



# STEM CELL LABORATORY (STCL)



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**DOCUMENT TITLE:**

Thawing Human Cord Tissue-derived MSCs for Infusion - IMPACT Study JA4

**DOCUMENT NOTES:**

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# STCL-SOP-028 JA4

## THAWING HUMAN CORD TISSUE-DERIVED MESENCHYMAL STEM CELLS (MSCs) FOR INFUSION – IMPACT STUDY

### 1 PURPOSE

- 1.1 The purpose of the procedure is to describe the process for thawing and preparing hCT-MSCs or Placebo (*consisting of Plasmalyte-A + 1% DMSO*) doses for patients randomized to participate in IMPACT Protocol IND 17313 “*A Phase II Study of hCT-MSC, An Umbilical Cord-Derived Mesenchymal Stromal Cell Product, in Children with Autism Spectrum Disorder*” and other similar MSC Studies.

### 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 and cryopreserved in multi-compartment cryobags in vapor phase or under liquid nitrogen. In the future, these products maybe cryopreserved in MedSep 80/20 bags, vials, etc. On the day of administration, the product is thawed and counted to include TNC and viability. The appropriate dose is prepared and transported to the patient bedside for administration.
- 2.2 The age range of patients enrolled on this study is  $\geq 4$  to  $\leq 8$  years of age.
- 2.3 Phase II, prospective, randomized, blinded, cross over, clinical trial designed to access the efficacy of IV dosing of hCT-MSC for improving social communication abilities in young children with ASD.
- 2.4 Arm A – Single dose of  $\leq 6 \times 10^6$  hCT-MSC/kg followed by placebo in 6 months
- 2.5 Arm B – Single dose of placebo followed by  $\leq 6 \times 10^6$  hCT-MSC/kg in 6 months
- 2.6 Volume of infusions could vary from 10 – 50 mL
- 2.7 Placebo consists of Plasmalyte-A + 1% DMSO-Dextran (*do not have to freeze*)

### 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
- 4.9 C of A Certificate of Analysis

## **5 MATERIALS**

- 5.1 60 inch ext Set, apv 2.4 ml
- 5.2 Albumin – Human 25%
- 5.3 Alcohol Pads
- 5.4 BacT Alert SN
- 5.5 BacT/Alert SA
- 5.6 ChloroPrep® SEPP® applicators
- 5.7 Clamp, rubber shod
- 5.8 Intralock 3 way stopcock
- 5.9 Double stopcock
- 5.10 Needles 16 Gauge
- 5.11 Plasmalyte-A
- 5.12 Syringe 10 mL
- 5.13 Syringe 30 mL
- 5.14 Syringe 60 mL
- 5.15 Sterile Access
- 5.16 Sterile bag
- 5.17 Needle-free Spike (DMSO-resistant)
- 5.18 Cellometer AOPI Staining Solution
- 5.19 Cell Counting Chambers Cellometer Auto 2000
- 5.20 Steri-Perox® 6% Wipe
- 5.21 CryoSolve DMSO USP / EP 55% w/v Dextran-40 USP / EP 5% w/v Akron

## **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.
- 7.2 A technologist will only handle/process one cellular therapy product (*ie. HPC, Apheresis, HPC, Marrow, HPC, Cord, MSCs, etc*) in the biological safety cabinet at any given time.

**NOTE:** This is specific for the processing section of the laboratory; HPCA and Flow Cytometry testing sections of the laboratory perform batch testing whenever practical.

- 7.3 When using ChloroPrep® SEPP® applicators (*or equivalent*) to disinfect the septum of the culture bottles, swab thoroughly and allow at least 30 seconds (*per supply instructions*) drying time **BEFORE** inoculating the sample into the bottles.

