



STEM CELL LABORATORY (STCL)



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Processing Granulocytes for Infusion

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STCL-PROC-004 PROCESSING GRANULOCYTES FOR INFUSION

1 PURPOSE

- 1.1 To describe the necessary steps for processing irradiated granulocytes to be infused to recipients in need of this cellular support.

2 INTRODUCTION

- 2.1 Granulocyte transfusions may be used to support aplastic recipients at increased risk for invasive fungal or bacterial infections through their neutropenic phase. Granulocytes may also be given to support high risk transplant recipients and/or recipients who have a history of fungal infections.
- 2.2 Given the nature of directed donation, granulocyte products are not ABO cross-matched. Instead, the product is manipulated as needed to render the product safe for infusion regardless of the ABO/Rh type. The ABO/Rh type of the recipient, the transplant product, and the granulocyte donor are all taken into consideration when determining if or how the irradiated granulocyte product will be processed prior to infusion. Products may require red cell depletion, plasma depletion, red cell and plasma depletion, or no depletion.
- 2.3 All granulocyte products are irradiated at 4000 rads in the Transfusion Services prior to infusion.

3 SCOPE AND RESPONSIBILITIES

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

4 DEFINITIONS/ACRONYMS

- | | | |
|------|------|---|
| 4.1 | STCL | Stem Cell Laboratory |
| 4.2 | mL | Milliliter |
| 4.3 | ISBT | International Society for Blood Transfusion |
| 4.4 | RN | Registered Nurse |
| 4.5 | QC | Quality Control |
| 4.6 | RPM | Revolutions per Minute |
| 4.7 | RBC | Red Blood Cell |
| 4.8 | WBC | White Blood Cell |
| 4.9 | BSC | Biological Safety Cabinet |
| 4.10 | HSA | Human Serum Albumin |
| 4.11 | QSU | Quality Systems Unit |

5 MATERIALS

- 5.1 300 mL transfer bags
- 5.2 600 mL transfer bags
- 5.3 Sampling site couplers
- 5.4 6% Hetastarch, if applicable
- 5.5 60 mL syringes
- 5.6 30 mL syringes
- 5.7 3 mL syringes
- 5.8 25% Albumin, if applicable
- 5.9 Plasmalyte-A® or equivalent, if applicable
- 5.10 Alcohol prep pads, Chloraprep, Steri Perox 6% wipes, or equivalent
- 5.11 16 or 19 gauge needles
- 5.12 Aerobic and Anaerobic Culture bottles
- 5.13 Pipet tips
- 5.14 12 x 75 test tubes
- 5.15 Sterile snap-cap tube
- 5.16 Permanent marker
- 5.17 Irradiation labels
- 5.18 Tie tags
- 5.19 ISBT labels
- 5.20 Demand 128 labels
- 5.21 Benzonase, if needed
- 5.22 Blood filter, if needed

6 EQUIPMENT

- 6.1 Sorvall RC3C Plus Centrifuge or equivalent
- 6.2 Plasma Expresser
- 6.3 Heat Sealer (Sebra or equivalent)
- 6.4 Sterile Tubing Welder (Terumo or equivalent)
- 6.5 Pipet
- 6.6 Demand 128 label printer
- 6.7 Blood irradiator (transfusion service)
- 6.8 Automated hematology analyzer
- 6.9 Biological Safety Cabinet (BSC)

6.10 Balance / Scale

7 SAFETY

- 7.1 Wear all applicable personal protective equipment when 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:

- Post collection and prior to infusion, the granulocyte product must be irradiated at **4,000 rads** for a pre-determined amount of time set by the Transfusion Services Department.
- Perform ALL work that requires manipulation of a cellular product in a biological safety cabinet using aseptic/sterile technique in order to maintain sterility of the product.
- To minimize cross-contamination of products, technologists will work with only one product at a time in the BSC.

8.1 Receipt of product in the laboratory

- 8.1.1 The RN, or tech, delivering the product to the lab must sign, date, and record the time on the *STCL-GEN-009 FRM2 Cellular Product/Sample Chain of Custody Form*.
- 8.1.2 The tech who receives the product in the lab must also sign, date, and record the time on the *STCL-GEN-009 FRM2 Cellular Product/Sample Chain of Custody Form*.
- 8.1.3 Visually confirm that all labeling, including the ISBT 128 barcode and donor and recipient information of the product and the accompanying paperwork is accurate.

