



# STEM CELL LABORATORY (STCL)



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**DOCUMENT TITLE:**

Thawing Human-CT-derived Mesenchymal Stem Cells (MSCs) for Infusion JA3

**DOCUMENT NOTES:**

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# **STCL-SOP-028 JA3**

## **THAWING HUMAN CORD TISSUE-DERIVED MESENCHYMAL STEM CELLS (MSCs) FOR INFUSION**

### **1 PURPOSE**

- 1.1 The purpose of the procedure is to describe the process for thawing and preparing hCT-MCs for patient administration.

### **2 INTRODUCTION**

- 2.1 Human cord tissue derived mesenchymal stromal cells are being manufactured in the Duke GMP cell manufacturing laboratory from consented, unrelated donor, healthy baby cord tissue and cord blood donors to manufacture hCT-MSC for use in regenerative and cell therapy trials in patients with various conditions amenable to this therapy. The cells are expanded to P2, cryopreserved in multi-compartment cryobags and frozen in the vapor phase or under liquid nitrogen. On the day of administration, they are thawed and counted. The appropriate dose is prepared and transported to the patient bedside for administration.

### **3 SCOPE AND RESPONSIBILITIES**

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

### **4 DEFINITIONS/ACRONYMS**

- |     |      |                           |
|-----|------|---------------------------|
| 4.1 | hCT  | human Cord Tissue         |
| 4.2 | MSC  | Mesenchymal Stem Cells    |
| 4.3 | CBT  | Cord Blood Tissue         |
| 4.4 | STCL | Stem Cell Laboratory      |
| 4.5 | mL   | Milliliter                |
| 4.6 | BSC  | Biological Safety Cabinet |
| 4.7 | N/A  | Not Applicable            |
| 4.8 | HSA  | human serum albumin       |

### **5 MATERIALS**

- 5.1 60 inch ext Set, apv 2.4 ml
- 5.2 Albumin – Human 25%
- 5.3 Alcohol Pads
- 5.4 BacT Alertt SN
- 5.5 BacT/Alert SA

5.6 ChloroPrep® SEPP® applicators

5.7 Clamp, rubber shod

5.8 Intralock 3 way stopcock

5.9 Needles 16 Gauge

5.10 Plasmalyte-A

5.11 Syringe 10 ml

5.12 Syringe 30 ml

5.13 Sterile Access

5.14 Sterile bag

## 6 EQUIPMENT

6.1 Biological Safety Cabinet

6.2 Water bath

6.3 Transport container (cooler)

6.4 Cellometer Auto 2000 (or equivalent)

## 7 SAFETY

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

## 8 PROCEDURE

### 8.1 Pre-Thaw

8.1.1 Delivery time of MSCs to the STCL by GMP Lab staff will be pre-arranged in advance of the infusion.

8.1.2 Frozen cells (CBT-derived MSCs) will be delivered in a transport container (cooler) to the Stem Cell Laboratory (STCL) already inside a sterile bag.

8.1.3 A chain of custody form, provided by the GMP Lab staff, will accompany the MSC product to the STCL. STCL staff will sign the chain of custody form (*CT2-MSC-009 FRM3 Chain of Custody for hCT Final Product*) and make a copy to place in the laboratory file.

8.1.4 Assign a STCL ISBT 128 barcode to each product so it can be traced back to the patient.

8.2 Plasmalyte-A and human albumin (USP) 25% will be prepared in the STCL on the day of infusion according to the following instructions:

8.2.1 For every 10 mL volume, use 8.0 mL of Plasmalyte-A and 2.0 mL of human albumin (USP) 25%. Make at least 30 mL of the solution for each 20% fraction thawed.