## 8 PROCEDURE

### 8.1 Pre-Thaw

- 8.1.1 Delivery time of MSCs to the STCL by GMP Lab staff will be arranged in advance or product may be stored on-site in a vapor LN2 freezer in the STCL to ensure product is available on short notice.
- 8.1.2 Frozen cells (CBT-derived MSCs) will be delivered in a transport container (cooler) already inside a sterile bag to the Stem Cell Laboratory (STCL).
- 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-MS-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 12 mL of the solution for each 20% fraction thawed. If thawing five (5) 20% bags, 60 mL of Plasmalyte-A/HSA should be prepared.

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

- 8.3 If patient randomizes to get **PLACEBO** instead of hCT-MSCs, add 49.5 mL of Plasmalyte-A and 0.5 mL of CryoSolve DMSO USP / EP 55% w/v Dextran-40 USP / EP 5% w/v Akron to a 60 mL syringe (attached to a stopcock).

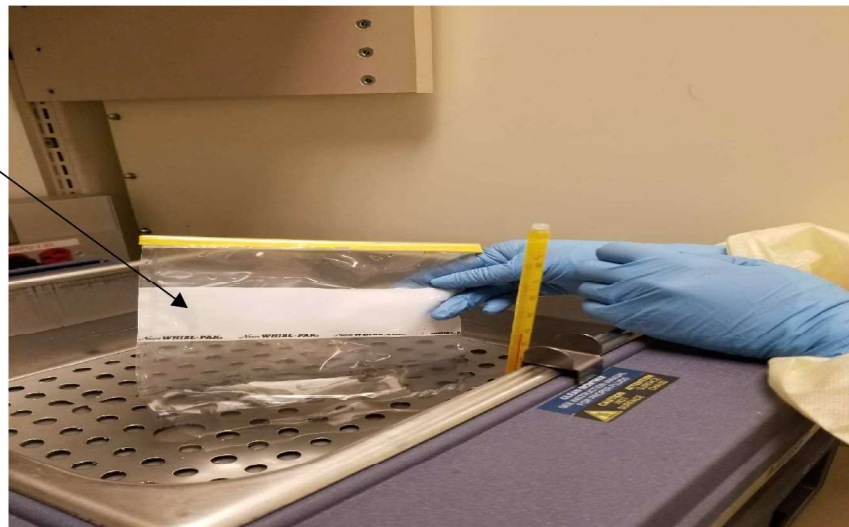
- 8.3.1.1 **Syringe A** will contain the Plasmalyte-A (*refer to pictures in sections 8.4.6 and 8.4.7*).

- 8.3.1.2 **Syringe B** will contain Plasmalyte-A + DMSO (1%) going to recipient
- 8.3.1.3 **Syringe C** will contain DMSO that will be added to **Syringe A**.
- 8.3.1.4 The patient will get ~ 40 mL of the placebo (Plasmalyte-A + 1% DMSO) remaining in **Syringe B**; new stopcock will be added and tubing will be primed.
- 8.3.1.5 The remaining ~ 10 mL (in **Syringe A**) will be used for 14-day culture; inoculate 5 mL of sample into each culture bottle.  
(**NOTE: Please follow instructions for inoculation of culture bottles reflected in Section 7.3**)
- 8.3.1.6 Page 2 of 2 of the C of A will be signed after results of the 14-day sterility testing are complete.
- 8.3.1.7 Randomization will be done in REDCAP the day before the scheduled infusion date by designated unblinded STCL staff to determine if recipient will get PLACEBO 1<sup>st</sup> or hCT-MSCs 1<sup>st</sup>. After six months, the recipient will get an infusion of the product that was not given on the first infusion date. Recipients, after one year, will have received one infusion of Placebo and one infusion of hCT-MSCs.