NOTE: All transfer bags used in the manipulation of the product **MUST** be labeled with the recipient's last name and/or ISBT 128 barcode.

8.2 Review signed doctor's order, *STCL-FORM-056 Cellular Therapy Infusion Request Form*, for processing instructions. Unless otherwise noted on the doctor's orders, granulocyte doses will not exceed 60 mL/dose or 5×10^9 total nucleated cells/kg. Products will be processed as follows based on ABO/Rh compatibility:

- 8.2.1 Unmanipulated (straight split)
- 8.2.2 RBC Reduced
- 8.2.3 Plasma Reduced
- 8.2.4 Plasma and RBC Reduced

8.3 Processing Requirements

- 8.3.1 Refer to the signed doctor's orders for processing instructions. Although the ABO/Rh type of the recipient, the transplant product, and the granulocyte donor are all taken into consideration when determining how the irradiated granulocyte product will be processed prior to infusion, the chart below only reflects the ABO/Rh types of the granulocyte donor and the recipient.

O Donor / O Recipient	NO manipulation required
O Donor / A Recipient	Plasma reduce
O Donor / B Recipient	Plasma reduce
O Donor / AB Recipient	Plasma reduce
A Donor / O Recipient	RBC reduce
A Donor / A Recipient	NO manipulation required
A Donor / B Recipient	RBC and plasma reduce
A Donor / AB Recipient	Plasma reduce
B Donor / O Recipient	RBC reduce
B Donor / A Recipient	RBC and plasma reduce
B Donor / B Recipient	NO manipulation required
B Donor / AB Recipient	Plasma reduce
AB Donor / O Recipient	RBC reduce
AB Donor / A Recipient	RBC reduce
AB Donor / B Recipient	RBC reduce
AB Donor / AB Recipient	NO manipulation required

8.4 Product that requires no manipulation

- 8.4.1 Heat seal the tubing on three labeled 300 mL transfer bags with a double seal, 1 to 2 inches from the base.
- 8.4.2 Insert a sampling site coupler into each 300 mL bag.
- 8.4.3 Insert a sampling site coupler into the collection bag containing granulocytes.
- 8.4.4 Remove 4 mL for QC
- 8.4.4.1 1 mL - to perform cell count, viability, and ABO/Rh
- 8.4.4.2 2 mL - for bacterial cultures (1 mL/bottle)
- 8.4.4.3 1 mL - to be used as a retention sample
- 8.4.4.4 Remove 60 mL of irradiated granulocytes from the collection bag and add to each of the three 300 mL transfer bags.
- 8.4.4.5 Refer to 8.8 for labeling instructions.

- 8.4.4.6 Discard any remaining cells in the collection bag to the biohazard trash. In the event that more cells are available then will be used, complete a *STCL-SOP-045 FRM1 Record of Discard* and get signatures from the medical director (or designee), lab manager, and QSU representative.

8.5 Product that requires RED CELL Reduction only

- 8.5.1 Transfer the collected product to a labeled 300 or 600 mL transfer bag and heat seal.
- 8.5.2 Sterile weld a second labeled 300 or 600 mL transfer bag onto the product-containing bag.
- 8.5.3 Insert a sampling site coupler into the labeled 300 or 600 mL transfer bag containing the product.
- 8.5.4 Add 20% (of the total product volume) of 6% hetastarch to the 300 or 600 mL product-containing bag (ie. If the product bag contains 200 mL, add 40 mL of 6% hetastarch).
- 8.5.5 Hang the product / hetastarch transfer bag on a plasma expresser undisturbed for 30 to 60 minutes.

NOTE: If a good separation is not achieved after 30 to 60 minutes, the product can be spun at 700 RPM for 10 minutes with the brake OFF in a Sorvall RC3 centrifuge (or equivalent).
- 8.5.6 Express off the WBC/plasma product into the attached 300 or 600 mL transfer bag. If the volume exceeds 180 mL, the product will need to be spun in the Sorvall RC3 (or equivalent) centrifuge at 1800 RPM for 20 minutes with the brake OFF to volume reduce the product.
- 8.5.7 Heat seal the tubing to remove the red cell containing bag and retain for QC testing.
- 8.5.8 Refer to step 8.8 for performance of QC and labeling of product for infusion.

