STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-PROC-015 JA4

DOCUMENT TITLE:

CliniMACS® CD34+ Cell Selection CTN 1301

DOCUMENT NOTES:

Document Information

Revision: 01	**Vault:** STCL-Processing-rel
Status: Release	**Document Type:** Processing

Date Information

Creation Date: 15 Sep 2015	**Release Date:** 05 Oct 2015
Effective Date: 05 Oct 2015	**Expiration Date:**

Control Information

Author: WATE02	**Owner:** WATE02
Previous Number: None	**Change Number:** STCL-CCR-308

STCL-PROC-015 JA4
CliniMACS® CD34+ Cell Selection CTN 1301

1 PURPOSE

1.1 This document describes the processing requirements for CD34-enrichment using the CliniMACS CD34 Reagent System procedure under conditions specified for CTN 1301, A Randomized, Multi-Center, Phase III Trial of Calcinerin Inhibitor-Free Interventions for Prevention of Graft-versus-Host Disease.

1.2 This document should be used in conjunction with *STCL-PROC-015* CD34 Positive Selection Using Miltenyi CliniMACS.

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	kg	kilogram
4.2	TNC	Total Nucleated Cells
4.3	mL	milliliter
4.4	IVIg/IgG	Immune Globulin
4.5	g	gram

4.6	mg	milligram
4.7	HPCA	Hematopoietic Progenitor Cell Assay
4.8	RPM	Revolutions per Minute
4.9	°	Degree
4.10	C	Celsius

5 MATERIALS

5.1 Immune Globulin Intravenous (Human) 10% GAMMAGARD S/D

5.2 PlasmaLyte A

5.3 Syringes (1 mL, 5 mL, 10 mL, 20 mL, 30 mL, 60 mL)

5.4 Sterile needles; 16G or 19G

5.5 Alcohol prep pads

5.6 600 mL transfer pack(s)

5.7 Sterile Tubing Welding Wafers

6 EQUIPMENT

6.1 CliniMACS

6.2 Temperature controlled centrifuge

6.3 Plasma extractor

6.4 Biological safety cabinet (BSC)

6.5 Sterile Tubing Welder

6.6 Balance

6.7 Tubing heat sealer

6.8 Tubing stripper

6.9 Hemostats

6.10 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 Specimen:

8.1.1 HPC, Apheresis product(s) with a target dose of CD34+ cells after processing of 5.0×10^6/kg containing $\leq 1.0 \times 10^5$ CD3+ T cells/kg.

8.1.2 Assuming a conservative recovery of 50%, up to two apheresis collection procedures are allowed to obtain $\geq 10 \times 10^6$ CD34+ cells/kg in starting product.

8.1.3 A $\geq 4.0 \times 10^6$ CD34+ cells/kg is required to achieve the minimum infusion dose of 2.0×10^6 CD34+ cells/kg. See Table 1.

Table 1: Collection decisions based on Cumulative CD34+ cell content in product at receipt

Collection Number	CD34/kg $\geq 4 \times 10^6$ & $\leq 10 \times 10^6$	CD34/kg $\geq 10 \times 10^6$	CD34/kg $< 4 \times 10^6$
Collection #1	Collect again	Stop	Collect Again
Collection #2	Stop	NA	Collect Again[1]

Note: [1] if $< 2 \times 10_6$ CD34+ cells per kg are available after processing of second collection, a third collection is allowed that will not undergo CD34-enrichment.

8.2 Products expected to start processing within 24 hours of collection may be stored at room temperature. Products that will start processing >24 hours after collection should be stored at refrigerator temperatures. When possible, processing should begin within 36 hours of the end of collection.

8.2.1 Dilute products to be stored overnight prior to processing to $< 200 \times 10^6$ TNC/mL with concurrent plasma obtained from donor at time of apheresis collection. If sufficient plasma is not available, use PBS/EDTA/HAS selection buffer to dilute the cells.

8.2.1.1 Caluculate volume of diluent required. (Volume required = TNC $\div 200 \times 10^6$ – current volume).

8.2.1.2 Add calculated plasma and mix. If necessary, transfer product to a larger transfer bag for storage.

8.2.2 Products stored overnight in the refrigerator must be brought to room temperature prior to processing.

8.2.3 Remove product from 1-10 °C storage conditions minimally 1 hour prior to starting selection procedure.

8.2.4 Resample stored products for TNC, platelet count, flow assays and sterility.

8.3 Prepare infusion media by adding 50 mL of 25% HAS to 500 mL PlasmaLyte A (acceptable to add 25 mL of 25% HAS to 250 mL PlasmaLyte A) and store at 1-6 °C until use. Use within 24 hours of preparation.

