# STEM CELL LABORATORY (STCL)

**DOCUMENT NUMBER:** STCL-PROC-028

**DOCUMENT TITLE:**

Standard Peripheral Stem Cell Processing and Preparation for Infusion or Cryopreservation

**DOCUMENT NOTES:**

FACT # 7D.201.

## Document Information

| | |
|---|---|
| **Revision:** 06 | **Vault:** STCL-Processing-rel |
| **Status:** Release | **Document Type:** STCL |

## Date Information

| | |
|---|---|
| **Creation Date:** 11 Mar 2021 | **Release Date:** 31 May 2021 |
| **Effective Date:** 31 May 2021 | **Expiration Date:** |

## Control Information

| | |
|---|---|
| **Author:** WATER002 | **Owner:** WATER002 |
| **Previous Number:** STCL-PROC-028 Rev 05 | **Change Number:** STCL-CCR-520 |

# STCL-PROC-028
# STANDARD PERIPHERAL STEM CELL PROCESSING AND PREPARATION FOR INFUSION OR CRYOPRESERVATION

## 1 PURPOSE

1.1 To describe the process by which peripheral blood progenitor cells are processed and prepared for immediate infusion or cryopreservation

## 2 INTRODUCTION

2.1 Isolation of circulating hematopoietic progenitor cells by leukapheresis and subsequent infusion of these progenitor cells has been shown to shorten the duration of aplasia following myeloablative therapy. These cells may be infused as a fresh product or cryopreserved for long time storage.

## 3 SCOPE AND RESPONSIBILITIES

3.1 The Medical Directors, Laboratory Manager, and the Stem Cell Laboratory (STCL) staff are responsible for ensuring that the requirements of this procedure are successfully met.

## 4 DEFINITIONS/ACRONYMS

| | | |
|---|---|---|
| 4.1 | STCL | Stem Cell Laboratory |
| 4.2 | HIS | Hospital Information System |
| 4.3 | PBPC | Peripheral Blood Progenitor Cell |
| 4.4 | PSC | Peripheral Stem Cell |
| 4.5 | HPCA | Hematopoietic Progenitor Cell Assay |
| 4.6 | DMSO | dimethyl sulfoxide, 99% |
| 4.7 | ISBT | International Society for Blood Transfusion |
| 4.8 | Demand 128 | Full-face, four quadrant, base label |
| 4.9 | mL | mililiter |

## 5 MATERIALS

Supplies

5.1 Sampling site couplers

5.2 Syringes; various sizes

5.3 Needles; 16 and 19 gauge

5.4 Alcohol wipes

5.5 Dilution tubes, plastic

5.6 Culture bottles, aerobic and anaerobic



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5.7 Cryogenic freezing bags

5.8 Stopcocks, double 3-way

5.9 Stopcocks, nylon double, (99% DMSO-resistant)

5.10 Plasma transfer sets with spike and needle adapter

5.11 1.8 mL cryo vials

5.12 ISBT Demand 128 Labels

5.13 Tie Tags

5.14 Barcode labels

5.15 Pipettor tips

Reagents

5.16 Trypan Blue Stain (0.4%)

5.17 DMSO

5.18 25% Albumin

5.19 Plasmalyte-A

5.20 ChloraPrep Applicators, Steri Perox 6% wipes, or equivalent

## 6 EQUIPMENT

6.1 Sebra bag heat sealer

6.2 Sysmex XS-1000i Hematology Analyzer (or equivalent)

6.3 Biological Safety Cabinet

6.4 Scale (0-2000g)

6.5 Calculator

6.6 Digital Pipettor

6.7 Microscope

6.8 Bag presses

6.9 Cryomed Freezing Chamber

## 7 SAFETY

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

7.2 In order to minimize cross-contamination, a **technologist will only handle/process one cellular product in the biological safety cabinet at any given time**.



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# 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 supervisor or designee to ensure that we follow steps outlined in *STCL-QA-007 Non-Conforming Products - Receipt, Processing, Distribution, and Disposition* procedure and initiate a *STCL-QA-007 FRM1 Non-Conforming Products* form.

