



STEM CELL LABORATORY (STCL)



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Thawing of Peripheral Blood Progenitor Cells (PBPCs) or Bone Marrow for Direct Administration

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STCL-PROC-021

THAWING OF PERIPHERAL BLOOD PROGENITOR CELLS (PBPCs) OR BONE MARROW FOR DIRECT ADMINISTRATION

1 PURPOSE

- 1.1 To describe the process by which cells are thawed in a 37 °C water bath and prepared for immediate infusion to patient following chemotherapy and/or radiation therapy regimen.

2 INTRODUCTION

- 2.1 Cryopreserved bags of peripheral blood progenitor cells and/or bone marrow are thawed rapidly in a 37 °C water bath in the laboratory or at the patient's bedside, for immediate infusion following preparative therapy.

3 SCOPE AND RESPONSIBILITIES

- 3.1 The Medical Directors, Stem Cell Laboratory Manager, 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 ABMTU Adult Bone Marrow Transplant Unit
- 4.2 BM Bone Marrow
- 4.3 BSC Biological Safety Cabinet
- 4.4 DHIS Duke Hospital Information System
- 4.5 HPC Hematopoietic Progenitor Cells
- 4.6 ISBT International Society of Blood Transfusion
- 4.7 N9200 9th Floor, Duke North Hospital, Adult Bone Marrow Transplant Unit
- 4.8 PBPCs Peripheral Blood Progenitor Cells
- 4.9 QSU Quality Service Unit
- 4.10 STCL Stem Cell Laboratory

5 MATERIALS

- 5.1 Supplies
 - 5.1.1 60 mL syringes
 - 5.1.2 10 mL syringes
 - 5.1.3 3 mL syringe or equivalent
 - 5.1.4 Double 4-way stopcock
 - 5.1.5 Plasma transfer set w/coupler & needle adapter

- 5.1.6 Alcohol wipes and spray bottle containing 70% isopropanol
- 5.1.7 3 small test tubes (for cell count and viability)
- 5.1.8 Male/Female Sterile Caps
- 5.1.9 Sterile thaw bags
- 5.1.10 Alcohol prep pads, SEPP applicators, Steri-Perox 6% wipes or equivalent
- 5.2 Additional supplies for N9200 (*or other off-site location*) reinfusions:
 - 5.2.1 Sterilized chux - (*these items are found on the Transplant Unit or in the transplant clinic*)
 - 5.2.2 Sterile water or equivalent - (*these items are found on the Transplant Unit or in the transplant clinic*)

6 EQUIPMENT

- 6.1 In laboratory thaw:
 - 6.1.1 37°C water bath
 - 6.1.2 Biological safety cabinet (BSC)
 - 6.1.3 Light microscope
 - 6.1.4 Hematology analyzer
 - 6.1.5 Insulated cryo gloves
 - 6.1.6 Small cup or beaker
 - 6.1.7 Crushed ice
 - 6.1.8 Pair of gloves
- 6.2 Bedside thaw:
 - 6.2.1 Small flat cart (i.e. – rolling cart)
 - 6.2.2 Charged dry shipper
 - 6.2.3 37°C water bath
 - 6.2.4 Light microscope
 - 6.2.5 Hematology analyzer
 - 6.2.6 Insulated cryo gloves
 - 6.2.7 Small cup or beaker
 - 6.2.8 Crushed ice
 - 6.2.9 Pair of gloves

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, goggles, etc.
- 7.2 **NOTE:** A technologist will only handle/process one cellular product in the biological safety cabinet at any given time.

8 PROCEDURE

- 8.1 Prior to day of reinfusion: confirm date patient will be Day 0, and assemble reinfusion paperwork:
 - 8.1.1 Cryopreservation packet from files (should include EPIC (*or equivalent*) verification of name, history number, and ABO/Rh type from Beaker (*or equivalent* Laboratory Information System) and the completed *STCL-PROC-033 (FRM1) Cellular Product Storage Location Confirmation* form.
 - 8.1.2 *STCL-FORM-056 Cellular Therapy Infusion Request Form* – fill in as much as possible, including available CD34+'s or CD3+'s, if applicable. .
 - 8.1.3 Note the freezer locations on *STCL-FORM-043 Thawing and Infusion Worksheet* (either autologous or allogeneic) for the bags being thawed. Place a barcode in the designated location on the *Thawing and Infusion Worksheet* for each bag being thawed.

