STEM CELL LABORATORY (STCL)

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Cryopreservation and Storage of CBU

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STCL-PROC-045
CRYOPRESERVATION AND STORAGE OF CBU

1 PURPOSE

1.1 Umbilical cord blood is an important source of stem cells for hematopoietic reconstitution. Cord blood can be collected shortly after birth but must be cryopreserved for long-term storage.

2 INTRODUCTION

2.1 Maintenance of the transplantable hematopoietic cells found in umbilical cord blood is achieved by storage in liquid nitrogen. This protocol details the steps involved in preparing cord blood for long-term storage at this temperature, with minimal loss of cell viability.

2.2 Specimen Requirements

2.2.1 Twenty-one (21) mL ($\pm$ 1.5 mL) of buffy coat from cord blood processed according to *STCL-PROC-042 UCB Processing Using the Automated Sepax 2 S-100 Cell Processing System with UCB_HES Protocl or STCL-PROC-044 CBU Processing via Manual Method.* Cryopreservation must be initiated within 60 minutes of addition of cryoprotectant (DMSO/Dextran). Before, during and after addition of DMSO, cells must be maintained at refrigerated temperatures at all times until cryopreservation.

2.3 Product Endpoint

2.3.1 Cryopreserved cord blood unit which contains ~25 mL of post processed cord blood containing ~5 mL of cryoprotectant. Unit should be successfully frozen and stored in liquid nitrogen.

3 SCOPE AND RESPONSIBILITIES

3.1 The Stem Cell Laboratory Medical Director, Stem Cell Laboratory Manager and representatives of the Stem Cell Laboratory staff are responsible for ensuring the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	mL	milliliter
4.2	DMSO	Dimethyl Sulfoxide
4.3	CBU	Cord Blood Unit
4.4	UCB	Umbilical Cord Blood
4.5	BSC	Biological Safety Cabinet
4.6	NaCl	Sodium Chloride
4.7	CRF	Control Rate Freezer
4.8	°C	Degrees Celsius

5 MATERIALS

5.1 Reagents

5.1.1 DMSO/Dextran (55% w/v DMSO 5% w/v Dextran 40) 0.9% NaCl single use vials (Protide) or pre-filled syringe (Akron)

5.2 Supplies

5.2.1 Processing Kit

5.2.1.1 Sepax CS-530 single-use kit (if processed using Sepax) or MedSep 791-02 Transfer/Freezing Bag Set

5.2.2 Cryovials

5.2.3 3 mL Syringe

5.2.4 10 mL Syringe

5.2.5 DMSO Extension Set (Sepax processing)

5.2.6 Needles

5.2.7 Ice Packs (frozen)

5.2.8 Gloves

5.2.9 Alcohol Prep Pads

5.2.10 ChloraPrep® SEPP®

5.2.11 Demand 128 Cryopreservation Labels

5.2.12 ISBT 128 Labels

5.2.13 ThermoGenesis® overwrap bags

5.2.14 Tissue Wipes

5.2.15 Thermogenesis® aluminum storage canister

5.2.16 Permanent Marker (if applicable)

6 EQUIPMENT

6.1 Biological Safety Cabinet/ Laminar Flow Hood

6.2 ThermoGenesis® BioArchive® Control Rate Freezer

6.3 ThermoGenesis® BioArchive® Liquid Nitrogen Freezer

6.4 Bar Code scanner

6.5 BioSafe® Coolmix AS-210 or Orbital Rocker

6.6 Medfusion/Protégé 3010/3010a syringe pump

6.7 Tubing Heat Sealer

6.8 Overwrap Heat Sealer

6.9 -20 °C Freezer

6.10 -80 °C Freezer

6.11 1-10 °C Refrigerator

6.12 Scissors

6.13 Manual Tubing Strippers

6.14 Sealing Jig

6.15 LN2 vapor freezer

7 SAFETY

7.1 Use all appropriate personal protective equipment when handling potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coats, etc.

8 PROCEDURE

NOTES:

- Unit should have been refrigerated for at least 15 minutes but no longer than 2 hours before continuing with the following steps.

- Buffy coat UCB bag should have already been labeled with Demand 128 barcode label.

8.1 Retrieve Demand 128 canister labels (printed during UCB processing) and adhere the label to an aluminum storage canister on the metal tab/clip piece.

