



STEM CELL LABORATORY (STCL)



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Peripheral Blood Progenitor Cell-HPC Apheresis-Concentration and Preparation for Cryopreservation

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STCL-PROC-007

PERIPHERAL BLOOD PROGENITOR CELL (HPC, APHERESIS) CONCENTRATION AND PREPARATION FOR CRYOPRESERVATION

1 PURPOSE

- 1.1 Cells are concentrated in an attempt to limit the amount of Dimethyl Sulfoxide (DMSO) that a recipient is exposed and to decrease the number of bags required to freeze the cellular product which in turn decreases the amount of freezer space required to house those cryopreserved cellular products.

2 INTRODUCTION

- 2.1 DMSO is the primary cryoprotectant used when cryopreserving cells for transplantation. The infusion of DMSO can precipitate various side effects to include nausea, vomiting, SOB, chills, fever and occasionally cardiovascular events. DMSO is added as 10% of the final volume of the infused product. If the volume of the product can be reduced, the amount of DMSO given to the recipient can also be reduced. As the product becomes more concentrated, however, the cells become crowded. Guidelines are in place to ensure that cell death due to cell crowding does not occur. With pediatric recipients, a cell count of no greater than $200 \times 10^6/\text{mL}$ is desired. With adult recipients, a cell count of approximately $\leq 500 \times 10^6/\text{mL}$ and/or 25×10^9 total nucleated cells per bag and a hematocrit $< 5\%$ is acceptable. With pediatric recipients, a cell count of approximately $\leq 200 \times 10^6/\text{mL}$ is desired. No significant cell death has been seen as a result of freezing at these concentrations.

3 SCOPE AND RESPONSIBILITIES

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

4 DEFINITIONS/ACRONYMS

- | | | |
|-----|------|-----------------------------------|
| 4.1 | DMSO | Dimethyl Sulfoxide |
| 4.2 | SOB | Shortness of Breath |
| 4.3 | SOP | Standard Operating Procedure |
| 4.4 | STCL | Stem Cell Laboratory |
| 4.5 | DUMC | Duke University Medical Center |
| 4.6 | BSC | Biological Safety Cabinet |
| 4.7 | PBPC | Peripheral Blood Progenitor Cells |
| 4.8 | TNCF | Total Number of Cells Frozen |
| 4.9 | mL | milliliter |

5 MATERIALS

- 5.1 Supplies/Reagents
- 5.2 Transfer packs
- 5.3 Transfer set (with needle adapter)
- 5.4 Syringes (various sizes)
- 5.5 Sampling site couplers (3)
- 5.6 Stopcock, double, 3-way
- 5.7 Alcohol prep pads
- 5.8 Tubes for dilutions
- 5.9 1.8 mL CRYO vials
- 5.10 Tie Tags
- 5.11 Barcode Labels
- 5.12 Pipettor Tips
- 5.13 Cryogenic Freezing bags
- 5.14 Needles 16 gauge
- 5.15 Needle, 19 or 20 gauge
- 5.16 Dimethylsulfoxide (DMSO)
- 5.17 Trypan Blue
- 5.18 Plasmalyte
- 5.19 Cellpack
- 5.20 ABO Rh reagents
- 5.21 ISBT Demand 128 Labels
- 5.22 Culture bottles
- 5.23 ChloroPrep Applicators or Steri Perox 6% wipes or equivalent

6 EQUIPMENT

- 6.1 Biological Safety Cabinet (BSC)
- 6.2 Centrifuge
- 6.3 Plasma Extractor
- 6.4 Sebra Heat Sealer
- 6.5 Digital scale
- 6.6 Cell counter
- 6.7 BacTec Instrument
- 6.8 Microscope

- 6.9 Serofuge
- 6.10 Bag Presses
- 6.11 Cryomed Freezing Chamber
- 6.12 Calculator
- 6.13 Cellometer (*if applicable*)