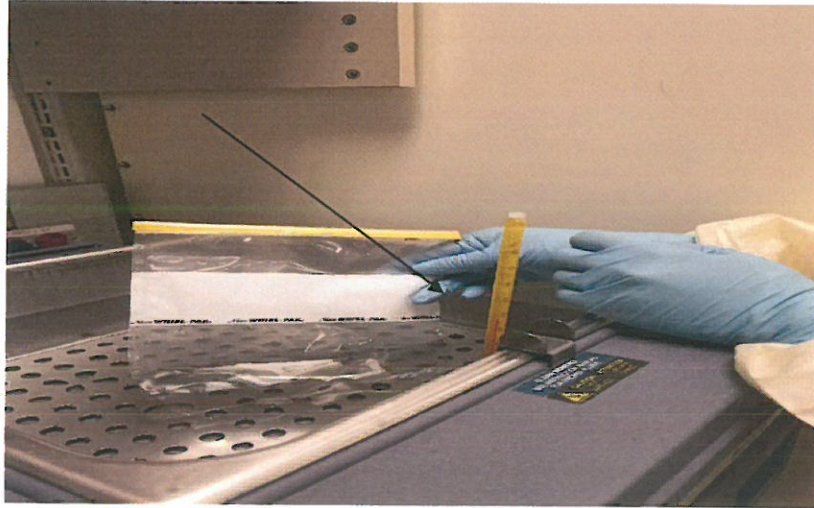
**(NOTE: This dilution will yield a final concentration of 5% HSA).**



8.3 After labeling of the product has been verified by STCL and GMP staff:

8.3.1 Confirm the water bath temperature is 37°C (+/- 1°C). Start thawing the cells (*already in a sterile bag*) in the water bath.

Thawing  
MSCs in a  
sterile bag

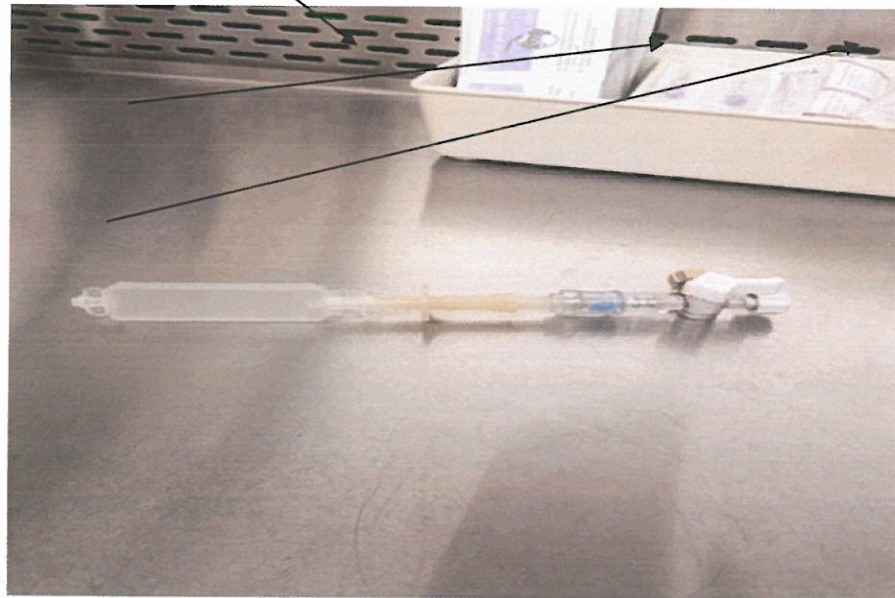


- 8.3.2 Thaw product until it is a slushy consistency. The time the bag is removed from the water bag is considered the “thaw time” and expiration time will be assigned at that point.
- 8.3.3 Check carefully to ensure there are no leaks in the bag. **If a leak is noted, do NOT infuse the product. Call the GMP Lab immediately so they can identify another fraction for the patient and notify the medical director (or designee).**
- 8.3.4 Post thaw, take the product to the biological safety cabinet (BSC), remove it from the sterile bag, and wipe the outside of the thawed product bag.
- 8.3.5 Insert a needless adapter into the port of the bag. Attach a stop cock to the needless adapter.