8.1 Prior to processing the cells, confirm that the physician orders for processing have been signed and a copy is available in the patient's laboratory file. A printed copy of the patient's (*and donor's if applicable*) information and blood type from Duke Hospital Information System (HIS) should also be in the file along with laboratory generated patient (and donor) labels containing the patient (and donor) name, history number, date of birth, blood type, product type and facility location.

8.2 On day of apheresis, assemble the following paperwork:

8.2.1 STCL-FORM-040 Peripheral Blood Progenitor Cell Worksheet Adult

8.2.2 FLOW-GEN-012 FRM5 STCL Flow Cytometer Worksheet

8.2.3 STCL-PROC-033 FRM1 Cellular Product Storage Location Confirmation

8.2.4 STCL Billing Log

8.2.5 STCL-PROC-022 FRM1 Stem Cell Laboratory Clinical HPCA Worksheet, if applicable

8.2.6 STCL-FORM-049 Processing Lot Numbers– Incoming Cellular Product Processing

8.2.7 STCL-EQUIP-005 JA2 Control Rate Freezer Checklist



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8.3 The peripheral stem cells and patient plasma will be delivered in a cooler by the apheresis nurse or designee in transfer packs that have been clamped or sealed. Check the cellular product to ensure that it meets all labeling criteria to include patient name, history number, date, AC (anticoagulant) volume, patient weight, etc. and that there are no leaks in or damage to the collection bag. Complete *STCL-GEN-009 FRM1 Cellular Product Chain of Custody FRM1* with the person delivering the product.

8.4 Product specific barcodes will arrive with the product. Place a barcode label on all worksheets and the charge sheet generated in the STCL.

8.5 Label the following with patient name, history number, and barcode label.

8.5.1 2 cryo vials, two 0.4 mL saline dilution tubes (for x 5 dilution), 1 empty tube, 1 set of culture bottles, and a cryo vial for HPCA testing if requested (for allogeneic products).

**NOTE**: Be sure to label tubes with the proper dilution used (ie. x 5) so the dilution is reflected on the tube.

8.6 Label three 5 mL polystyrene tubes for ABO testing. First tube should have patient name, history number, barcode, and "A" for the antibody that is to be added. The following 2 tubes must be labeled with patient initials and the Antibody that is to be added.

8.7 Weigh the PSC bag and record the value on the worksheet. Subtract 43.3 g (*weight of an empty bag*) from the weight of 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, the volume may be measured directly using a syringe if only a small volume was collected.

8.8 Place the bag under the hood and insert a sampling site coupler in one of the ports. Clean the sampling site coupler with an alcohol prep pad. Mix the PSCs well and withdraw 1.5 mL or 2.5 mL if HPC testing is requested with a 3mL or 5mL syringe (or equivalent) and 16 or 19 gauge needle (or equivalent). Transfer 1.0 mL to an empty plastic tube and 1.0 mL into HPC sterile cryo vial, if testing was requested.

8.9 Use a digital pipettor to add 0.l mL to each of two 0.4 mL saline dilution tubes. Use one of these tubes for the cell count and flow cytometry sample; use the other tube for viability sample. An additional sample is removed for HPC assay, when indicated for allogeneic products. Add 1 drop with a plastic pipette into each of the ABO designated tubes.

8.10 Add 3 mL of autologous plasma to the remaining 0.5 mL in the syringe. Set this sample aside; it will be used for cultures and cryo vials.

8.11 Run the cell count on the automated cell counter. Multiply the results by the dilution factor of 5. Calculate the total number of cells and the cells/kg. Record these values on the *STCL-FORM-040 Peripheral Blood Progenitor Cell Worksheet Adult.*



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**NOTE**: If product is being prepared for an allogeneic donor, the RECIPIENT'S weight is used in all calculations.

8.12 Initial and date the automated hematology print-out. Indicate that it is an x 5 dilution and ***show the dilution multiplication on the tube***.