8.2 The tab will be scanned by the BioArchive during cryopreservation for label verification purposes. Make sure that the label is aligned on the tab/clip. Do not touch the adhesive on the label while affixing it to the tab/clip as this could cause the label to fall off during freezing.

8.3 If the CBU is a directed or autologous unit, also write auto/directed, UCB, the name of the birth mother and date of processing on the barcode label affixed to the cryopreservation bag as well as the side of the aluminum storage canister. See photos 1 and 2. If the CBU is remaining back up cells from an original UCB reinfusion, also write the recipient's name, history number, date of processing/cryopreservation and UCB B/Up on the barcode label as well as the side of the aluminum storage canister.

Photo 1

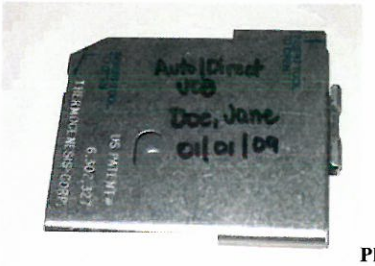


Photo 2

8.4 Obtain on ice pack from the -20 °C freezer. Retrieve pre-cooled UCB product cryobag and insert it into the ice pack.

8.5 Retrieve vial or pre-filled syringe of Cryoprotectant (55%DMSO/5%Dextran) from the refrigerator. Insert into ice pack to keep cool during these steps.

8.6 These steps must be performed using BSC and aseptic technique:

8.6.1 **Cryoprotectant – vial** (Protide, 7 mL vial):

8.6.1.1 Attach a needle to a 10 mL syringe.

8.6.1.2 Remove snap-cap and disinfect rubber stopper at the top of the bottle. Insert needle and withdraw contents of DMSO/Dextran solution.

8.6.1.3 Remove any air bubbles from the syringe.

8.6.2 **Cryoprotectant - pre-filled syringe** (Akron, 7 mL syringe):

8.6.2.1 Remove any bubbles from the syringe.

8.6.2.2 Verify that syringe contains adequate volume of cryoprotectant ($\geq$ 7 mL).

8.7 Record DMSO/Dextran lot number, manufacturer and expiration date on *STCL-FORM-045 FRM1 CBU Cryopreservation.*

Note: The following steps will be determined by whether the CBU was processed via Sepax method or by manual method. If Sepax method was used, continue with the next step. If manual method was used, skip down to manual method processed units, step 8.9.

8.8 Cryoprotectant and bag preparation steps for CBU processed via Sepax CS530 kit:

8.8.1 Using a BSC and aseptic technique, attach the DMSO filled syringe to the white capped end of the DMSO line (provided in CS-530 kit) and prime with DMSO.

8.8.2 Attach the DMSO filled line to the CBU buffy coat product cryobag by attaching the white capped luer port on the cryobag to the clear cap luer port on the DMSO line. Be sure to keep cryobag in the ice pack to keep the unit cold.

8.8.3 Open clamp on DMSO line and port line to allow for further priming to the base line of the tubing of the cryobag where it meets the syringe port. See Figure A. This should leave ~5 mL of DMSO in the syringe.

Figure A

8.8.4 Close clamp on the DMSO line to prevent exchange and ensure accurate volume while transporting syringe and unit to the infusion pump.

8.8.5 Demand 128 Cryopreservation label should already be adhered to the buffy coat bag. Visually verify labels on both the 20% and 80% portions on the bag.

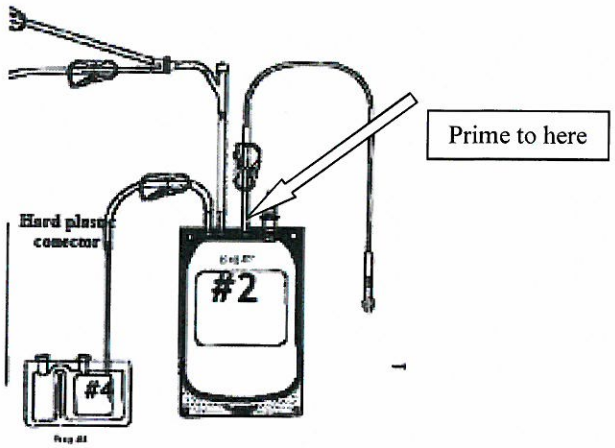
8.8.6 Proceed to cryopreservation infusion steps below, 8.10.