8.6 Product that requires Plasma Reduction only

- 8.6.1 Transfer cells to a labeled 300 or 600 mL transfer bag and heat seal tubing.
- 8.6.2 Sterile weld a second labeled 300 or 600 mL transfer bag onto the product containing bag.
- 8.6.3 Place transfer bag inside the appropriate insert inside a centrifuge bucket and balance.
- 8.6.4 Centrifuge at 1800 RPM for 20 minutes with the brake OFF in the Sorvall RC3C Plus centrifuge or equivalent.
- 8.6.5 After centrifugation, express the plasma off, and heat seal the tubing to remove plasma-containing bag and retain for QC.

- 8.6.6 Insert a sampling site coupler into the RBC/WBC containing transfer bag.
- 8.6.7 Using a 60 mL syringe measure the volume of RBC/WBC in the bag.
- 8.6.8 Add 25 mL of 25 % human serum albumin (HSA).
- 8.6.9 Add the appropriate volume of Plasmalyte-A to achieve a final product volume of 180 mL (180 mL of RBC/WBC + HSA + Plasmalyte-A).
- 8.6.10 Refer to step 8.8 for performance of QC and labeling of product for infusion.
- 8.7 Product that requires Red Cell and Plasma Reduction
 - 8.7.1 Refer to steps 8.5.1 through 8.5.7 for red cell reduction.
 - 8.7.2 Refer to steps 8.6.2 through 8.6.10 for plasma reduction.
- 8.8 QC and labeling of product for infusion
 - 8.8.1 MIX the final cell-containing product and remove 0.5 mL for QC to include cell count and viability.
 - 8.8.2 Remove 0.2 mL from the RBC containing bag and perform ABO/Rh testing. Record results in the ABO/Rh log and on the processing worksheet.
 - 8.8.3 Remove sample volumes for sterility testing as follows:
 - 8.8.3.1 RBC Reduced products; remove 2 mL of RBC (1 mL/bottle)
 - 8.8.3.2 Plasma Reduced products; remove 5 mL of plasma (2.5 mL/bottle)
 - 8.8.3.3 RBC and Plasma Reduced products; remove 2 mL from the RBC containing bag and 5 mL from the plasma containing bag (3.5 mL/bottle).

NOTE: The inoculation volumes differ because of the WBC content. False positives may occur if there is a large number of WBCs present in the sample.
 - 8.8.4 Remove 1 mL to be used as a "retention sample." Place the sample in a sterile snap cap tube and label with the ISBT 128 barcode and the collection date. Place the "retention sample" in the designated monitored refrigerator in the sample rack labeled "Granulocyte Retention Samples" and store for a minimum of 7 days.
 - 8.8.5 Once all QC and retention samples have been removed, mix the product again and split the product into 3 equal doses (not to exceed 60 mL/dose and/or 5 x 10⁹ cells/kg/dose). One dose of cells will be infused to the recipient over three consecutive days unless otherwise ordered by the attending physician.
 - 8.8.6 Prepare Demand 128 labels per procedure *COMM-PAS-003 Labeling Cellular Therapy Products* and affix onto each bag. The date and time of collection, ABO/Rh type, and product type must be entered. Assign

a 3-day expiration date counting the collection day as day one (i.e. Collection on 10/17/13; expiration date of 10/20/2013 at time product collected).

NOTE: For American Red Cross (ARC)-collected granulocytes, refer to *COMM-PAS-003 JAI Storage Temperature and Expiration of Cellular Products*. Given the processing required in the Stem Cell Laboratory, the expiration time will be extended by the medical director (or designee), if needed, based on Urgent Medical Need.