8.13 The product must have a final cell count < 500 x $10^6$/mL for adult products and <200 x $10^6$/mL for pediatric products. If the final product count is > 500 x $10^6$/mL (for adult products) or >200 x $10^6$/mL (for pediatric products), the product must be diluted to meet this criteria.

8.13.1 For cellular products requiring DILUTION:

8.13.1.1 Calculate the amount of 25% albumin and autologous plasma that needs to be added. If you do not have enough autologous plasma, use Plasmalyte-A (*you may mix the two*). An example is followed with each step in the calculation.

8.13.1.2 Original WBC count = 535.33 x $10^6$

8.13.1.3 Original Volume = 330 mL

8.13.1.4 (Original WBC count) (x) = 450 x $10^6$/mL, solve for x (factor required for the equation to achieve concentration of < 500 x $10^6$/mL)

8.13.1.5 (535.44) (x) = 450, x = 0.84

8.13.1.6 (1/x) (Original volume) = final volume you need

8.13.1.7 (1/0.84) (330 mL) = 392.86 mL

8.13.1.8 Final Volume – Original volume = amt. of plasma/albumin to add

8.13.1.9 Your ratio is 80% Plasma and 20% albumin.

8.13.1.10 392.86 mL– 330 mL = 62.86 mL to be added (round up), so 63 mL.

8.13.1.11 63 mL x 0.8 = 50.4, 50 mL of Plasma/Plasmalyte-A

8.13.1.12 63 mL x 0.2 = 12.6, 13 mL of Albumin

8.13.1.13 Add your calculated amounts of Albumin and Plasma to your collection bag; mix well. Use appropriate sized syringes and gauge needles for your albumin and plasma.

8.13.1.14 Prepare new tubes for your cell count as in step 8.5 and repeat step 8.11.

8.13.1.15 Ensure your count is now < 500 x $10^6$; if not, add more albumin and plasma then repeat cell count.

8.14 Prepare the remaining dilution tube 150 uL for trypan blue viability analysis and perform the viability count, following *STCL-SOP-022 Viability Counts via*



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*Trypan Blue Dye Exclusion* or other applicable procedure for viability testing. Record the viability on the Peripheral Stem Cell Cryopreservation worksheet.

8.15 Record the requested information on the flow cytometry worksheet and deliver the tube used for the cell count to flow for analysis. If product was diluted, use the original cell count and tube for flow cytometry and HPCA testing. If indicated, complete the HPCA worksheet and deliver it and the HPCA specimen to the cell plating area. Deliver with five (*or amount requested*) barcodes.

8.15.1 Bags must be in the Cryomed freezer within 4 hours from the time the cell count was reported or count must be repeated.

8.16 If the product is not for immediate cryopreservation, add 0.5 mL DMSO to the 5 or 3 mL syringe prepared in step 8. Use 1 ChloraPrep applicator, Steri Perox 6% wipes, or equivalent 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). Place the 2 vials in a rack for the freezing chamber.

8.17 Sandwich the 2 cryo vials between two Styrofoam tube "racks" and place them in a minus 80 degree freezer overnight. These vials will be placed in permanent storage in LN2. Document their LN2 storage location on the cryo vial log and the patient's processing records.

8.18 Record the requested patient and product information on the BacT/Alert 3D log; enter the information into the BacT/Alert 3D system and insert the bottles in the BacT/Alert 3D incubator.

8.19 If the product is to be cryopreserved for future infusion with no further manipulation, calculate the approximate number of freezing bags needed by dividing the collection volume by 63. A target total cell number per bag not to exceed is **$25 \times 10^9$**. Prepare the correct number of bags for the cryopreservation procedure. The maximum volume for each 250 mL cryogenic bag is 70 mL of product.

8.20 Prepare tie tag labels for freezing bags using the prepared labels and barcodes; tie one to each freezing bag. Attach 2 tie tag labels to any bag which would contain more than 60 mL. Prepare the freezer bags by heat sealing or roll clamp the 2 side ports, leaving only the middle port available for use.