NOTE: If the thaw is being performed on N9200 (*or other off-site location*), note on the back of *STCL-FORM-043 Thawing and Infusion Worksheet*, in the comments section, that the thaw was performed on N9200 (*or other off-site location*) and no BSC was available for use.
 - 8.1.4 Obtain the patient's weight at admission if the product was cryopreserved more than two weeks before the infusion. If the cells were cryopreserved within two weeks, it is appropriate to use the patient's weight at the time of collection for the calculations (*if a current weight is not available or accessible*).
 - 8.1.5 Billing log form
 - 8.1.6 Copy of *APBMT-COMM-001 FRM2 Summary of Donor Eligibility and Infectious Disease Testing* for the product being thawed
 - 8.1.7 M0226 form
 - 8.1.8 *STCL-FORM-044 Processing Lot Numbers- 37 Degree Celsius Thaw*
 - 8.1.9 *STCL-DIST-003 Cellular Product Distribution Form*
 - 8.1.10 *STCL-SOP-050 Infusion Form*
 - 8.1.11 In an effort to minimize bags from cracking, cells stored in liquid phase of LN2 should be allowed to equilibrate in vapor phase for at least 4 hours prior to being thawed.

- 8.1.12 Call or email the CCBB Laboratory the day before the infusion to request a dry shipper be available, programmed, and delivered to the STCL on the day of infusion (*if the infusion will be performed on N9200 or other off-site location or if cells are located in a freezer outside of the STCL (ie. 2309B Pratt Street, etc).*
- 8.1.13 Dry shippers are the property of the CCBB Laboratory and are located in Suite 1400 of the North Pavilion. As much advance notice as possible should be provided to the CCBB Lab when a dry shipper is needed so they have ample time to prepare one for use. They will complete the log attached to the lid of the shipper when the shipper is filled. Monitor the temperature displayed on the logger lid to ensure that the temperature of the shipper is registering $\leq -150^{\circ}\text{C}$ while in use.
- 8.1.14 If the thaw is being performed on N9200 (*or other off-site location*), call the patient's nurse the day before the scheduled thaw to coordinate an approximate infusion time, which can be no earlier than 24 hours after the patient's Melphalan has finished.

NOTE: Coordinating the infusion time in advance will be especially helpful if/when the patient has been given Melphalan late in the day resulting in a **LATE** infusion time.

8.2 Day of Reinfusion:

8.2.1 Prior to thaw

- 8.2.1.1 Call the clinic, or N9200, to setup/confirm a time for reinfusion. If the thaw is being performed on N9200 (*or other off-site location*), ask the nurse to set up a water bath containing 3 liters of sterile water or equivalent approximately 30 minutes prior to the infusion time (so it is ready upon arrival).

- 8.2.1.2 Confirm water bath temperature is $37^{\circ}\text{C} \pm 1.0^{\circ}\text{C}$.

NOTE: If the thaw will take place on N9200 (*or other off-site location*), use the thermometer in the storage box to confirm the temperature on the display **BEFORE** thawing cells. Record the temperature of both the thermometer and the digital reading on the water bath on *STCL-FORM-014 Water Bath Temperature and Maintenance Log* located in the duffle bag that is taken to off-site infusion locations.

- 8.2.1.3 Pull designated canisters and place them in vapor phase inside a designated freezer if they were stored in liquid phase or in a charged dry shipper if the thaw is being performed on N9200 (*or other off-site location*).