- 8.8.7 The designated ISBT 128 barcode will be placed on each bag and/or scanned directly onto the Demand 128 label.
- 8.8.8 An irradiation sticker, obtained in the Transfusion Services at the time the product was irradiated, will also be placed on each bag.
NOTE: The standard irradiation sticker reflects 25 Gy to reflect 2500 rads; since the granulocytes are irradiated at 4000 rads, the 25 Gy should be crossed out and replaced by "40 Gy" on the label with tech's initials and date change was made.
- 8.8.9 A tie tag with both the recipient and donor information and an ISBT 128 barcode (with letter designation A, B and C) must be affixed to each product bag.
- 8.8.10 Prepare a copy of the HPC Infusion Form to accompany each of the three split products. This form will be photocopied to allow for one copy to remain on the infusion unit and the original form to come back to the lab for placement in the laboratory file.
- 8.8.11 *APBMT-COMM-001 FRM2 Summary of Donor Eligibility and Infectious Disease Testing* must be copied and provided to accompany each bag to the infusion unit. An ISBT 128 barcode will be placed on this form along with the product's collection date.
- 8.8.12 Place one bag of cells in a biohazard zip lock bag along with 2 copies of the infusion form, a copy of the donor eligibility form and one *STCL-GEN-009 FRM2 Cellular Product-Sample Chain of Custody Form*. Repeat for the remaining 2 bags of cells.
- 8.8.13 Write the recipient's name and history number on the log sheet along with the product ISBT 128 barcode and letter designation and place the product in the monitored refrigerator.
- 8.8.14 The designated infusion site should be contacted to arrange an infusion time. Courier services are available to the lab until 4:30 pm. Every effort should be made to deliver the product before 5:00 pm. Call the courier cell phone **(919-812-4284)** and inform them of the time the cells need to be on the floor. If cells cannot be infused before the courier leaves for the day, alternate arrangements for delivery of the product will have to be made.

8.9 Problems Encountered

8.9.1 CLOTTING

- 8.9.1.1 If the product clots during processing, contact the medical director immediately for modified processing order. If instructions are given to add benzonase (200 IU units/vial) to the product, be sure to note the number of vials added as per the physician's order.
- 8.9.1.2 Rotate the product for at least 30 minutes.
- 8.9.1.3 Filter the product using a 150-170 micron filter.
- 8.9.1.4 Perform cell count, and aliquot into three transfer bags according to the original orders. Record all pertinent information on the infusion form.

8.9.2 LEAKS

- 8.9.2.1 If product should leak, identify the source of the leak and make every effort to maintain product sterility. If sterility has been compromised, contact medical director immediately to get modified orders to proceed with processing/infusion or discard.
- 8.9.2.2 Complete *STCL-QA-007 Non-Conforming Products* form or *STCL-SOP-045 FRM1 Record of Discard*, as deemed appropriate.

9 RELATED DOCUMENTS/FORMS

- 9.1 APBMT-COMM-001 FRM2 Summary of Donor Eligibility and Infectious Disease Testing
- 9.2 COMM-PAS-003 Labeling Cellular Therapy Products
- 9.3 COMM-PAS-003 JA1 Storage Temperature and Expiration of Cellular Products
- 9.4 COMM-QA-042 Deviations and Investigations
- 9.5 COMM-QA-042 (FRM1) Deviation and Investigation Report
- 9.6 STCL-GEN-009 FRM1 Cellular Product Chain of Custody
- 9.7 STCL-GEN-009 FRM2 Cellular Product-Sample Chain of Custody
- 9.8 STCL-EQUIP-011 Sterility Culture Using the BACT-ALERT Microbiology System
- 9.9 STCL-FORM-056 Cellular Therapy Infusion Request Form
- 9.10 STCL-SOP-045 FRM1 Record of Discard
- 9.11 STCL-SOP-049 ABO-Rh Typing
- 9.12 STCL-QA-007 Non-Conforming Products – Receipt, Processing, Distribution, and Disposition

9.13 STCL-QA-007 (FRM1) Non-Conforming Products

10 REFERENCES

10.1 AABB Technical Manual, Current Edition

10.2 Standards for Blood Banks and Transfusion Services, Current Edition

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
10	Barb Waters-Pick	<ul style="list-style-type: none"> • Section 5.10 added Chloro Prep, Steri Perox 6% wipes, or equivalent • Added “Balance / Scale” to section 6.10 • Section 8.4.4.6 added document # to the title • Section 8.8.6 added document # to the title • Section 8.8.11 updated the names of the documents • Section 8.8.12 added document # to the title • Section 8.9.2.2 added document # to the titles • Updated Section 9 so documents are in alphabetical order • Sections 9.2, 9.9, and 9.10 updated document names • Removed STCL-PROC-029 HPC Infusion Request Form and added STCL-FORM-056 Cellular Therapy Infusion Request form to Section 9 • Sections 10.1 and 10.2 – updated to reflect current edition of documents

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STCL-PROC-004 Processing Granulocytes for Infusion**Author**

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		17 May 2021, 03:42:11 PM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATER002)		17 May 2021, 03:42:30 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		17 May 2021, 04:54:29 PM	Approved

Quality

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Isabel Storch De Gracia (IMS19)		20 May 2021, 08:44:25 AM	Approved

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