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

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

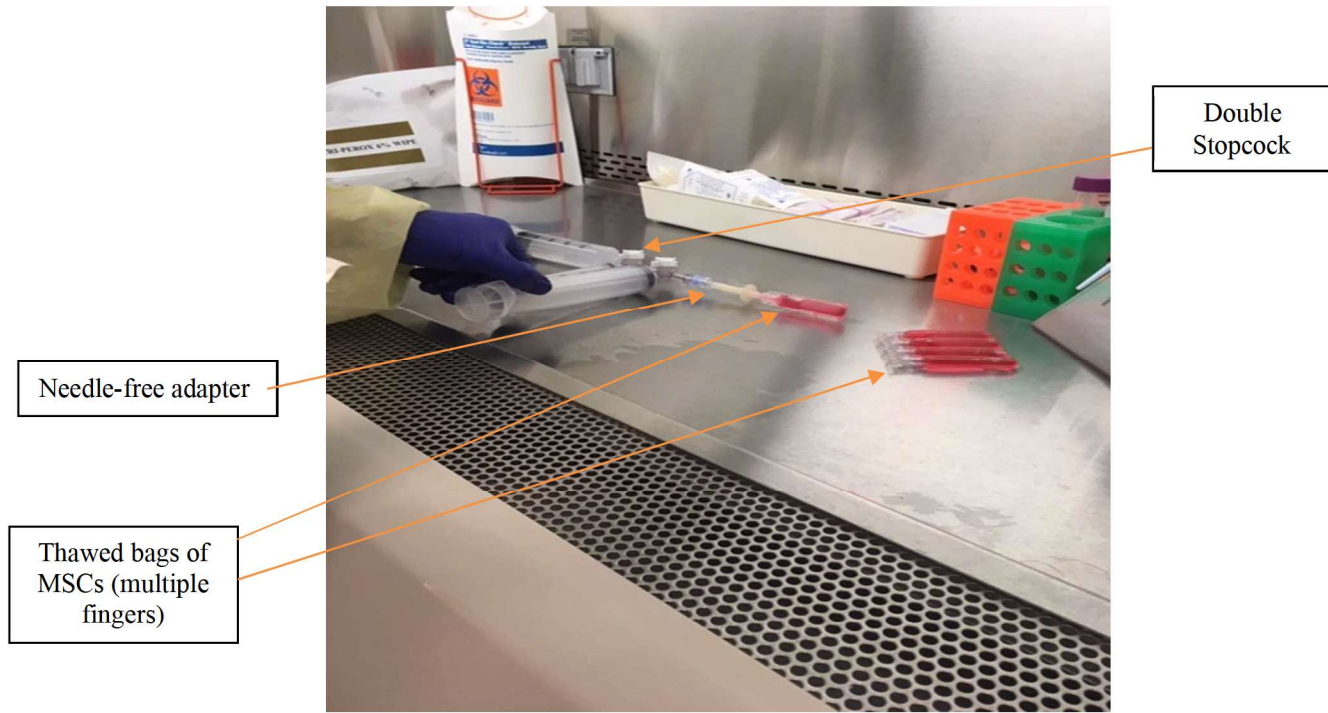
Thawing  
MSCs in a  
sterile bag



- 8.4.2 Thaw product until it is a slushy consistency. The time the bag is removed from the water bath is considered the “thaw time” and expiration time will be assigned at that point (**4 hour** expiry post thaw).
- 8.4.3 Check carefully to ensure there are no leaks in the bag. **If a leak is noted, do NOT infuse the product. Call the medical director immediately who will call the GMP Lab so another fraction for the patient can be identified, if appropriate.**