7 SAFETY

- 7.1 Wear all appropriate personal protective equipment whenever handling potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coat, etc.
- 7.2 **NOTE: A technologist will only handle/process one cellular product in the biological safety cabinet at any given time.**

8 PROCEDURE

NOTES:

- Perform all work that requires manipulation of the cell bag in a biological safety cabinet (BSC) using aseptic/sterile technique. Document supplies, reagents and equipment used in processing of the cells on form *STCL-FORM-049 Processing Lot Numbers – Incoming Cellular Product Processing*.
- If necessary, the cellular product may be held overnight and processed the following day but must be stored at 1-6 °C in a monitored refrigerator. The cellular product should be cryopreserved within 48 hours from the time of collection unless otherwise authorized by the medical director or designee.
- Inspect the product to verify that :
 - the product is properly labeled
 - all necessary paperwork accompanies the product
 - the paperwork and the product labels match one another and match the barcode assigned to the product
 - the appropriate temperature was maintained during transport
 - there are no visible problems such as leaks, tears, clumps, or flaws in the product container
- If there are any discrepancies found in labeling, product container appears to be compromised, product is clumping, etc, notify the laboratory manager or designees to ensure that we follow steps outlined in *STCL-QA-007 Non-Conforming Products* procedure and initiate a *STCL-QA-007 FRM1 Non-Conforming Products* form.

8.1 Prepare the following:

- 8.1.1 Three 1:5 cell count dilution tubes by adding 0.4 mL of saline to each tube. Label these tubes appropriately to include at a minimum the ISBT 128 barcode. One tube will be used for cell count and flow, one tube will be used for viability analysis, and one tube will be saved for post concentration cell count.

- 8.1.2 2 CRYO vials labeled with patient's name, history number and barcode.
- 8.1.3 2 BacT/Alert bottles; each labeled an ISBT 128 barcode and recipient's name and history number.
- 8.1.4 Label three (3) 5mL polystyrene tubes for ABO testing. The first tube should be labeled with the patient name, history#, barcode, and "A" for the antibody to be added. The following two tubes must be labeled with patient initials and the antibody that is to be added (ie, "B", "AB", "D").
- 8.2 Weigh the PSC bag and record the value on the worksheet. Subtract 43.3 g (*weight of an empty bag*) from the weight of the bag and record the remaining volume as the collection volume or tare with empty collection bag and tie tag.
NOTE: For pediatric cellular products collected in very small volumes, the volume may be directly measured using a syringe.
- 8.3 Using a 1mL syringe (or equivalent), remove 1mL of volume from the well mixed product to perform all required testing to include ABO/Rh, cell count, flow, viability and any required research samples.
- 8.4 Examine the volume to determine the appropriate size transfer collection bag to use.
NOTE: 300mL bags will hold up to a 350mL volume; a 600mL transfer bag can also be used. Label transfer bag with patient's name, history number, and barcode label. Transfer the product from the collection bag to a transfer pack of the appropriate size. Be sure to transfer the entire product into the transfer pack and then tie off the transfer tubing.
- 8.5 Place the transfer pack with attached collection bag in a centrifuge bucket. Place the insert on a digital scale and tare the scale to the weight of the packed insert. Place a matching insert on the scale and adjust the weight to balance the product, using a zip closure biohazard bag filled with water.
- 8.6 Place the inserts in balanced centrifuge buckets and centrifuge at the appropriate rpms for either the Sorvall centrifuge or the Allegra centrifuge for 20 minutes with the brake OFF.
 - 8.6.1 Sorvall RC 3C Centrifuge – 1800 rpms
 - 8.6.2 Allegra 6KR Centrifuge – 1873 rpms
- 8.7 After centrifugation, carefully remove the transfer pack from the insert and hang the pack on the plasma extractor. Give the cells 10-15 minutes to "settle", then express the plasma back into the collection bag and heat seal the tubing.
 - 8.7.1 To determine amount of plasma to extract: $TNCF / 25 = A$; $30 \times A = B$; Calculated volume – B = plasma amount to be extracted
 - 8.7.2 A = amount of bags to concentrate the product;
 - 8.7.3 B = final amount needed to cryopreserve the product
 - 8.7.4 TNCF = Total Number of Cells Frozen
 - 8.7.5 Example: $TNCF = 45.44 \times 10^9$; Calculated volume = 358.4 mL