8.9 Cryoprotectant and bag preparation steps for CBU processed using Pall 791-02 Transfer/Freezing Bag Set:

8.9.1 Remove the needle/cap from syringe and attach the syringe to the female luer adapter on the end of the microbore tubing attached to processing bag (bag #2). (See Figure 1, Processing Set.)

8.9.2 Prime the tubing with DMSO to the point where the tubing enters the bag. This should leave ~5 mL DMSO still remaining in the syringe.

Figure 1 Processing Set



8.9.3 Close clamp on DMSO line to ensure accurate volume while transporting syringe and unit to infusion pump.

8.10 Cryoprotectant can be infused through syringe pump and mixer combo:

8.10.1 Select the Medfusion/Protégé 3010/3010a Syringe Pump to be used. See *STCL-EQUIP-020 Operation of Medfusion-Protégé 3010-3010a Syringe Pumps.*

8.10.2 To cool and mix the unit while infusing DMSO/Dextran, select either the Coolmix AS-210 or the orbital rocker.

8.10.2.1 For operation and procedure for Biosafe® Coolmix AS-210 use *STCL-EQUIP-015 Operation of Coolmix AS-210.* **NOTE:** Coolmix AS-210 cannot be used for MedSep 791-02 kits as DMSO infusion takes place in bag #2 instead of cryobag.

8.10.2.2 For operation of the orbital rocker in conjunction with ice packs use *Operation of Medfusion-Protégé 3010-3010a Syringe Pumps.*

8.10.3 Record syringe pump, mixer serial numbers, time/date of start of DMSO infusion on *STCL-PROC-045 FRM1 CBU Cryopreservation.* **NOTE:** Time between end of processing and addition of DMSO should be within two hours.

Following completion of DMSO/Dextran Infusion:

8.11 For units processed using the Sepax CS-530 kit, complete the following:

8.11.1 Close DMSO line clamp on bag port.

8.11.2 Place cryobag into ice pack to keep cold.

8.11.3 Using BSC and aseptic technique, attach a needle to a 3 mL syringe.

8.11.4 Disinfect syringe port of the CBU product buffy coat cryobag and insert needle of syringe.

8.11.5 Mix the cells and remove any air from the cryobag using a syringe. Remove 1 mL of cryoprotected cells.

8.11.6 With remaining air in the syringe, push the cells down in the tubing to the point below the syringe port where the last seal will be made. This will ensure that these cells remain with the unit instead of being discarded. (See Photo 3.)

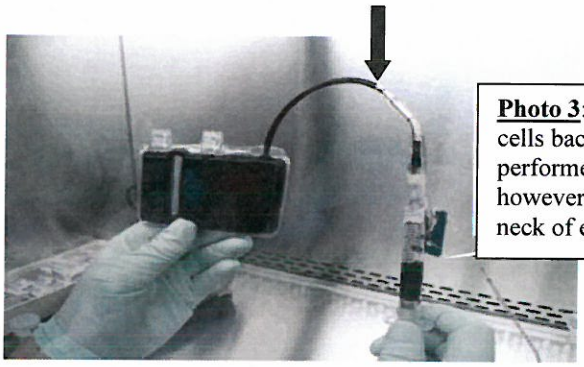


Photo 3: Leave 1 mL in syringe, use air to push cells back in tubing to where last seal will be performed on segments. Pall bag shown in photo, however, use air to push blood back to the joint neck of either Pall or Biosafe cryobags.

8.11.7 Dispense 0.5 mL each into the two cryovials. The cryovials will be referred to as "DMSO nuncs".

8.11.8 Put DMSO nuncs onto ice pack to keep cold until storage.

8.11.9 Place the CBU cryobag in the appropriate sealing jig to evenly distribute the volume between the two fractions and to prevent overfilling of the bag.

8.11.10 Dry the tubing with tissue wipes.

8.11.11 For sealing of the cryobag and attached tubing use the following steps and in sequential order.

8.11.11.1 Make first seal close to CBU cryobag and move away from the bag to make subsequent seals. Leave segments intact and cut (at seal #4) remainder of the tubing (syringe/port side) off and discard into biological waste.

NOTE: Sealing the tubing closest to the bag first alleviates the buildup of pressure inside the tubing. These steps need to be done carefully but quickly to ensure that unit is not warming during these steps.