8.4 Follow *STCL-PROC-015* CD34 Positive Selection Using Miltenyi CliniMACS with the following exceptions/additions.

8.4.1 Add the additional pre-processing assays to step 8.3

8.4.1.1 14 day sterility

8.4.1.2 Platelet count

8.4.2 For pooled products (determined by tables 1 and 2):

8.4.2.1 Transfer both products into a single transfer pack.

8.4.2.2 If flow was not performed on the second product, remove a sample for TNC, platelet count, viability and flow.

8.4.2.3 If flow was performed on the second product, flow from the pool is not required. Starting values will be the sum of the individual products.

8.4.2.4 Weigh and record product bag volume (1 g of product = 1 mL).

8.4.3 Refer to Table 2 below to determine limits of TNC and CD34 for the CliniMACS tubing sets.

Table 2: Requirements for CD34-Enrichment Procedures

TNC Limits	CD34 Limits	Tubing Set	Bottle CD34 Reagent	Volume at Label
$\leq 6 \times 10^{10}$	$\leq 6 \times 10^{8}$	161-01 (NS)	1	95 ± 5
$\leq 6 \times 10^{10}$	$> 6 \times 10^{8}$	162-01 (LS)	2	190 ± 5
6×10^{10} to 12×10^{10}	$\leq 6 \times 10^{8}$	162-01 (LS)	2	190 ± 5
6×10^{10} to 12×10^{10}	$> 6 \times 10^{8}$	162-01 (LS)	2	190 ± 5

Note: If more than one tubing set is needed, split products equally for best CD34+ cell recovery. The column is bigger in the 162-01 large-scale (LS) set and can hold more CD34+ cells. Therefore, even if TNC is $< 6.0 \times 10^{10}$, if the number of CD34+ cells exceed 6×10^{8} the LS set must be used. The LS tubing set may be used for products meeting the parameters for use of the NS tubing set. A product may be held overnight and pooled with a subsequent collection so long as capacities are not exceeded.

8.4.4 For first wash (Platelet Wash), step 8.5:

8.4.4.1 Add no more than 200 mL of product to each transfer pack.

8.4.4.2 Add buffer, centrifuge and express. If more than one bag was used for the platelet wash, combine and adjust volume to appropriate volume for labeling. See Table 2.

8.4.4.3 Obtain a small sample for a platelet count.

8.4.5 IVIg Addition, between step 8.12 and step 8.13

8.4.5.1 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.

8.4.5.2 Allow IgG to reach room temperature before reconstitution if refrigerated.

8.4.5.3 To reconstitute a 10% solution of IgG, remove 48 mL from the 96 mL included sterile water diluent and discard.

8.4.5.4 Add the remaining 48mL of sterile water diluent to the concentrate bottle and immediately swirl the concentrate bottle gently to thoroughly mix contents.

8.4.5.5 Rotate gently until all concentrate is dissolved. Do NOT shake in order to avoid foaming.

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

8.4.5.7 Add 1.5 mg IgG per mL of cellular product.

8.4.5.8 The calculated volume of IVIG added should be included in the final labeling weight; do not exceed 95 g or 190 g.

8.4.5.9 If target volume for addition of CD34 reagent is 95 mL, multiply 1.5 mg times 95 mL to determine that 14.2 mg of IgG is needed.

8.4.5.10 In a 5g bottle, there are 5000 mg reconstituted in 48 mL. Use a proportion to calculate the desired volume needed.

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

8.4.5.11 Add to bag and mix contents thoroughly.

8.4.5.12 Incubate at room temperature using a gentle rotating motion a minimum of 5 minutes before adding CD34+ reagent.

8.4.6 In preparation for the column after completion of step 8.18:

8.4.6.1 Filter the cells into a new, labeled 600 mL transfer pack through a standard blood filter.

8.4.6.2 Use PBS/EDTA/HSA buffer to flush filter (~25 mL).

8.4.6.3 Add additional buffer to achieve final volumes of 150 mL for $\leq 6 \times 10^{10}$ cells or 300 mL for 6-12 x 10^{10} cells.

8.4.6.4 Mix contents and retrieve a sample for TNC, platelet count, viability, HPCA and flow.

8.5 Preparation for Infusion

8.5.1 The volume at the end of selection is expected to be ~ 40–50 mL for the standard selection tubing set and ~ 75-80 mL for the LS tubing set.

8.5.2 Obtain samples for product analysis, including TNC, viability, HPCA, gram stain, endotoxin and 14 day sterility (sample may be taken from final wash supernatant after preparation for infusion).