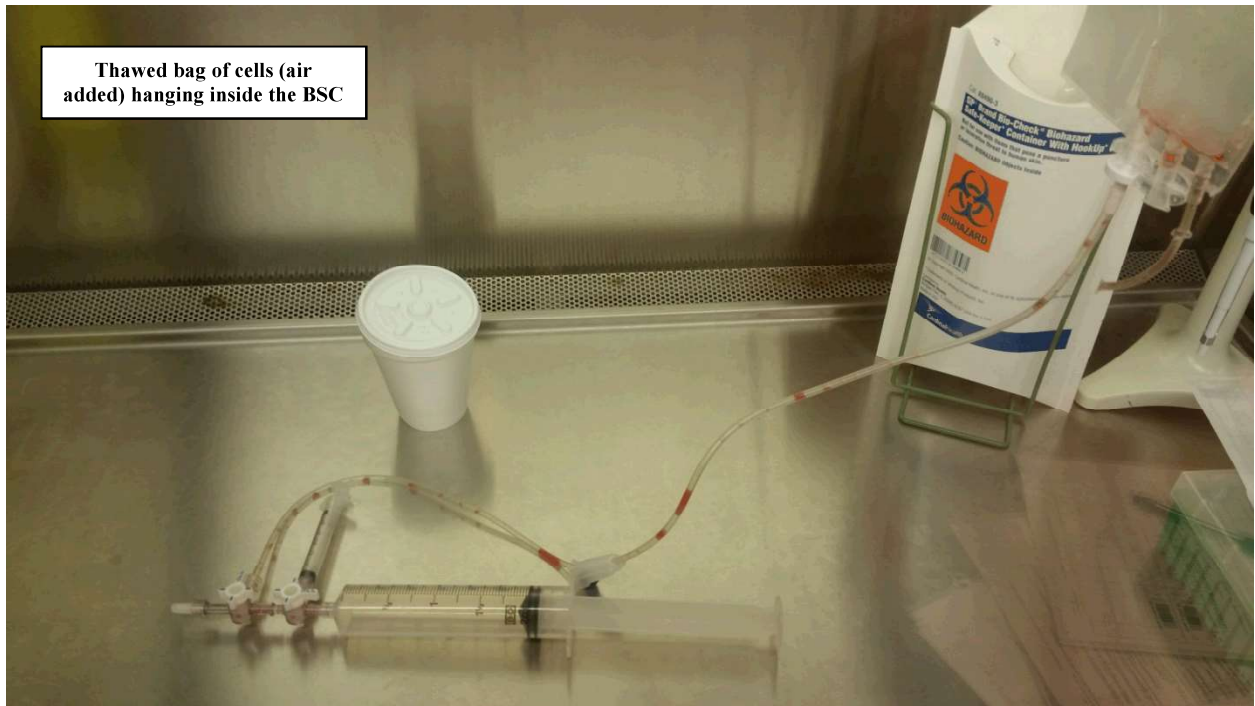
NOTE: If the thaw is being performed in the laboratory and the cells were originally stored in vapor phase, do NOT pull the canister(s) until 15 minutes before the time the clinic wants to infuse the thawed cells.

- 8.2.1.3.1 If removing canisters to place in the dry shipper or in vapor phase, confirm with a second technologist or designee, the patient's name, history number, and ISBT barcode on each bag of cells being thawed for transplant. Both individuals must initial the "Freezer" column on *STCL-FORM-043 Thawing and Infusion Worksheet* to reflect this confirmation. If placing canisters in a dry shipper, press and hold the **START** button on the data logger until it reads **ARRIVED**
- 8.2.1.4 Prepare Demand ISBT 128 labels for each bag to be thawed and label with corresponding letter designation. If the volume of the bag(s) exceeds 60 mL, prepare two (2) Demand 128 labels for the product. One Demand 128 label should be attached to the 60 mL syringe and the other Demand 128 label to be attached to the second syringe (10 mL syringe).
- 8.2.1.5 If the thaw is being performed on N9200 or other off-site location:
 - 8.2.1.5.1 Fill out and assemble the materials listed on *STCL-PROC-021 JA1 Checklist for 37 Degree Thaws Performed Off-Site* and place in the duffle bag to go to N9200 (*or other off-site location*).
 - 8.2.1.5.2 Call the courier at least one (1) hour before the scheduled infusion time so a ride can be provided from the North Pavilion to N9200 (*or other off-site infusion location*).
 - 8.2.1.5.3 Once the courier arrives, press and hold the **START** button on the data logger again until it reads **IN TRANSIT**.
 - 8.2.1.5.4 Once on N9200 (*or other off-site location*), wash your hands and get a cup of ice from the galley located just before entering the front doors to the Adult BMT Unit (N9200) after passing the receptionist.