- 8.7.6 $45.44 \times 10^9 / 25 = 1.82 \times 10^9$ (round up to the nearest whole number - 2×10^9); $30 \times 2 = 60$; $358.4 \text{ mL} - 60 = \underline{298.4 \text{ mL}}$ to be extracted
- 8.8 Tare the scale, to accommodate the weight of the transfer pack, and weigh the pack containing the cells to determine an approximate volume. Divide the approximate volume by a correction factor of 1.06 to give the post extraction volume. Aseptically add approximately 120 mL of air to the bag.
- 8.9 Mix the cells thoroughly and remove 0.8 mL using a 5mL syringe. Dispense 0.3 mL into an empty tube. Use this aliquot to make a 1:9 dilution and perform a post concentration cell count. Calculate a predetermined cell recovery percentage. If the percent recovery is **less than or equal to 85%**, troubleshooting should be initiated to determine why the recovery is low (ie. repeat post cell count, check the setting on centrifuge to make sure the correct program was used to spin the product, etc).
- 8.9.1 New TNCF = Post WBC x Post extraction volume
- 8.9.2 Estimated Percent Recovery = New TNCF/Old TNCF
- 8.10 The remaining 0.5 mL in the 5 mL syringe will be used during cryopreservation for cultures and cryovials. Q.S. this sample to 2 mL with autologous plasma from the collection bag. "Cap" the syringe with a sterile needle, using the *one-handed scoop technique*, and set aside.
- 8.11 Place the cell bag in the refrigerator to chill prior to cryopreservation.
- 8.12 Prepare the freezing solution as follows:
- 8.12.1 40% Plasmalyte-A
- 8.12.2 40% autologous plasma
- 8.12.3 20 % DMSO
- Pull into syringe/transfer pack in this order: 1st DMSO, 2nd Plasmalyte-A, 3rd autologous plasma. Always make about 5 mL more than what is calculated.
- 8.13 Make freezing solution in a transfer pack or syringes depending on the volume needed. Label syringes or transfer pack with patient's name, history number, and barcode label. Refrigerate for exactly 15 minutes. **Set a timer for 15 minutes.** When refrigerating for less than 15 minutes, the solution will not be adequately chilled; storage in a refrigerator for more than 15 minutes could result in the formation of cryoprecipitate.
- 8.14 Be sure to start the control rate freezer ahead of time so **the chamber is pre-chilled to 4°C** before placing product inside the presses to freeze. Refer to *STCL-EQUIP-005 Control Rate Freezing Using CryoMed* for details.
- 8.15 Proceed with a standard cryopreservation of the cells, using an equal volume of the cells and freezing solution.
- 8.16 Add 2 mL of freezing solutions to your 2 mL of blood/plasma for cultures and cryo vials after your last bag. Use 1 ChloraPrep applicator, Steri Perox 6%, or equivalent wipes to clean each BacT bottle. Using sterile technique, inject 1 mL

of the PSC mixture into both an aerobic and anaerobic blood culture bottle. Place the remaining 2 mL into the 2 CRYO vials (1 mL each).