Thawed bag  
of MSCs

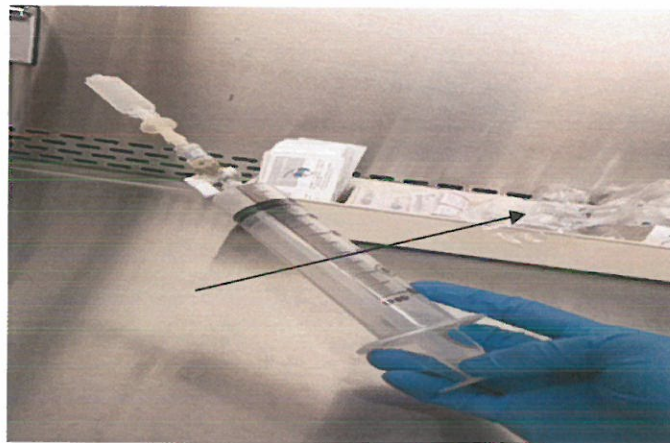
Needless adapter

Stopcock



- 8.3.6 Pull up 5 mL of Plasmalyte-A/albumin solution in a syringe and attach to the stopcock. Slowly add the Plasmalyte-A/albumin solution to the thawed product bag. Gently mix the diluted product between the bag and syringe. Pull the total volume (~10 mL) into the syringe.

Syringe containing  
Plasmalyte-A and  
Human Albumin (25%)





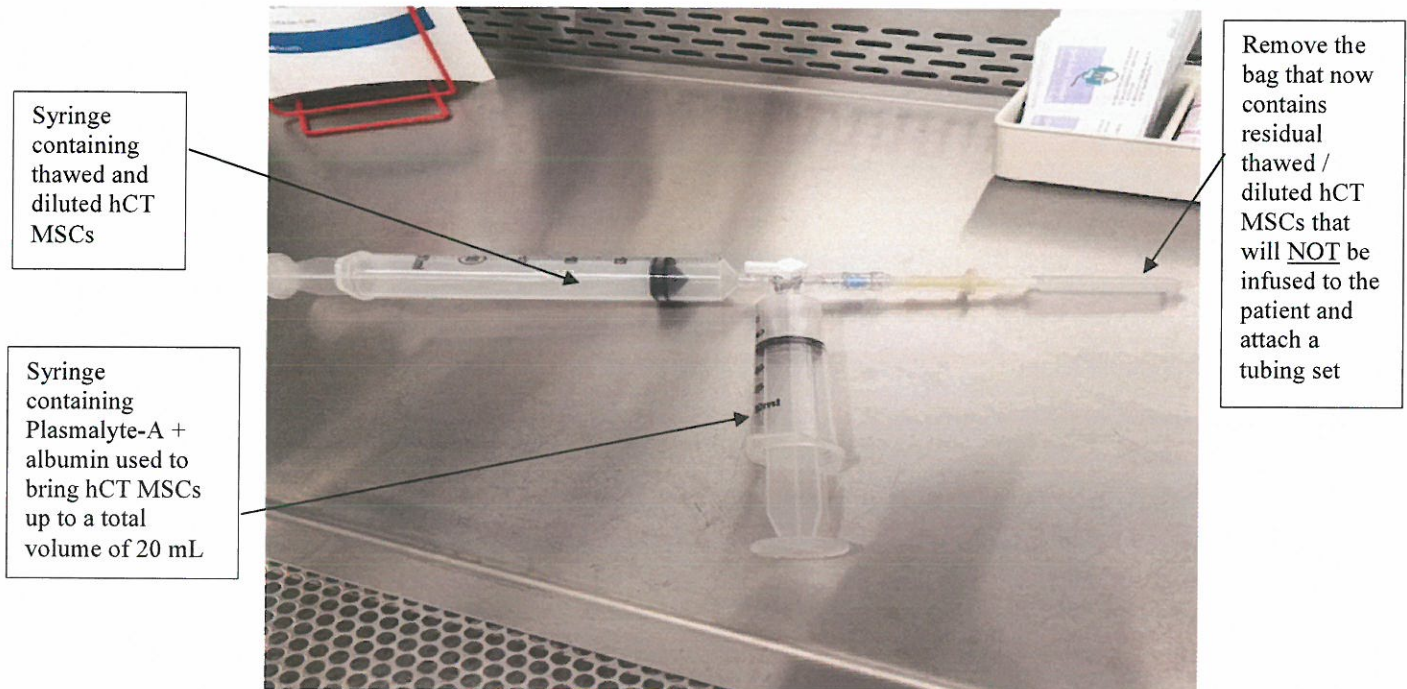
- 8.3.7 Remove 0.2 mL from the syringe using the stop cock and add to test tube. Perform cell count and viability in duplicate using the Cellometer instrument.