NOTE: If infusing cells at another off-site location, ask the nurse if a cup of ice can be provided to transport the sample back to the lab.
- 8.2.1.6 Prepare the BSC or N9200 cart for thaw as follows:
 - 8.2.1.6.1 Obtain the number of 60 mL syringes, 10 mL syringes, and/or 3 mL syringes needed based

on the number of bags and their respective volumes that need to be thawed.

- 8.2.1.6.2 Open a double 4-way stopcock and attach a 60 mL syringe full of air to one port; attach a 3 mL syringe, or equivalent, to a second port, and attach the needle adaptor end of the transfer tubing to a third port on the stopcock.



- 8.2.1.6.3 Lay the syringe/transfer tubing set inside the BSC or on the cart (*if the infusion is taking place on N9200*).

- 8.2.1.7 If the infusion is taking place in the Adult BMT Clinic, get a cup from above the water bath and aseptically fill it with ice from the ice machine (*located in the breakroom in Suite 1400*).

- 8.2.1.8 Place a test tube (*labeled with patient's name, history#, and ISBT barcode*) into the cup of ice and put the cup inside the BSC or on the cart if infusion is being performed on N9200 (*or other off-site location*). This sample will be used for cell count and viability after all of the bags have been thawed.

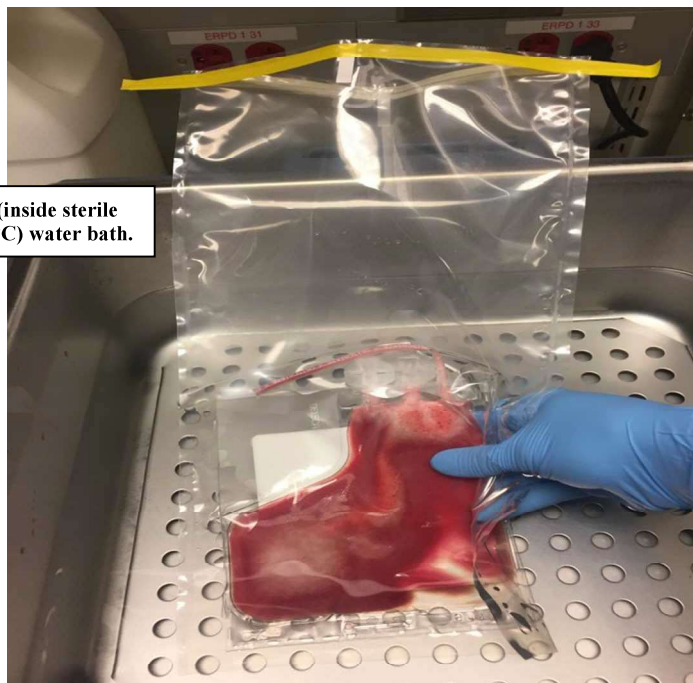
8.2.2 At time of thaw

- 8.2.2.1 Confirm, with a second technologist or designee, the patient's name, history number and ISBT barcode on each bag at time of thaw. Both individuals must initial the "Pre-Thaw" column (*and "Freezer" column if canisters were not moved to vapor phase or a charged dry shipper prior to this*

step) on *STCL-FORM-043 Thawing and Infusion Worksheet* to reflect this confirmation.

- 8.2.2.2 Remove the tie tag from the cryobag and spray the exterior with 70% isopropanol before placing it in the sterile thaw bag. Place the cell bag into the water bath and massage the bag gently until thawed. The final thawed product should be a “slushy” consistency.

A cryobag being thawed (inside sterile thaw bag) in a 37° C (+/- 1°C) water bath.