- 8.17 Next take the full bags and heat seal tubing on each bag, using the heat sealer to make at least 1 segment on each bag. Two segments must be made if only 1 bag is being cryopreserved.
- 8.18 Place the 2 CRYO vials in a rack for the freezing chamber.
- 8.19 Quickly place the bags into the bag presses in the CryoMed freezing chamber. Make sure the temperature probe is sandwiched between 2 bags (i.e., do not let the probe rest against the metal plate). Place the rack with vials in the freezing chamber.
- 8.20 Close the chamber door and press RUN again so the freezing program advances to **Step 2**. Refer to *STCL-EQUIP-005 Control Rate Freezing Using CryoMed* to proceed with the cryopreservation of the cells and complete *STCL-EQUIP-005 JA2 Control Rate Freezer Checklist*. At the completion of the freezing run, perform location checks as products are stored in the freezer, if time permits. Refer to *STCL-PROC-033 FRM1 Cellular Product Storage Location Confirmation*.
- 8.21 Record the date, time started Step 2, and initials on the top of the freezing graph. Record the same information on the *STCL-FORM-055 Control Rate Freezer Canister and Vial Storage Log*.
- 8.22 Calculate final bag count from volume cryopreserved.
 - New Volume x Post count = New TNC
 - New TNC/Old TNC= Actual % recovery
 - Actual % Recovery x CD34+/kg = Final CD34+/kg x 10⁶
- 8.23 For apheresis products, order a *HPCA Leukapheresis*, *HPC Phenotype*, *HPCA Basic (if applicable)* in the Laboratory Information System (LIS).
- 8.24 Enter results into EPIC Beaker under *HPC Phenotype* then select “Pend Final”; the medical director will render an interpretation and select “Final Verify” when the results are ready to be released in EPIC.
- 8.25 Enter PBPC worksheet results into EPIC under *HPCA Leukapheresis* orderable and select “SAVE” until Sterility results are available, entered, and results “Final Verified”. *HPCA Basic* results will be entered by designated laboratory staff as results are available and then verified as “Pend Final” so Dr. Kurtzberg can render an interpretation before “Final Verify” is selected.
- 8.26 Call final CD34+ x 10⁶/kg results to designated apheresis voicemail or phone so clinical team can determine if additional apheresis procedures need to be performed (or not). Record result on the back of the Flow worksheet along with the time and initials of the person you reported the result.

9 RELATED DOCUMENTS/FORMS

9.1 COMM-PAS-003 (JA1) Storage Temperature and Expiration of Cellular Products

- 9.2 STCL-FORM-040 Peripheral Blood Progenitor Cell Worksheet Adult
- 9.3 STCL-FORM-049 Processing Lot Numbers – Incoming Cellular Product Processing
- 9.4 STCL-FORM-055 Control Rate Freezer Canister and Vial Storage Log
- 9.5 STCL-EQUIP-005 Control Rate Freezing Using CryoMed
- 9.6 STCL-EQUIP-005 JA2 Control Rate Freezer Checklist
- 9.7 STCL-PROC-033 (FRM1) Cellular Product Storage Location Confirmation
- 9.8 STCL-QA-007 Non-Conforming Products – Receipt, Processing, Distribution, and Disposition
- 9.9 STCL-QA-007 (FRM1) Non-Conforming Products FRM1

10 REFERENCES

- 10.1 Rowley SD, Bensinger WI, Gooley TA, Buckner CD. Effect of cell concentration on bone marrow and peripheral blood stem cell cryopreservation. Blood 83:2731-2736, 1994.
- 10.2 AABB Technical Manual, current edition.

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
06	B. Waters-Pick	<ul style="list-style-type: none"> • Section 5.23 added Steri Perox 6% wipes or equivalent • Added Section 7.2 • Section 8 Notes - added document # to title • Section 8 Notes - changed storage temperature range from 1-10°C to 1-6°C • Section 8.16 added Steri Perox 6% or equivalent wipes • Section 8.21 added document # to title • Section 9 added STCL-FORM-055 and revised section to put all documents in alphabetical order; updated document name in Section 9.8 • Section 10 removed bullet 10.2 “<i>Internal Peripheral Blood Progenitor Cryopreservation Procedure</i>”

Signature Manifest

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