8.11.11.2 With the CBU cryobag still in the sealing jig, heat-seal the chambers between the 80% portion and 20% portion (Figure 2, seal/arrow 5) of the cryobag.

8.11.11.3 Where the 1st seal was made, bend segments across at a 90° angle over top of ports. See Figure 2b.

8.11.11.4 Proceed to step 8.13, Overwrapping and Cryopreservation.

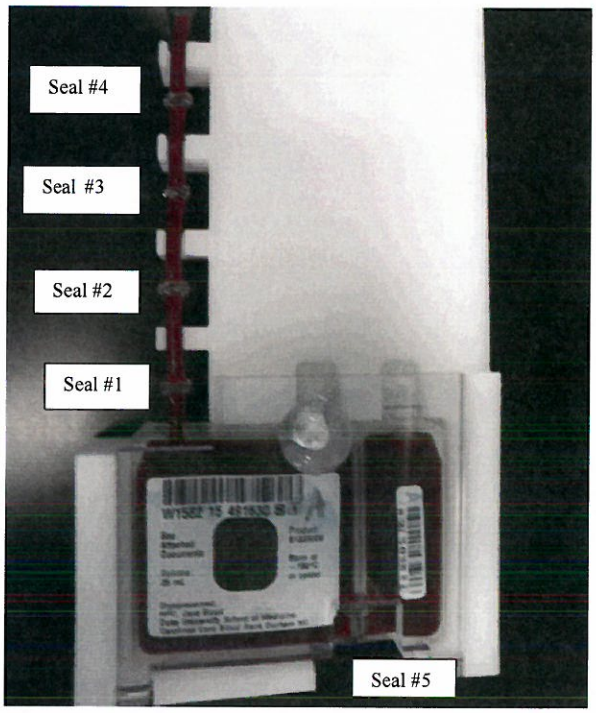


Figure 2a

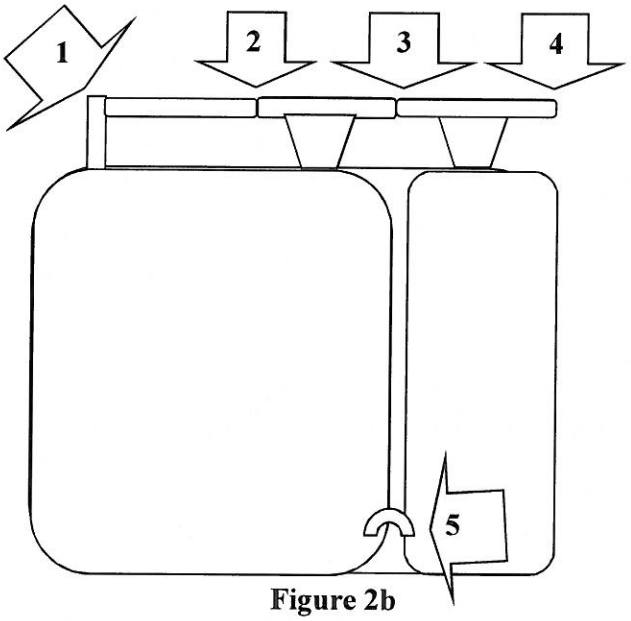


Figure 2b

FIGURE 2a/b: BIOSAFE CS530 CRYOBAG

8.12 For UCB units processed in Medsep 790-02 bag kit complete the following:

8.12.1 Close clamp on DMSO line.

8.12.2 Place bag 2 in an ice pack to keep cool while performing the next steps.

8.12.3 Allow bag #2 to drain into bag #4 (cryobag). Move freezer pack to bag #4 where the cells are.

8.12.4 Burp cryobag to remove any air and then let remaining cells drain back into bag #4, leaving cells in the tubing.

8.12.5 Place bag # 4 into appropriate jig to ensure proper volume and distribution of cells.

8.12.6 Using the heat sealer, seal tubing to create 3 segments between the cryobag and the hard plastic connector (see Figure 3, see arrows 1, 2, 3, & 4).

8.12.7 Make first seal close to cryobag and move away from the bag to make subsequent seals.

NOTE: Sealing the tubing closest to the bag first alleviates the buildup of pressure inside the tubing. These steps need to be done carefully but quickly to ensure that unit is not warming during these steps.