- 8.2.2.3 After confirming that there are no leaks or cracks in the thawed cryobag, remove it from the sterile thaw bag, dry the exterior, and place it in the BSC or on the cart if the thaw is being performed on N9200 (*or other off-site location*).
- 8.2.2.4 On *STCL-FORM-043 Thawing and Infusion Worksheet*, record the time each bag was placed in the water bath and the time each bag was removed from the water bath.
- 8.2.2.5 Once in the BSC or on the cart (*if the thaw is being performed on N9200 or other off site location*), attach the tie tag from the cryobag to the plunger of the 60 mL syringe full of air.
- 8.2.2.6 Remove the stopper from one of the entry ports of the thawed bag. Clean the port with an alcohol wipe, SEPP applicator, Steri-Perox 6% wipes or equivalent. Insert the coupler end of the transfer tubing into this port.
- 8.2.2.7 Remove the product label from the cryobag and attach it to the M0226 form. Completing this step now will prevent the label from tearing.

NOTE: Be sure the system is labeled with the tie tag **BEFORE** removing the product label.

8.2.2.8 Next, holding the cryobag upright to prevent the introduction of air bubbles, inject the 60 mL syringe full of air into the bag. Hang the cell bag inside the BSC or hold in one hand, if the thaw is being performed on N9200 (*or other off-site location*).

8.2.2.9 Mix cells adequately, and withdraw the cells into the 60 mL syringe. Gently rubbing the cryobag, try to get as many cells out of the bag as possible.

8.2.2.10 Remove the QC sample(s) into the 3 mL syringe, or equivalent, according to how many bags are being thawed. Detach and dispense the volume into the cell count tube on ice

NOTE: Be sure to turn the stopcock to the OFF position before detaching the syringe. Reattach the 3 mL syringe to the stopcock.

8.2.2.10.1 For one (1) bag, remove 0.4 mL of cells

8.2.2.10.2 For two (2) bags, remove 0.2 mL of cells from each bag

8.2.2.10.3 For three (3) bags, remove 0.1 mL of cells from bags 1 and 2, and 0.2 mL of cells from bag 3

8.2.2.10.4 For more than three (>3) bags, remove 0.1 mL of cells from each bag

8.2.2.11 Before detaching the syringe, confirm that the patient's name, history number, and ISBT on the product are the same as on the reinfusion orders, and initial the "BSC" column on *STCL-FORM-043 Thawing and Infusion Worksheet* to reflect this confirmation.

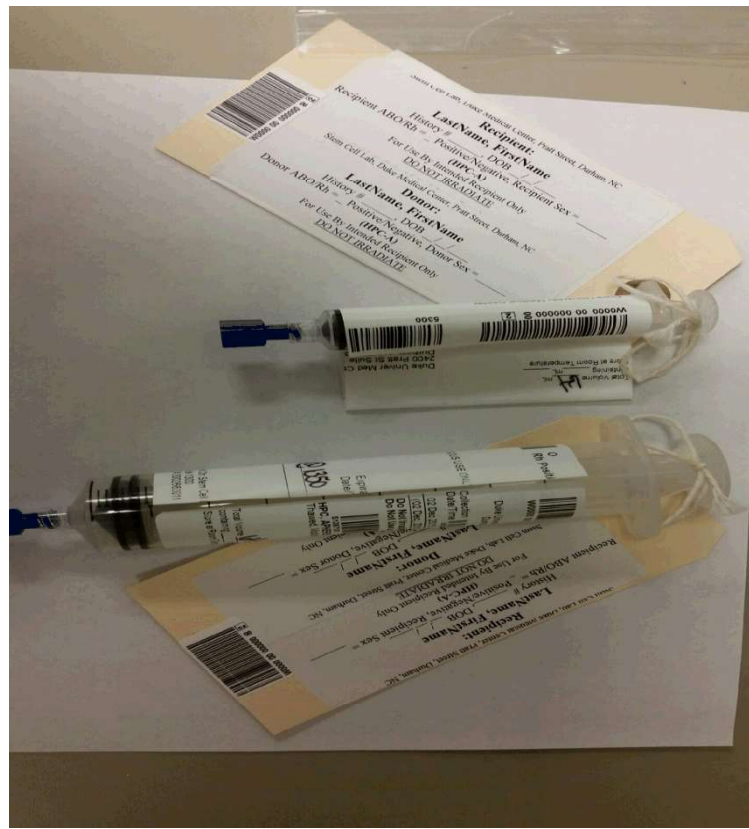
8.2.2.12 Turn the stopcock to the **OFF** position then remove the 60mL syringe, containing the cells to be infused, from the stopcock and attach a sterile cap.