8.21 Generate a Demand 128 label for each bag. Record the bag designation on each Demand 128 label. (*i.e. Bag A, Bag B, etc.*) Affix a Demand 128 Label to each freezing bag; refer to labeling procedure *COMM-PAS-003 Labeling Cellular Therapy Products*. Label to ensure that all pertinent information is included when labeling the cellular product(s).

8.22 Take 10% of the collection volume, plus 0.5 mL for cultures/cryo vials, plus 1-2 mL "safety margin" and aseptically draw up that amount of DMSO in appropriate size syringe. Do not use a syringe greater than 30 mL to draw up DMSO. Set these aside. Place a nylon double stopcock (99% DMSO resistant) on a 60 mL syringe and set aside.



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8.23 Insert the coupler end of a plasma transfer set with coupler and needle adapter into the second port on the collection bag. Place the needle adapter into the lower stopcock on the 60 mL syringe, and hang the collection bag on a ring stand or hook. Connect the syringe of DMSO to the upper stopcock.

8.24 Place appropriate amount of presses and cryo vial rack in the Cryomed freezing chamber. Ensure there is enough printing paper; if not change it before starting. Shut the door and select 5.1 for freezing 250 mL cryogenic storage bags. Select 1.1 for freezing 50mL cryogenic storage bags. Press RUN button once so that it says STEP 1.

8.25 Get an ice pack and take it back to the biological safety cabinet to use while preparing the product for cryopreservation.

8.26 Withdraw the calculated volume of peripheral stem cells into the syringe. Slowly, with constant mixing, add DMSO to the cells to result in a 10% by volume concentration of DMSO. Mix well and push into a freezing bag through the Luer adapter. Withdraw any air in the bag, leaving product in the first part of the tubing to make 1 contiguous segment. Place the bag on the ice pack.

8.27 Repeat step 8.26 for as many bags as needed. Since the volume of PSCs will be different each time, adjustments in the volume of the last freezing bag will be necessary. The goal is to mix 10% DMSO with the PSCs prior to freezing.

*For example*: If for the last bag there were only 27 mL of PSC, add 3 mL DMSO and push into a freezing bag.

**NOTE**: The minimum volume for the 250 mL cryogenic storage bag is 30 mL

8.28 Place the needle from your 5 or 3 mL syringe that was prepared in step 10 on the end of the double stopcock. Remove the 60 mL syringe from the double stopcock and attach the syringe of diluted PSCs prepared in step 10. Slowly add 0.5 mL of DMSO. Mix well. Use 1 ChloraPrep applicator, Steri Perox 6% wipes, or equivalent 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). Place the 2 vials in a rack for the freezing chamber.

8.29 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.

8.30 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. Close the chamber door and press RUN again so that now it says Step 2. Refer to *STCL-EQUIP-005 Control Rate Freezing Using Cryomed* to proceed with the cryopreservation of the cells.

8.31 For apheresis products, order a HPCA Leukapheresis, HPC Phenotype, HPCA Basic (*if applicable*) in the Laboratory Information System (LIS).

8.32 Enter flow results into EPIC under HPC PHENOTYPE and select “Pend Final”.



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8.32.1 # viable CD 34+ cells/kg

8.32.2 # viable CD 34+ cells/uL

8.32.3 # viable CD34% (*as percent of viable CD45*)

8.32.4 Enter Additional Flow results as applicable

8.33 Enter PBPC worksheet results into EPIC under Leukapheresis, "Saved" until Sterility results are confirmed. Enter preliminary HPCA Basic results and "Save" in EPIC until plates are read 14 – 16 days later.

8.34 Call final CD34+/kg (x $10^6$) to designated apheresis voice mail or phone. Record on the back of the Flow worksheet the time, initials and who you reported the result.