8.12.8 Seals should be made at 90° to the top seam of the bag to allow the tubing to bend parallel to the freezer bag for proper storage. See Figure 3.

8.12.9 With the CBU cryobag still in the sealing jig, heat-seal the chambers between the 80% portion and 20% portion of the cryobag (see Figure 3, arrows 5 & 6).

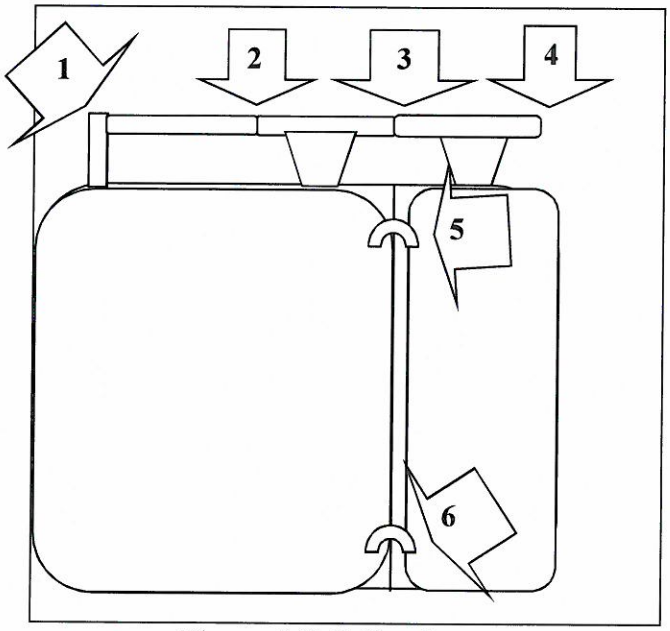


Figure 3 Pall Cryobag

8.12.10 Separate (bag #4) with attached segments from processing bag (bag #2) by cutting across the heat seal most distal to the stem cell cryobag (seal #4). Cryobag should now have three attached segments and be separated from all other bags and tubing.

8.12.11 Return the processing bag (bag #2) with remaining tubing back on an ice pack for the removal of residual cells and processing of the cryovials after the freezing program is underway. **NOTE:** This step is not performed on UCB supernatant back-up units.

8.12.12 Bend segments across at a 90° angle over top of ports where 1st seal was made. Then bend the last segment down to lay over where the chambers were sealed.

8.12.13 Proceed to step 8.13, Overwrapping and Cryopreservation.

8.12.14 Following completion of cryopreservation, return to a BSC to process the cryovials using aseptic technique:

8.12.14.1 Using a manual tubing stripper, strip any residual cells from the tubing back into bag #2.

8.12.14.2 Using a syringe and needle remove any remaining cells from bag#2 and tubing and distribute evenly between two labeled cryovials.

8.12.14.3 Place cryovials in designated syrofoam rack in designated -80 °C freezer. Cryovials will be transferred to MVE vapor phase freezer at a later point for long term storage. Record the freezer location on *STCL-PROC-045 CBU Cryopreservation Form.*

Overwrapping and Cryopreservation

8.13 Fold the cryobag between the two fractions. Place the cryobag with segments, top first, into an overwrap bag.

8.14 Maneuver the bags until the cryobag with non-bent segments is flat and pressing the top and sides of the overwrap bag. The non-bent segments should be resting on top of the cryobag.

8.15 Place the overwrapped cryobag onto the vacuum sealer with the sealing strip as close to the bottom edge of cryobag as possible without sealing the bottom of the bag in the overwrap seal.

8.16 Press the handle down. Hold until the light is out then hold for five additional seconds. Fold the excess overwrap under the unit.

8.17 Place labeled, vacuum-sealed overwrapped CBU cryobag into an ISBT bar code labeled aluminum storage canister.

8.18 Confirm barcode labels on the cryobag to the label on the canister. Check to see that the canister is closed tightly and none of the overwrap is protruding out of the canister.

8.19 Obtain a controlled-rate freezer (CRF). Verify that the CRF is completely dry.

NOTE: A CRF should sit in the CRF Drying Box a minimum of 15 minutes before re-use (Figure 4). The Drying Box is located on the control system electronics box. **CAUTION:** If the CRF or canister is wet, the freezing process may freeze the CRF doors closed or freeze the canister to a door. **These items must be dry**.