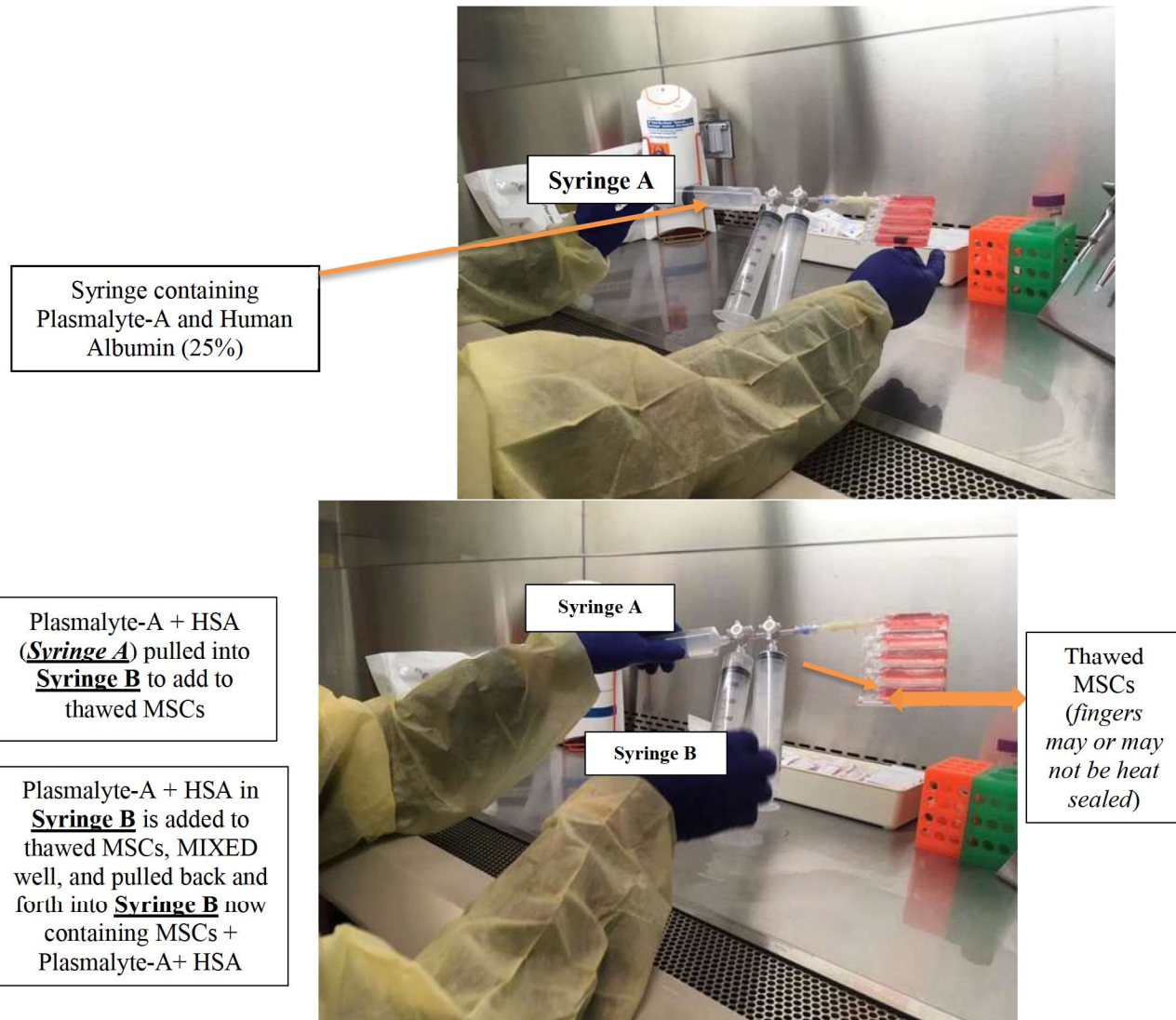


- 8.4.4 Post thaw, take the product to the biological safety cabinet (BSC), remove it from the sterile bag, and wipe the outside of the thawed product bag using Steri-Perox®6% Wipe or equivalent.
- 8.4.5 Insert a needleless adapter into the port of the bag. Attach a double stopcock to the needle-free spike (adapter).