## 9 RELATED DOCUMENTS/FORMS

9.1 COMM-PAS-003 Labeling Cellular Therapy Products

9.2 FLOW-GEN-012 FRM5 STCL Flow Cytometer Worksheet

9.3 Lab Billing Log

9.4 STCL-EQUIP-005 Control Rate Freezing Using Cryomed

9.5 STCL-EQUIP-005 JA2 Control Rate Freezer Checklist

9.6 STCL-FORM-040 Peripheral Blood Progenitor Cell Worksheet Adult

9.7 STCL-FORM-041 Doctors Orders Adult Stem Cell Transplant Program

9.8 STCL-FORM-049 Processing Lot Numbers – Incoming Cellular Product Processing

9.9 STCL-FORM-055 Control Rate Freezer Canister and Vial Storage Log

9.10 STCL-FORM-062 Stem Cell Laboratory Processing Order Form

9.11 STCL-GEN-009 FRM1 Cellular Product Chain of Custody Form

9.12 STCL-PROC-022 FRM1 Stem Cell Laboratory Clinical HPCA Worksheet, if applicable

9.13 STCL-PROC-033 FRM1 Cellular Product Storage Location Confirmation

9.14 STCL-QA-007 Non-Conforming Products - Receipt, Processing, Distribution, and Disposition

9.15 STCL-QA-007 FRM1 Non-Conforming Products FRM1.

9.16 STCL-SOP-022 Viability Counts via Trypan Blue Dye Exclusion

## 10 REFERENCES

10.1 Peters W, Olson G. Cryopreservation of bone marrow procedure. Sidney Farber Cancer Institute, 1985. Personal communication.

10.2 Technical Manual for Makel 1010A Micro Computer Programmable Freezing Controller. CryoMed, New Baltimore, Michigan.



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10.3 Edwards S, Shpall E. Bone marrow cryopreservation procedure, Cryopreservation Laboratory Procedure Manual, Autologous Bone Marrow Transplant Program, Duke University Medical Center, 1988.

10.4 Korbling M, Martin H. Autologous blood stem cell transplantation: A new treatment concept for patients with malignant lymphohematopoietic disorders, Autologous Bone Marrow Transplantation, 1985.

10.5 Peters W. Amendment to the protocol #05, A Phase I/II clinical trial of recombinant human granulocyte macrophage colony stimulating factor (rGM-CSF) in autologous bone marrow transplantation, March 1989.

## 11 REVISION HISTORY

| Revision No. | Author | Description of Change(s) |
|---|---|---|
| 06 | B. Waters-Pick | • Section 5.20 added Steri Perox 6% wipes or equivalent<br>• Section 7.2 added<br>• Section 8 last bullet point in Notes added document # and corrected document titles<br>• Section 8.2.1 and 8.2.3 corrected document titles<br>• Section 8.3 added document title<br>• Section 8.11 corrected document title<br>• Sections 8.16 and 8.28 added Steri Perox 6% wipes or equivalent to these sections<br>• Section 9 added # and titles, where needed, and organized so document are listed alphabetically |



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## Signature Manifest

**Document Number:** STCL-PROC-028 **Revision:** 06

**Title:** Standard Peripheral Stem Cell Processing and Preparation for Infusion or Cryopreservation

**Effective Date:** 31 May 2021

All dates and times are in Eastern Time.

### STCL-PROC-028 Standard Peripheral Stem Cell Processing and Prep for Infusion or Cryopreservation

### Author

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Barbara Waters-Pick (WATER002) | | 17 May 2021, 03:43:51 PM | Approved |

### Management

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Barbara Waters-Pick (WATER002) | | 17 May 2021, 03:44:09 PM | Approved |

### Medical Director

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Joanne Kurtzberg (KURTZ001) | | 17 May 2021, 04:53:55 PM | Approved |

### Quality

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Isabel Storch De Gracia (IMS19) | | 20 May 2021, 08:44:53 AM | Approved |

### Document Release

| Name/Signature | Title | Date | Meaning/Reason |
|---|---|---|---|
| Sandra Mulligan (MULLI026) | | 25 May 2021, 08:01:07 PM | Approved |