8.2.2.13 Attach the next sterile syringe full of air to the stopcock setup (*use a 10mL syringe, if there is still product left in the bag or use a 60mL syringe if all of the product has been removed from the current bag*).

8.2.2.14 Record the volume on the worksheet and on the Demand 128 label, and then attach the label to the 60 mL syringe of cells.

NOTE: Record the expiration date and time on each Demand 128 label(s). The **EXPIRATION** time should be **20**

minutes from the time the product was removed from the water bath).



- 8.2.2.15 Deliver the cells to the treatment nurse for reinfusion. Sign and date the *STCL-FORM-056 Cellular Therapy Infusion Request Form* at the bottom as the “person delivering cells for patient infusion”. With the infusion nurse and a second verifier, recite the patient’s name, history number, date of birth, and ISBT barcode from the product; include donor information, if applicable.
- 8.2.2.16 The nurses should confirm this information on the *STCL-FORM-056 Cellular Therapy Infusion Request Form*. Have the infusion nurse sign and date the bottom of the form as the “Receipt Person’s Signature” and “ID verification signature #1”; have the second verifier sign and date the bottom as the person for “ID verification signature #2”. This should only be done for the 1st bag. Also have both nurses initial the “Distribution” column on *STCL-FORM-043 Thawing and Infusion Worksheet* and document the time of distribution for each syringe once the name, history number, and ISBT barcode number have been confirmed.
- 8.2.2.17 Repeat steps 8.2.2.1 – 8.2.2.16 for each subsequent bag being thawed.

8.2.3 After all bags have been thawed

- 8.2.3.1 If the thaw is being performed on N9200 (*or other off-site location*), following the final bag, press and hold the **STOP** button on the data logger on the dry shipper until it shows **ARRIVED**. Also, put a cap on the cell count/viability tube and leave it on ice. Put a lid on the cup containing the ice and place the sample in a biohazard bag to go back to the lab. After emptying the water bath and wiping it down with germicidal wipes, gather all items to include the duffle bag, paperwork, ice cup, and dry shipper, and return to the laboratory immediately.
- 8.2.3.2 Label two (2) dilution tubes with the patient's last name and history number for cell count and viability. Add 0.4 mL of Cell Pack to both tubes and add 0.1 mL of albumin to the viability tube only.
- NOTE:** *Tubes can be labeled and prepared before starting the thawing procedure).*
- 8.2.3.3 Following the thaw of the final bag, mix the test tube of cells thoroughly (*upon return to the lab if thaw is being performed on N9200 [or other off-site location]*). Add 0.1 mL of cells to both dilution tubes. Using the cell count tube, perform a cell count on the Sysmex hematology analyzer or equivalent. Add 0.15 mL of trypan blue to the viability tube, set a timer for five (5) minutes, and perform and record the results of the viability.
- 8.2.3.4 Label the cell count printout with the patient's name, history number, and dilution value.
- 8.2.3.5 Complete the paperwork including the necessary calculations. Make a copy of the *STCL-FORM-056 Cellular Therapy Infusion Request Form* and the *Summary of Donor Eligibility and Infectious Disease Testing* for the lab records. Deliver a copy of these documents along with *STCL-SOP-050 Infusion Form* to the infusion nurse for inclusion in the patient's medical record.
- NOTE:** If the thaw was performed on N9200 (*or other off-site location*), leave a copy of the *APBMT-COMM-001 FRM2 Summary of Donor Eligibility and Infectious Disease Testing* form and *STCL-SOP-050 Infusion Form* with the patient's nurse. Once back to the laboratory, after the cell count and viability testing has been performed, fax a copy of the *STCL-FORM-056 Cellular Therapy Infusion Request Form* to the patient's nurse so that the information can be included in the patient's medical record).
- 8.2.3.6 Attach an EPIC reinfusion label containing the patient's name and history number to the top of the billing log. Add

the date of infusion, and fill out the form to include the number of bags infused.

