



STEM CELL LABORATORY (STCL)



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Preparation of Bone Marrow before CD34+ Selection Procedure JA2

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STCL-PROC-015 JA2

PREPARATION of BONE MARROW BEFORE CD34+ SELECTION PROCEDURE

1 PURPOSE

- 1.1 This document describes the procedure for obtaining a mononuclear cell preparation and volume reduction of bone marrow (HPC-MARROW) along with the addition of human immune globulin (IgG) in preparation for the selection and enrichment of human CD34 positive hematopoietic progenitor cells utilizing the CliniMACS magnetic cell separation system.

2 INTRODUCTION

- 2.1 The CliniMACS system is a fully automated device designated for cell processing and selection. It is used for the positive selection of CD34 positive cells or for simultaneous CD34 positive cell selection with purging of unwanted cells from a heterogeneous cell population. The CliniMACS system utilizes highly specific CD34 monoclonal antibodies conjugated to super-paramagnetic particles. Super-paramagnetic particles are small in size (about 50nm in diameter) and are composed of iron oxide and dextran. The magnetic particles form a stable colloidal suspension and do not precipitate nor aggregate in magnetic fields. The CD34 positive cells are specifically labeled for selection during incubation with the CliniMACS CD34 Reagent. After unbound reagent is washed from the suspension, the cells are ready to be selected in the automated continuous flow separation system. The CliniMACS system passes the antibody labeled cell suspension through a column in which strong magnetic gradients are generated. The targeted cells are retained in the selection column, where they are washed several times to remove any extraneous material. After the final wash, the column is removed from the magnetic field and the targeted cells are eluted to the collection bag.
- 2.2 When processing cellular products for transplantation, sterile technique should be used whenever feasible. Every precaution should be taken to minimize the possibility of contaminating cellular products.

3 SCOPE AND RESPONSIBILITIES

- 3.1 The Medical Directors, Laboratory Manager, Quality Manager, and applicable laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

- | | | |
|-----|-----|------------------------------|
| 4.1 | IgG | Immune Globulin |
| 4.2 | BSC | Biological Safety Cabinet |
| 4.3 | SOP | Standard Operating Procedure |
| 4.4 | mL | milliliter |

4.5	mg	milligram
4.6	G	gauge
4.7	QC	Quality Control
4.8	HPCA	Hematopoietic Progenitor Cell Assay
4.9	RFLP	Restriction Fragment Length Polymorphism
4.10	RPM	Revolutions per Minute
4.11	RBC	Red Blood Cell
4.12	°	Degree
4.13	C	Celsius

5 MATERIALS

- 5.1 Immune Globulin Intravenous (Human) GAMMAGARD S/D
- 5.2 Syringes (1 mL, 5 mL, 10 mL, 20 mL, 30 mL, 60 mL)
- 5.3 Sterile needles; 16G or 19G
- 5.4 Alcohol prep pads
- 5.5 600 mL transfer pack(s)
- 5.6 Sterile Tubing Welding Wafers

6 EQUIPMENT

- 6.1 Temperature controlled centrifuge
- 6.2 Plasma extractor
- 6.3 Biological safety cabinet (BSC)
- 6.4 Sterile Tubing Welder
- 6.5 Balance
- 6.6 Tubing heat sealer
- 6.7 Tubing stripper
- 6.8 Hemostats
- 6.9 Timer