8.5.3 Obtain sample for flow analysis to include:

8.5.3.1 Viability using 7-AAD

8.5.3.2 Total number and percentage (purity) of CD34+ cells

8.5.3.3 Total number and percentage of CD3+ cells

8.5.3.4 Total numbers and percentages of monocytes ($CD45^{bright}CD14^{bright}$), B cells (CD19 or CD20+), NK cells (CD3-CD56+)

8.5.3.5 % Recovery of CD34+ cells from product prior to processing

8.5.3.6 CD3 log depletion from product prior to processing

8.5.4 Prior to infusion to the patient, the CD34 enriched fraction must be removed from the CliniMACS PBS/EDTA Buffer and suspended in a medium suitable for clinical use (PlasmaLyte A + HSA).

8.5.4.1 Transfer cells from the collection bag to 50 mL sterile conical tubes with not more than 25 mL added to each tube.

8.5.4.2 Rinse collection bag with ~50 mL of infusion medium and add to the tubes.

8.5.4.3 Fill tubes to capacity with infusion medium.

8.5.4.4 Centrifuge the capped tubes at 500-750 g for 10 minutes.

8.5.4.5 Remove supernatant up to the pellet, combine the tubes rinsing each to ensure recovery of all cells and suspend cells in $\geq$ 50 mL infusion medium and transfer cells to appropriate size transfer pack or syringe for infusion.

8.5.4.6 Count the washed cells and use count to determine volume needed to archive 2 vials of cells each containing 2.0×10^6 cells.

8.5.4.7 Store cells during release testing at refrigerator temperatures (1-10 °C)

8.6 Release Requirements

8.6.1 Products may not be released from the lab until all criteria for release are met and a protocol certificate of analysis is completed. Criteria include:

8.6.1.1 TNC viability $\geq$ 70% by 7-AAD

8.6.1.2 Cumulative CD3+ T cell dose $\leq 1.0 \times 10^5$/kg

8.6.1.3 Cumulative CD34+ cell dose $\geq 2.0 \times 10^6$/kg (Individual products may be less than this amount.)

8.6.1.4 Negative gram stain

8.6.1.5 No excessive hemolysis or clumping

8.6.1.6 Bag integrity – no visible leaks or tears.

8.6.1.7 Endotoxin: Post release final endotoxin infused should not exceed 5.0 EU/kg

8.6.1.8 Sterility: Post release final sterility culture results should be negative

8.6.1.9 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.

8.6.2 Products should be infused on the day of processing.

8.7 Expected Results

8.7.1 CD34 recovery from 30 – 100% with an average recovery of ~ 66%

8.7.2 CD34 purity ranging from 62% - 99% with an average of > 90%

8.7.3 CD3 log reduction ranging from 3.2 to 5.9 with an average of >4.8

8.7.4 Viability ranging from 74-100%, but expected to exceed 96%

8.7.5 Target dose of 5.0×10^6 CD34+/kg for adult donors and recipients should be attainable in a single collection for 45% of patients and for 89% of patients with 2 collections.

8.7.6 Recipients are expected to receive $\geq 2.0 \times 10^6$ CD34 enriched cells/kg

8.7.7 Recipients are expected to receive the targeted and minimum CD34 cell dose with fewer than 1.0×10^5 CD3+ T cells/kg.

9 RELATED FORMS

9.1 STCL-PROC-015 CD34 Positive Selection Using Miltenyi CliniMACS

9.2 STCL-PROC-015 (FRM1) CliniMACS Worksheet

9.3 STCL-PROC-015 (FRM4) Cell Selection – Product at Receipt/Pre-Processing/Pre-Column Form

9.4 STCL-PROC-015 (FRM5) Graft Evaluation Form (GRF)

9.5 STCL-PROC-015 (FRM6) Certificate of Analysis for CTN1301 Protocol

9.6 COMM-PAS-003 Labeling Cellular Therapy Products

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

Revision No.	Author	Description of Change(s)
01	B. Waters-Pick	New document

Signature Manifest

Document Number: STCL-PROC-015 JA4 **Revision:** 01
Title: CliniMACS® CD34+ Cell Selection CTN 1301

All dates and times are in Eastern Time.

STCL-PROC-015 JA4 CliniMACS® CD34+ Cell Selection CTN 1301

Author

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		16 Sep 2015, 04:01:29 PM	Approved

Manager

Name/Signature	Title	Date	Meaning/Reason
Barbara Waters-Pick (WATE02)		16 Sep 2015, 04:01:42 PM	Approved

Medical Director

Name/Signature	Title	Date	Meaning/Reason
Joanne Kurtzberg (KURTZ001)		16 Sep 2015, 05:46:19 PM	Approved

Quality

Name/Signature	Title	Date	Meaning/Reason
John Carpenter (JPC27)		21 Sep 2015, 04:39:17 PM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		27 Sep 2015, 04:24:55 PM	Approved