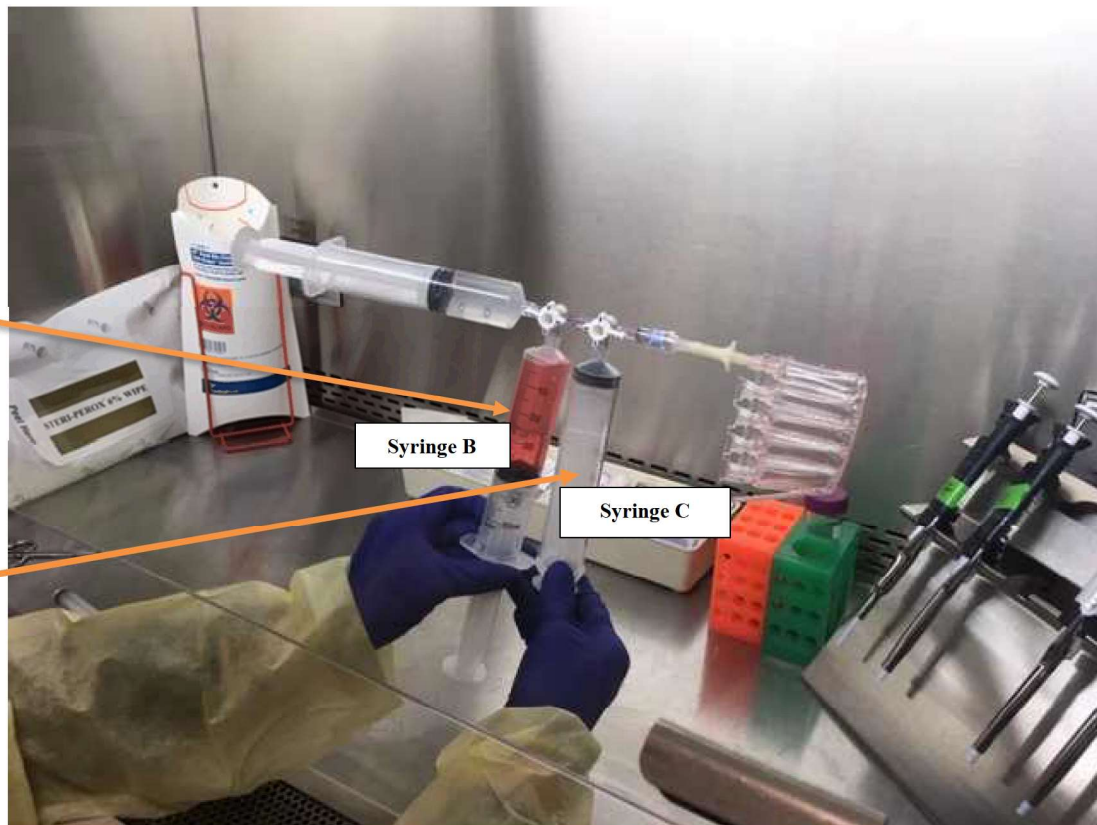
- 8.4.6 Pull up the appropriate volume of Plasmalyte-A/albumin solution in a syringe to provide a 1:1 dilution and attach to the stopcock. Slowly add the Plasmalyte-A/albumin solution to the thawed product bag(s). Gently and thoroughly mix the diluted product back and forth between the bag(s) and **Syringe B**. Pull the total volume into the syringe after mixing. *(In the picture below, ~ 20 mL of MSCs (4 mL per bag x 5 bags) + ~ 20 mL of Plasmalyte-A + HSA (to provide 1:1 dilution) would total of volume of ~ 40 mL).*

**NOTE:** Dilution may vary from 1:1 depending on the patient's weight and/or based on instructions from the medical director.



- 8.4.7 Once MSCs are mixed thoroughly with Plasmalyte-A + HSA, quickly remove 0.2 mL from the syringe using the stop cock and add to test tube. Perform cell count and viability using the Cellometer instrument.