7 SAFETY

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

8 PROCEDURE

- 8.1 All work in this procedure should be performed in a BSC whenever possible using aseptic technique at all times.
- 8.2 Record lot numbers and expiration dates for all appropriate reagents and disposables on the worksheet (*STCL-PROC-015 FRM1 CD34+ CliniMACS Worksheet*) and/or on the appropriate lot sheet (*STCL-FORM-045 Processing Lot Numbers – Bone Marrow Processing*).
- 8.3 Obtaining a mononuclear cell preparation and performing the volume reduction of the bone marrow product can be achieved by a few methods including the use of the Sepax 2 RM instrument, by performing a manual hard spin via centrifugation, or by other method as deemed appropriate by the medical director.
- 8.4 To obtain a mononuclear cell preparation and achieve volume reduction of a bone marrow product using the Sepax 2 RM, refer to *STCL-PROC-046 Processing Bone Marrow Using the Sepax 2 Instrument* for processing instructions and steps.
- 8.5 To obtain a mononuclear cell preparation and achieve volume reduction of a bone marrow product by performing a manual hard spin via centrifugation, proceed to step 8.5.1.
 - 8.5.1 Weigh the bone marrow collection bag and record the value on the worksheet.
 - 8.5.2 In a BSC, obtain a sample (~1.5 mL) to perform initial pre-processing QC to include cell count, trypan blue viability, ABO/Rh, manual differential, flow analysis, and RFLP (*if indicated*); record results on the appropriate laboratory worksheets.
 - 8.5.3 Obtain a sample to perform pre-processing sterility (~2 mL).
 - 8.5.4 Obtain a sample (~5 mL in a syringe) for HPCA analysis.
 - 8.5.5 To obtain a mononuclear cell preparation:
 - 8.5.5.1 Label a 600 mL transfer pack(s) and transfer the entire product from the collection bag to the transfer pack(s).
 - 8.5.5.2 Place the transfer pack(s) in a centrifuge insert(s). Balance the inserts.
 - 8.5.5.3 Place inserts into the centrifuge and centrifuge at the appropriate rpm for either the Sorvall or the Allegra centrifuge for 20 minutes with the brake OFF.
 - 8.5.5.4 Sorvall RC 3C Plus Centrifuge – 1200 rpm
 - 8.5.5.5 Allegra 6KR Centrifuge – 1249 rpm
 - 8.5.5.6 After centrifugation, remove the inserts from the centrifuge and using the sterile welder, attach an empty 600 mL transfer pack(s) to the transfer pack(s) containing the bone marrow.
 - 8.5.5.7 Carefully remove the transfer pack from the insert and hang the pack(s) on the plasma extractor. Give the cells 10-15

- minutes to “settle”; then express the plasma, the buffy coat layer and ~5 mL of the RBC layer.
- 8.5.5.8 Heat seal the tubing between the bags; leave enough tubing for several more sterile welds.
- 8.5.5.9 Mix the cells thoroughly.
- 8.5.6 To volume reduce the buffy coat-enriched product:
 - 8.5.6.1 Place the transfer pack(s) containing the mononuclear cell preparation in a centrifuge insert(s). Balance the inserts.
 - 8.5.6.2 Place inserts into the centrifuge and centrifuge at the appropriate rpm for either the Sorvall or the Allegra centrifuge for 20 minutes with the brake OFF.
 - 8.5.6.3 Sorvall RC 3C Plus Centrifuge – 1800 rpm
 - 8.5.6.4 Allegra 6KR Centrifuge – 1873 rpm
 - 8.5.6.5 After centrifugation, remove the inserts from the centrifuge and using the sterile welder, attach an empty 600 mL transfer pack(s) to the transfer pack(s) containing the mononuclear cell preparation.
 - 8.5.6.6 Carefully remove the transfer pack from the insert and hang the pack(s) on the plasma extractor. Give the cells 10-15 minutes to “settle”; then express off the plasma.
 - 8.5.6.7 Mix the cells carefully and thoroughly.
 - 8.5.6.8 Using a sterile welder, transfer all of the cells into one of the transfer packs.
- 8.5.7 Mix the cells carefully and thoroughly.
- 8.5.8 In a BSC, obtain a sample (~ 0.7 mL) to perform QC to include cell count, trypan blue viability, manual differential, flow analysis, and HPCA analysis, recording results on the appropriate worksheets.
- 8.6 The bone marrow product post mononuclear cell preparation and/or volume reduction is now ready to further process for CD34 positive selection, refer to *STCL-PROC-015 CD34 Positive Selection Using Miltenyi CliniMACS* for processing steps, see step 8.7 for instructions for the addition of immune globulin.