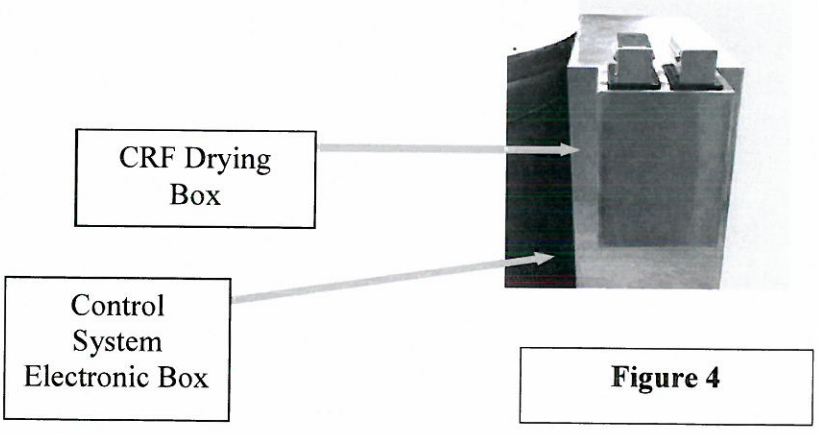


Figure 4

8.20 Place the labeled canister containing the filled CBU cryobag into the CRF.

8.20.1 Holding the CRF as oriented in Figure 5, insert the canister in the open slot at the forward side of the doors

NOTE: The doors of the CRF will open slightly during this process.

8.21 Push the canister back until the canister is flush with the forward face of the CRF (Figure 5). The doors will snap closed.

NOTE: Be sure that the canister barcode label is facing you.

8.22 Make sure the CRF doors are fully closed.

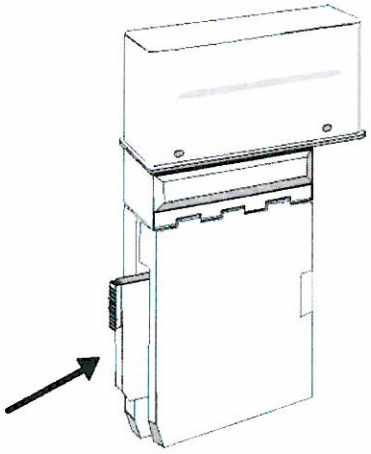


Figure 5: Placing the Canister into the CRF

Storing a sample in the BioArchive System

8.23 After the canister containing the overwrapped filled cryobag is placed in the CRF, the freezing process can be initiated.

8.24 To initiate the freeze:

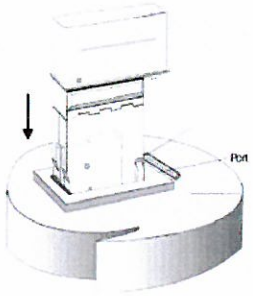
8.24.1 Select the port to be used. If both are available, either one can be used. Two units can be frozen in the same freezer at one time, one in each port.

8.24.2 Remove the port plug from that port.

8.24.3 Gently insert the CRF in a port with the exposed side of the canister facing the periscope shaft (Figure 6).

NOTE: Only insert CRF into port if Motor System is idle. Loading the CRF while periscope or other motor parts are moving can damage the freezer, the CRF and the unit itself.

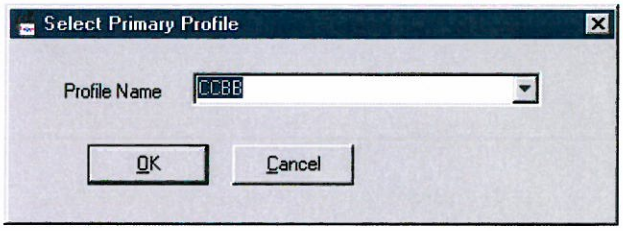
Figure 6: Inserting the CRF into the Port.



8.25 Fully seat the CRF gently in the port. If the CRF is seated correctly the indicator light will change from green to red and will not be blinking. Within a few seconds, the system will move to read the barcode label on the canister. When complete, the ID of the sample will appear in the sample ID part of the display and the port status will indicate idle. Only the sample ID will be displayed at this point.

8.26 Log onto the computer associated with the chosen BioArchive by clicking onto the "open door" icon and signing in with a user name and password. Wait for the system status to return to "idle".