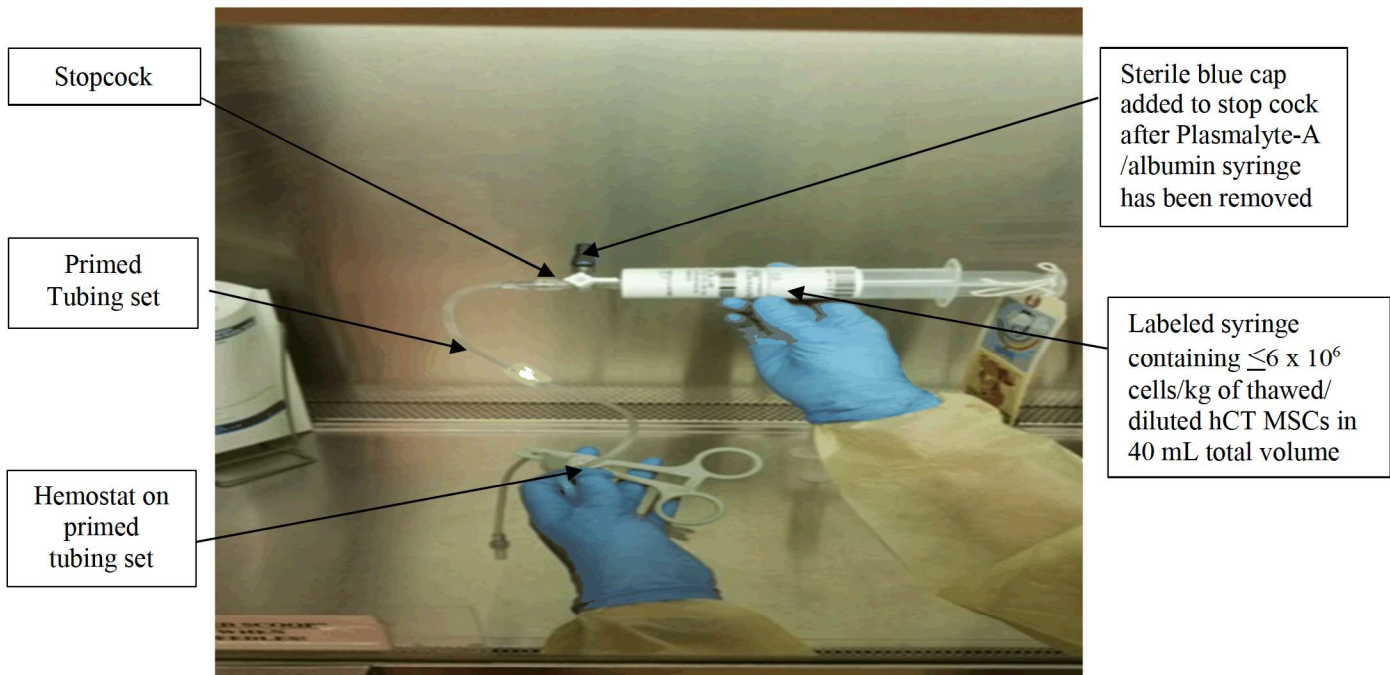
**Syringe B** (is used to remove QC sample for cell count and viability) and then the appropriate volume of thawed/ diluted MSCs (needed to provide dose of  $\leq 6 \times 10^6$  hCT-MSCs/kg) will be moved into **Syringe C**.

- 8.4.8 **NOTE:** In order to meet release criteria, the viability must be  $\geq 70\%$  and  $\leq 6 \times 10^6$  hCT-MSCs/kg (based on medical director's instruction).
- 8.4.9 Calculate the volume required to give patient an infusion dose of  $\leq 6 \times 10^6$  cells/kg based on the total nucleated cell count /mL obtained from the Cellometer.
- 8.4.10 Send an e-mail to Dr. Kurtzberg (or designee) to include the following: cell count, viability, patient's current weight, and calculation of volume required to administer a dose of  $\leq 6 \times 10^6$  cells/kg so the information can be **VERIFIED** before the dose is distributed for infusion.
- 8.4.11 Dr. Kurtzberg (or designee) will verify the math to ensure the dose was calculated accurately and provide confirmation via e-mail.
- 8.4.12 Unless otherwise instructed, remove the volume of thawed product needed to provide a dose of  $\leq 6 \times 10^6$  cells/kg into a 60 mL syringe (Syringe C) (**EXAMPLE:** If 26 mL of the 30 mL volume is calculated to yield a dose of  $\leq 6 \times 10^6$  cells/kg, 4 mL will not be infused to the patient).
- 8.4.13 Any residual, thawed/diluted sample, that is **NOT** infused to the recipient, will be remain in Syringe B; add Plasmalyte-A/Albumin to Syringe B yielding a total of 10 mL to be used for 14-day sterility testing.



