thawed, washed, and resuspended in a saline solution containing 4.2 % (w/w) human serum albumin and 10% (v/w) Dextran 40 in a volume of 21 ml.

5.2 Reagents

5.2.1	Cryoprotectant ambot containing	MedSep Corporation
5.2.2	55% (v/w) DMSO 5% (v/w) Dextran 40	Protide or equivalent
5.2.3	DMSO (Cryoserv) and	Research Industries Inc.

	5.2.4	Dextran 40	Hospital Pharmacy
5.3	Supplies		
	5.3.1	Stem Cell Transfer/Freezing bag set	MedSep Corporation
	5.3.2	3 ml Syringe	Hospital Storeroom
	5.3.3	10 ml Syringe	Hospital Storeroom
	5.3.4	Alcohol Wipes	Hospital Storeroom
	5.3.5	Cryopreservation Labels	Custom MedSep Corp
	5.3.6	Large Rubber Bands	Hospital Storeroom
	5.3.7	Study Bar Code Labels	CompuType
	5.3.8	Overwrap bags	ThermoGenesis Inc.
	5.3.9	BioArchive System Canister	ThermoGenesis Inc.
	5.3.10	Plastic Sealing Jig	ThermoGenesis Inc.

6 EQUIPMENT

6.1	Type II Laminar Flow Hood	Baker Co.
6.2	ThermoGenesis BioArchive System	Biogenic Systems
6.3	Bar Code scanner	CompuType
6.4	Heat Sealer	Sebra Model 2100, modified
6.5	Syringe Pump	Medex Inc.
6.6	Orbital Rocker or Rotator	Nutator - Clay Adams Co.
6.7	Cool Packs (0 - 4°C)	Fisher #03528D
6.8	Freezer Gloves	Fisher Co.
6.9	Aluminum Storage Cassette	Custom, Biogenic Systems
6.10	Vacuum Sealer	Fuji Impulse
6.11	Kim Wipes	Kimberly Clark Corporation
6.12	LN2 Freezer - MVE xlc1520	MVE, Inc.

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, lab coats, goggles, etc.

8 PROCEDURE

8.1 Procedure Notes

8.1.1 Turn on the biological safety cabinet at least 15 minutes before initiation of procedure.

8.1.2 Clean working area inside the hood with 70% alcohol or 10% Na hypochloride.

8.1.3 Use aseptic technique and biological safety cabinet for all processing steps, all blood bag-spiking operations and all open-container sampling.

8.2 Sample Preparation

8.2.1 Before centrifugation (second wash) sterile dock the waste bag from thawing/infusion set to a transfer/freezing bag set.