Syringe used to remove QC sample for cell count and viability



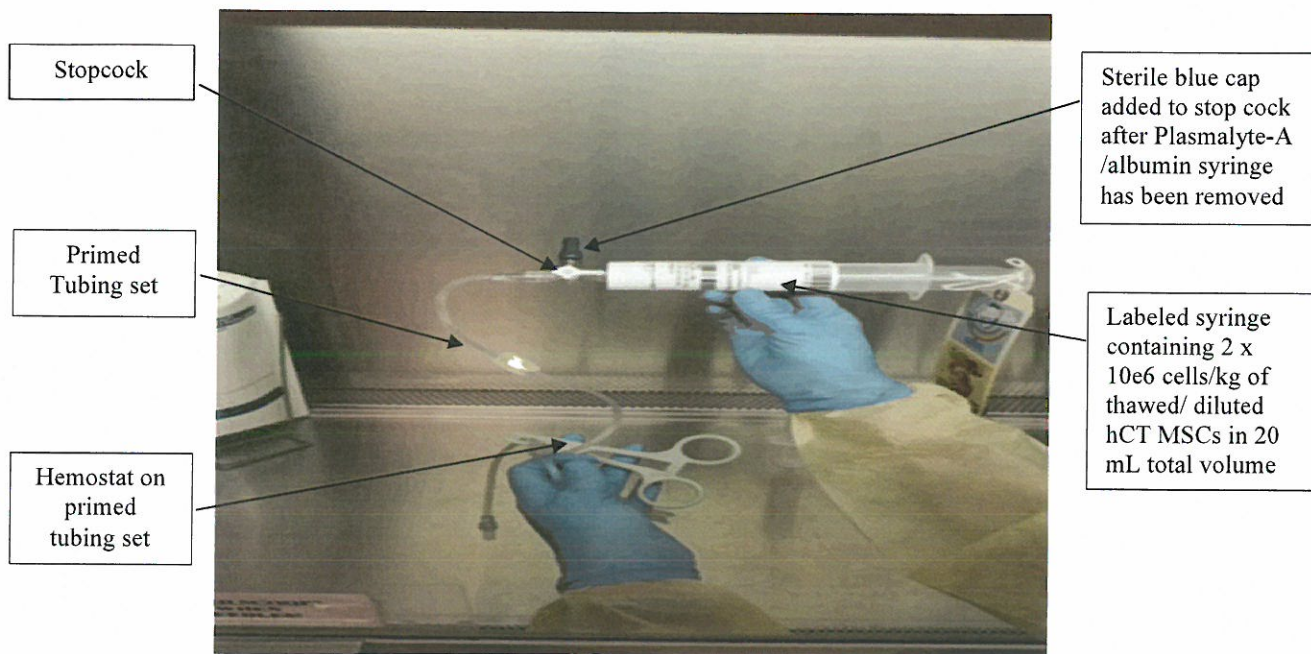
- 8.3.8 Duplicate cell count and viability results will be averaged for reporting purposes.
- 8.3.9 **In order to meet release criteria, the viability must be  $\geq 70\%$ .**
- 8.3.10 Calculate the volume required to give patient an infusion dose of  $2 \times 10^6$  cells/kg based on the total nucleated cell count /mL obtained from the Cellometer.
- 8.3.11 Send an e-mail to Dr. Kurtzberg (*or designee*) to include: cell count, viability, patient's current weight, and calculation of volume required to administer a dose of  $2 \times 10^6$  cells/kg so the information can be VERIFIED before the dose is distributed for infusion.
- 8.3.12 Dr. Kurtzberg (*or designee*) will verify the math to ensure the dose was calculated correctly and respond via e-mail.
- 8.3.13 Unless otherwise instructed, remove the volume of thawed product needed to give a dose of  $2 \times 10^6$  cells/kg into a 60 mL syringe.  
(**EXAMPLE:** If 6 mL of the 10 mL volume is calculated to yield a dose of  $2 \times 10^6$  cells/kg, 4 mL will not be infused to the patient).
- 8.3.14 The residual thawed/diluted sample that will NOT be infused to the recipient should be diverted back into the bag using the stopcock; this sample will be used for sterility testing.