NOTE: The product may also be held overnight at 4 °C for CD34 selection the following day. Allow product to come to room temperature prior to beginning selection procedure.
- 8.7 Instructions for the addition of immune globulin (IgG):
 - 8.7.1 Follow *STCL-PROC-015 CD34 Positive Selection Using Miltenyi CliniMACS* until step 8.12.
 - 8.7.2 When working with bone marrow, IgG needs to be added prior to adding the CD34 reagent.

- 8.7.3 IgG is ordered through the pharmacy or directly from Baxter: Immune Globulin Intravenous (Human) GAMMAGARD S/D 5g, 96 mL, store at a temperature not to exceed 25° C.
- 8.7.4 Allow IgG to reach room temperature before reconstitution if refrigerated.
- 8.7.5 To reconstitute a 10% solution of IgG, remove 48 mL from the 96 mL of the included sterile water diluent and discard.
- 8.7.6 Add the remaining 48 mL of sterile water diluent to the concentrate bottle and immediately swirl the concentrate bottle gently to thoroughly mix contents.
- 8.7.7 Rotate gently until all concentrate is dissolved. Do NOT shake to avoid foaming.

NOTE: Use within 24 hours of reconstituting and then discard any remaining solution.

- 8.7.8 Add 1.5 mg human IgG per mL of cellular product.
 - 8.7.8.1 If target volume for addition of CD34 reagent is 95 mL, multiply 1.5 mg times 95 mL to determine that 142.5 mg of IgG is needed.
 - 8.7.8.2 In a 5g bottle, there are 5000 mg reconstituted in 48 mL. Use a proportion to calculate the desired volume needed.

$$\frac{5000 \text{ mg}}{48 \text{ mL}} = \frac{142.5 \text{ mg}}{x} \quad x = 1.4 \text{ mL of IVIg needed}$$

- 8.7.9 Mix the contents thoroughly.
- 8.7.10 Incubate for 5 minutes at controlled room temperature by using a gentle rotating motion.
- 8.8 Return to *STCL-PROC-015 CD34 Positive Selection using Miltenyi CliniMACS*, step 8.13 to proceed with selection of cellular product.

9 RELATED FORMS

- 9.1 STCL-PROC-015 FRM1 CD34+ CliniMACS Worksheet
- 9.2 STCL-PROC-015 FRM2 Certificate of Analysis COA for Autologous CD34+ Selections
- 9.3 STCL-PROC-015 FRM3 CD34+ Certificate of Analysis (COA) for CliniMACS Allogeneic Donors
- 9.4 STCL-PROC-015 JA1 Auto CD34-Selected PBPCS for Treatment of Severe Chron's Disease
- 9.5 COMM-PAS-003 Labeling Cellular Therapy Products
- 9.6 STCL-FORM-045 Processing Lot Numbers – Bone Marrow Processing (*Use if bone marrow is being processed*).

- 9.7 STCL-FORM-049 Processing Lot Numbers – Incoming Cellular Product Processing (*Use if peripheral blood progenitor cells are being processed*).

10 REFERENCES

- 10.1 CliniMACS System User Manual, Miltenyi Biotec, Presidential Way, Woburn, Massachusetts, 01801.
- 10.2 Adult Bone Marrow Transplant Program Protocol Notebooks – internal protocols, Duke University Medical Center, Durham, NC.

11 REVISION HISTORY

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02	B. Waters-Pick	Updated document numbers throughout the procedure

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STCL-PROC-015 JA2 Preparation of Bone Marrow before CD34+ Selection Procedure**Author**

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		16 Aug 2020, 07:46:48 PM	Approved

Management

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		16 Aug 2020, 07:47:04 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		17 Aug 2020, 08:59:32 AM	Approved

Quality

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Isabel Storch (IMS19)		17 Aug 2020, 02:57:47 PM	Approved

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