**NOTE: If no residual sample remains available in Syringe B, add 10 mL of Plasmalyte-A/Albumin to Syringe B and inoculate 5 mL to each culture bottle following the instructions outlined in Section 7.3.**

- 8.4.14 Add Plasmalyte-A/albumin solution to thawed product volume calculated to yield an infusion dose of  $\leq 6 \times 10^6$  cells/kg to yield a final volume of 40 -50 mL. (**EXAMPLE:** If 26 mL of thawed/diluted hCT MSCs will yield a dose of  $\leq 6 \times 10^6$  cells/kg, add 14 - 24 mL of Plasmalyte-A/albumin to give a final volume of 40 – 50 mL).
- 8.4.15 Remove the **Syringe C** (containing calculated thawed hCT-MSC dose containing  $\leq 6 \times 10^6$  cells/kg), add a NEW stopcock and a tubing set. Prime the tubing set to the end of the line to ensure there are no air bubbles. Add hemostat and CLAMP tightly so there are no leaks during transport.
- 8.4.16 Replace the other stopcock cap with a sterile cap to ensure it doesn't come off during transport to the infusion location.



- 8.4.17 Label the final product with the demand 128 label assigned custom product code # D0020400 (*IMPACT Blinded Placebo Controlled*), 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 Study-related details (*ie. IND# , etc.*).
- 8.5 Assign a **four (4) hour expiration time** to the final product starting at the time the thawed product was removed from the water bath.
- 8.6 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 TNCs/kg and viability only IF the recipient was randomized to get a hCT-MSC infusion.

- 8.7 If the recipient randomized to get a placebo infusion, QSU will sign page 2 of the C of A once the 14-day sterility results are available.
- 8.8 The C of A will remain in the recipient's lab file and will **NOT** accompany the product at the time of distribution to ensure the "blind is not compromised". STCL staff is aware of the randomization for each recipient but the clinical staff is NOT supposed to know whether the recipient is getting hCT-MSCs or placebo.
- 8.9 To serve as a **visual reminder** for the STCL staff **NOT** to release the C of A from the laboratory (*which could result in "breaking the blind" for a patient*), a NOTE has been attached to the C of A in an effort to ensure the blind is not compromised (*see picture below*)

InfoCard #: STCL-FORM-071 Rev. 03 Effective Date: 31 Dec 2020

**CERTIFICATE of ANALYSIS**  
**MSC, Umbilical Cord - Post Thaw**

Patient Demographic Label <b>(PLACE HERE)</b> <small>(Name, History #, DOB, Blood Type, Sex)</small>	Product Demographic Label <b>(PLACE HERE)</b> <small>(Lot #, Product Description, etc)</small>
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Date of Infusion: \_\_\_\_\_ Patient weight (kg): \_\_\_\_\_  
 Lot #: \_\_\_\_\_ Dose #: \_\_\_\_\_ Study: \_\_\_\_\_  
 Date of Cryopreservation: \_\_\_\_\_ ISBT 128 Barcode: \_\_\_\_\_  
 Date of Thawing: \_\_\_\_\_

*Specifications for Final Release of MSC, Umbilical Cord for Infusion - Post Thaw*

**Do NOT release the Certificate of Analysis  
(C of A) from the Stem Cell Laboratory**

☐ Pro00102894 (IMPACT) IND #17313: Infusion dose =  $\leq 6 \times 10^6$  cells/kg x 1 dose  
☐ Pro00105410 (MASC) IND #19968: Infusion dose =  $\leq 1 \times 10^6$  cells/kg/dose (MAX of 100 x 10e6 total cells per dose) daily x 3 doses  
☐ Pro00106044 (MISTIC) IND #23378: Infusion dose =  $\leq 2 \times 10^6$  kg/dose (MAX dose of 100 x 10e6 total cells per dose) daily x 3 doses  
☐ Other: \_\_\_\_\_