- 8.3.15 Add Plasmalyte-A/albumin solution to thawed product volume calculated to yield an infusion dose of  $2 \times 10^6$  cells/kg to yield a final volume of 20 mL. (**EXAMPLE:** If 6 mL of thawed/diluted hCT MSCs will yield a dose of  $2 \times 10^6$  cells/kg, add 14 mL of Plasmalyte-A/albumin to give a final volume of 20 mL).



- 8.3.16 Remove the empty bag and attach a tubing set. Prime the tubing to the end of the line to ensure there are no air bubbles. Add hemostat and CLAMP tightly so there are no leaks.
- 8.3.17 Remove the syringe containing Plasmalyte-A/albumin and replace it with a sterile cap.





8.3.18 Residual product that will not be infused to the recipient (*now in the bag*) is used to set up the 14-day sterility culture on the final product.

8.3.19 Label the final product with the demand 128 label assigned product code # S3385 (*MSC-Umbilical Cord Blood*), the ISBT128 barcode assigned to this patient product, and tie tag containing the patient's demographic information (*ie. name, history #, DOB, blood type, sex, etc.*) and MSC-related details (*ie. Lot #, Dose #, Study Strata #, etc.*).

8.4 Assign a **four (4) hour expiration time** to the final product starting at the time the thawed product was removed from the water bath.

8.5 The Certificate of Analysis (C of A) will be completed by the lab staff and signed by a representative from the QSU to approve/release the final product for clinical use.

8.6 Once the 14-day sterility results are available, complete the second page of the C of A and obtain signature from QSU representative. File all related documentation in the patient's lab file.

## 9 RELATED DOCUMENTS/FORMS

9.1 **STCL-FORM-043** Thawing and Infusion Worksheet

9.2 **STCL-DIST-003** Cellular Product Distribution Form

9.3 **STCL-GEN-009 FRM2** Cellular Product-Sample Chain of Custody

9.4 **STCL-FORM—044** Processing Lot Numbers – 37 Degree Celsius Thaws

9.5 **STCL-FORM-071** Certificate of Analysis MSC, Umbilical Cord – Post Thaw

9.6 **CT2-MSC-009 FRM3** Chain of Custody for hCT Final Product



## 10 REFERENCES

10.1 N/A

## 11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
02	B. Waters-Pick	<ul style="list-style-type: none"><li>• Section 4 – Added HSA Human Serum Albumin</li><li>• Section 8.2.1 – Modified amount of HSA and plasmalyte-A needed to yield a 5% final concentration of HSA instead of 1% HSA as was previously reflected in the document.</li></ul>

**Signature Manifest****Document Number:** STCL-SOP-028 JA3**Revision:** 02**Title:** Thawing Human-CT-derived Mesenchymal Stem Cells (MSCs) for Infusion JA3

All dates and times are in Eastern Time.

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