8.2.2 Pellet the cells and express supernatant to the transfer/freezing bag set.

8.2.3 Heat seal tubing and detach bag containing the cell pellet and add the cell suspension into the infusion bag.

8.2.4 Place the bag containing the supernatant inside the same insert used for thawing.

8.2.5 Balance centrifuge cups and pellet the cells at 1200 RPM for 20 minutes at 10°C.

8.2.6 Place centrifuged bag in the auto volume expresser. Express the supernatant.

8.2.7 The automated expresser is calibrated to leave 21.5 ml of cell suspension inside the bag.

8.2.8 Heat seal tubing and detach bags at sealed point.

8.2.9 Place the bag with cells inside the hood and clean the access port with alcohol.

8.2.10 Draw 0.5 ml aliquot for cell counts and cell viability testing.

8.2.11 Place bag in the refrigerator for 15-20 minutes.

8.3 DMSO Injection

8.3.1 Attach printed bar code labels to a metal storage cassette.

8.3.2 Label cryobag with barcode identification numbers and one label documenting the contents.

8.3.3 Record the lot number and expiration date of the cryoprotectant mix on the worksheet and in the database.

8.3.4 Place inside the hood a 10ml syringe, cryoprotectant, and a 16G needle.

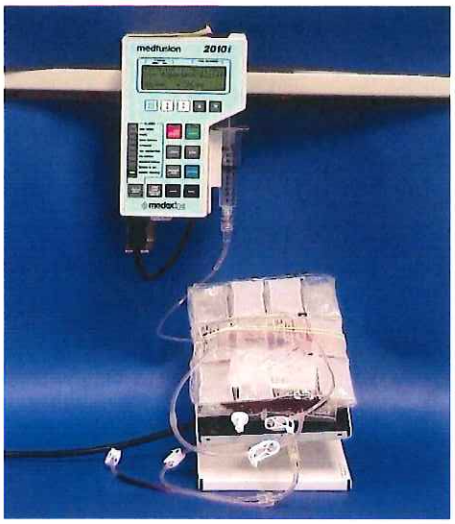
8.3.5 Load a 10ml syringe with 7ml of pre-cooled cryoprotectant.

8.3.6 Retrieve the cooled bag containing the cells, wrap the bag in a frozen ice mat, and place it inside the hood.

8.3.7 Remove the needle, attach the syringe to the female end of the tubing attached to the bag.

8.3.8 Open clamp and prime tubing.

Figure 1



8.4 Metered Flow Injection (***Figure 1***)

8.4.1 Place the ice wrapped cell suspension bag on the rocker.

8.4.2 Turn on the rocker.

8.4.3 Insert the syringe onto the syringe pump.

8.4.4 Turn on pump to initiate the metered flow of DMSO.

8.4.5 Maintain the rocker in continuous rotation to ensure adequate mixing between cells and cryoprotectant.

8.5 Preparation for freezing

8.5.1 Dry the tubing with kimwipes before sealing.

8.5.2 Heat-seal tubing at the far end from the cryobag.

8.5.3 Make three independent segments.

8.5.4 Cut tubing and detach cryobag.

8.5.5 Place the cryobag inside the heat-sealing plastic jig.

8.5.6 Align sealing positions in the jig groove.

8.5.7 Heat seal the cryo bag on both sides to create two independent compartments. Fold the cryobag between the two sections. Place the bag with segments, top first, into an overwrap bag.

8.5.8 Position the cryo bag inside the overwrap.

8.5.9 Place the overwrapped cryobag onto the vacuum sealer with the sealing strip as close as possible to the bag.

8.5.10 Press the handle down. Hold until the light is out, and then hold for five additional seconds.

8.5.11 Fold the excess of plastic under the overwrap bag.

8.5.12 Place labeled, vacuum-sealed cryobag into the barcode labeled metal canister.

8.5.13 Confirm the identity of the barcodes on the canister and on both compartments of the cryobag.

8.5.14 Label outside of the metal canister with patient and product information.

8.6 Controlled rate freezing and Storage

Place the metal cassette into a dry controlled rate freezer (CRF) device.

1. Log on and follow the protocol to initiate the desired freezing program.
2. From drop down menu click on store to open controlled rate freezing and storage application.
3. Remove the CRF device from BioArchive port once the freezing procedure is complete and the canister has been stored.
4. Place CRF device into warm air slot.
5. Place records and paper work in the appropriate recording folder.

NOTE: The product is automatically stored after freezing.
The freezing graph is automatically printed after storage.

8.7 Quality Control:

8.7.1 The trace of the controlled rate freeze showing the freeze curve/sample temperature must be within the program profile.

8.8 Controlled Rate Freezer Program

8.8.1 The program is designed to provide freezing at the rate of 10°C/min with 100% of fan power starting at 4°C until reaches -3°C. From -15°C to -50°C the rate decreases to 2°C/min with 50% of fan power. Once -50°C is reached the sample is automatically stored in the BioArchive system.

9 RELATED DOCUMENTS / FORMS

9.1 *Thawing and Infusion Worksheet*

9.2 *Processing Lot Numbers for DAT UCB Thaws*

10 REFERENCES

10.1 Areman E, Deeg HJ, Sacher RA. *Bone Marrow and Stem Cell Processing: A Manual of Current Techniques*. F.A. Davis Company, Philadelphia, PA, 1992.

10.2 Rubinstein P, Dobrila LI, Rosenfeld R, et al. Proc. Of the National Academy of Science USA, Volume 92, pp. 10119 – 10122, October 1995.

11 REVISION HISTORY

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Signature Manifest

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