Technologist Completing Specifications: \_\_\_\_\_ Date: \_\_\_\_\_

Review of Final Product for Suitability - Post Thaw:

Product meets or exceeds all release criteria (check ONE): ☐ YES ☐ NO  
 Product is released for clinical use (check ONE): ☐ YES ☐ NO

Released by: \_\_\_\_\_ Date: \_\_\_\_\_  
 Director, Quality Assurance (or designee)

STCL-FORM-071 Certificate of Analysis for MSC, Umbilical Cord – Post Thaw  
 Stem Cell Laboratory, DUMC  
 Durham, NC

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## 8.10 TROUBLESHOOTING

- 8.10.1 Option 1: If viability and TNC meet criteria, QSU can sign the C of A to release the product. (**NOTE:** *The C of A will remain in the lab file to ensure blinding is not compromised*).
- 8.10.2 Option 2: If viability meets release criteria but TNC does not, thaw additional hCT-MSC product, pool the thawed products together and perform viability and TNC counts. If both meet criteria, QSU will sign the C of A to release the product. (**NOTE:** *The C of A will remain in the lab file to ensure blinding is not compromised*).



- 8.10.3 Option 3: If the viability and TNC criteria fail, pull fresh hCT-MSCs products and starting from the beginning to achieve cell dose/kg until viability and TNC meet criteria reflected on the C of A. (**NOTE**: *The C of A will remain in the lab file to ensure blinding is not compromised*).

## 9 RELATED DOCUMENTS/FORMS

- 9.1 **STCL-DIST-003** Cellular Product Distribution Form
- 9.2 **STCL-FORM-043** Thawing and Infusion Worksheet
- 9.3 **STCL-FORM-044** Processing Lot Numbers – 37 Degree Celsius Thaws
- 9.4 **STCL-FORM-071** Certificate of Analysis MSC, Umbilical Cord – Post Thaw
- 9.5 **STCL-GEN-009 FRM2** Cellular Product-Sample Chain of Custody
- 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)
03	B. Waters-Pick	<ul style="list-style-type: none"> <li>Made minor edits and format changes throughout the document that had no impact on the content of the procedure</li> <li>Changed references throughout the document referring to “dose of 6 x 10e6 cells/kg” to reflect dose of “<math>\leq 6 \times 10^6</math> cells/kg” based on the instructions provided by the medical director when each dose is prepared for distribution</li> <li>Added section 7.3 to provide drying time after using chloraprep swab</li> <li>Section 8.3.1.5 added “inoculate 5 mL of sample into each culture bottle” and added NOTE</li> <li>Section 8.4.3 – modified wording to including calling the medical director before GMP Lab in the event that a leak occurs</li> <li>Section 8.4.6 – Added NOTE regarding the dilution of the product in the event the medical director provides instruction otherwise</li> <li>Section 8.4.8 – added “based on medical director’s instruction”</li> <li>Section 8.4.13 – changed wording to provide instructions IF no thawed/diluted sample remain available in syringe B for 14-day sterility testing</li> <li>Section 8.4.13 – Added NOTE providing additional detail regarding sterility sample</li> <li>Removed original 8.4.17 “Residual product that will not be infused to the recipient will be used to set up the 14 day sterility culture on the final product since information was included in the NOTE in Section 8.4.13.</li> </ul>

**Signature Manifest****Document Number:** STCL-SOP-028 JA4**Revision:** 03**Title:** Thawing Human Cord Tissue-derived MSCs for Infusion - IMPACT Study JA4**Effective Date:** 25 Nov 2022

All dates and times are in Eastern Time.

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Name/Signature	Title	Date	Meaning/Reason
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Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		14 Nov 2022, 05:06:05 PM	Approved

**Quality**

Name/Signature	Title	Date	Meaning/Reason
Isabel Storch De Gracia (IMS19)		18 Nov 2022, 12:20:34 PM	Approved

**Document Release**

Name/Signature	Title	Date	Meaning/Reason
Sandra Mulligan (MULLI026)		18 Nov 2022, 04:40:24 PM	Approved