8.27 Initiate the freezing process by clicking on the "store" icon. Choose the appropriate freezing profile (CCBB) and scan the entire barcode of the unit.



8.28 Click "OK". Wait for the system status to change to either "Finding Slope" or "Busy". The BioArchive will automatically freeze and store the unit per the profile below. Record date and start time for freezing on the *CBU Cryopreservation Form*.

Profile Name	CCBB
Pre-Freeze Rate	-10°C / minute
Start Freeze Temperature	-3°C
Fan Power	100%
End Freeze Temp	-12°C
Post-Freeze Rate	-2°C / minute
End Temp	-50°C

8.29 Log off of the computer by clicking on the "closed door" icon. Once the freezing procedure is complete and the canister has been stored under liquid nitrogen, the freezing graph and storage location will automatically print on the printer.

8.30 Transfer the cryovials to predetermined spaces in the MVE on the following day. **NOTE:** Make sure all locations are written on the forms and in the specific freezers' logbook.

8.31 Review curve for accuracy by ensuring the curve went from -20 °C to -40 °C in 7-18 minutes and file with unit paperwork.

8.32 The CBU's freezing curve is reviewed by the Medical Director/designee before unit is released.

NOTE: A freeze profile consists of defined cooling rates for the specimen while it is in a liquid state (pre-freeze) and after it has frozen (post freeze). The pre-freeze, freezing, and post-freeze states are described below.

State	Description
Pre-Freeze	The period of time after the cooling process has started during which the temperature steadily drops.
Freezing	The period of time during which the cooling rate slows (due to heat of fusion).
Post-Freeze	The period of time after freezing occurred during which the temperature steadily drops again

A typical freeze profile is shown here in **Figure 7**.

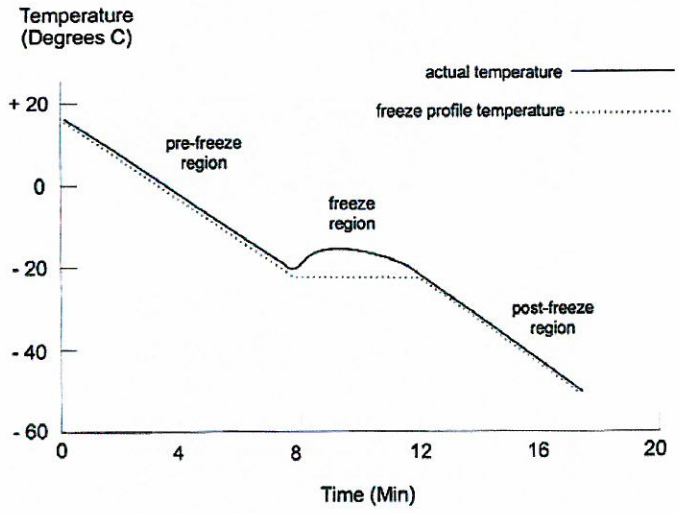


Figure 6 Typical Freeze Profile

8.33 Record the storage location along with storage date and time from freeze graph to the CBU Cryopreservation Form. Place the freezing graph into the CBU's working folder. Enter all information from the CBU Cryopreservation Form into the EMMES database, if applicable. CBU Cryopreservation form will be retained and stored with the corresponding paperwork for the unit.

8.34 It takes approximately 35-45 minutes to cryopreserve a CBU using the Bioarchive freezing system.

9 RELATED DOCUMENTS/FORMS

9.1 STCL-PROC-045 FRM1 CBU Cryopreservation

9.2 STCL-EQUIP-020 Operation of Medfusion- Protégé 3010-3010a Syringe Pumps

9.3 STCL-EQUIP-015 Operation of Coolmix AS-210

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.

10.3 American Association of Blood Banks. Standards for Hematopoietic Progenitor Cell and Cellular Product. Current edition.

10.4 Foundation for the Accreditation of Hematopoietic Cell Therapy (FACT) and Netcord. International Standards for Cord Blood Collection, Processing, Testing, Banking, Selection and Release Current edition.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
12	B. Waters-Pick	Section 8 - Updated pictures and information to support to change from preparing 4 segments back to 3 segments.

Signature Manifest

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Sandy Mulligan (MULLI026)		27 Sep 2015, 04:15:11 PM	Approved