- 8.2.3.7 If the thaw was performed on N9200 (*or other off-site location*), return the dry shipper to the CCBB Lab, Suite 1400. A copy of the data logger report will be faxed for inclusion in the patient's laboratory file.

8.3 SPECIAL PROCEDURE NOTES:

- 8.3.1 If a bag appears to be leaking during the thawing process, the patient's attending physician must be consulted immediately (*since you have a limited amount of time to infuse cells post thaw*) to obtain instructions regarding disposition of the unit (*i.e. infuse, discard, etc.*). Complete *STCL-QA-007 FRM1 Non-Conforming Products* form or *STCL-SOP-045 FRM1 Record of Discard*, as appropriate.
- 8.3.2 A **maximum of 10 bags** may be infused to a full adult in a 24 hour time period. If a patient has more than 10 bags to be infused, split them to be given over 2 days unless written doctor's orders dictate otherwise.
- 8.3.3 As a guideline, the maximum cell dose for AUTOLOGOUS infusions is $\sim 8 \times 10^6$ CD34+ cells/kg per Dr. Chao effective July 7, 2003.

9 RELATED DOCUMENTS/FORM

- 9.1 APBMT-COMM-001 FRM2 Summary of Donor Eligibility and Infectious Disease Testing
- 9.2 COMM-PAS-003 (JA1) Storage Temperatures and Expiration of Cellular Products
- 9.3 M0226 Form
- 9.4 STCL-DIST-003 Cellular Product Distribution Form
- 9.5 STCL-FORM-014 Water Bath Temperature and Maintenance Log
- 9.6 STCL-FORM-043 Thawing and Infusion Worksheet
- 9.7 STCL-FORM-044 Processing Lot Numbers- 37 degree Celsius Thaw
- 9.8 STCL-FORM-056 Cellular Therapy Infusion Request Form
- 9.9 STCL-PROC-021 (JA1) Checklist for 37 Degree Thaws Performed Off-Site
- 9.10 STCL-PROC-033 (FRM1) Cellular Product Storage Location Confirmation
- 9.11 STCL-QA-007 FRM1 Non-Conforming Products
- 9.12 STCL-SOP-045 FRM1 Record of Discard
- 9.13 STCL-SOP-050 Infusion Form

10 REFERENCES

- 10.1 Standards for Blood Banks and Transfusion Services, American Association of Blood Banks, Arlington, Virginia: Current Editions

- 10.2 Cohen, A., Tepperberg, M., Waters-Pick, B., Coniglio, D., Perfect, J., Peters, W.P., Gilbert, C., Morgan, C., Vredenburgh, J.J., The Significance of Microbial Cultures of the Hematopoietic Support for Patients Receiving High-Dose Chemotherapy. Journal of Hematotherapy, 5:289, 1996
- 10.3 Chao, N.J., Personal Communication, July 7, 2003

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
07	Barb Waters-Pick	<ul style="list-style-type: none"> Section 5.1.10 Added Steri Perox 6% wipes or equivalent Section 8.1.1 corrected document number Section 8.1.6 added document # and updated title of the document Section 8.2.2.6 added Steri Perox 6% or equivalent wipes Section 8.2.3.5 updated document # and titles Section 8.3.1 added document #s to the titles Section 9 corrected document titles and listed documents in alphabetical order General grammatical and formatting changes made throughout the document

Signature Manifest

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STCL-PROC-021 Thawing of PBPCs or Bone Marrow for Direct Administration

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Medical Director

Name/Signature	Title	Date	Meaning/Reason
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Quality

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Isabel Storch De Gracia (IMS19)		20 May 2021, 08:44:43 AM	Approved

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