STEM CELL LABORATORY (STCL)

DOCUMENT NUMBER: STCL-PROC-015

DOCUMENT TITLE:

CD 34 Positive Selection Using Miltenyi CliniMACS

DOCUMENT NOTES:

Document Information

Revision: 05	**Vault:** STCL-Processing-rel
Status: Release	**Document Type:** Processing

Date Information

Creation Date: 02 Sep 2015	**Release Date:** 21 Oct 2015
Effective Date: 21 Oct 2015	**Expiration Date:**

Control Information

Author: WATE02	**Owner:** WATE02
Previous Number: STCL-PROC-015 Rev 04	**Change Number:** STCL-CCR-308

STCL-PROC-015
CD34 POSITIVE SELECTION USING MILTENYI CliniMACS

1 PURPOSE

1.1 This document describes the procedure for the selection and enrichment of human CD34 positive hematopoietic progenitor cells from G-CSF mobilized Hematopoietic Progenitor Cells, Apheresis (HPC-A) utilizing the CliniMACS magnetic cell separation system.

1.2 If working with bone marrow as a cellular source, instead of a mobilized HPC-A product, in preparation for CD34 selection, follow the specific instructions outlined in *STCL-PROC-015 JA2 Preparation of Bone Marrow before CD34+ Selection Procedure*. This job aide will detail how the bone marrow product should be processed and it will list any additional reagents and/or supplies that are needed before initiating the selection procedure according to the steps outlined in this SOP.

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+ cells or for simultaneous CD34+ 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	BSC	Biological Safety Cabinet
4.2	PBS	Phosphate buffered saline
4.3	SOP	Standard Operating Procedure
4.4	HSA	Human Serum Albumin
4.5	HPCA	Hematopoietic Progenitor Cell Assay
4.6	Sample A	Pre-Processing sample
4.7	Sample B	Post Wash / Post Incubation / Pre-Selection sample
4.8	Sample C	Post selection (final) sample
4.9	mL	milliliter

5 MATERIALS

5.1 CliniMACS tubing set, Ref. No. 161-01 or 162-01

5.2 CliniMACS CD34 reagent, Ref. No. 171-01

5.3 CliniMACS PBS/EDTA buffer, Ref. No. 700-25

5.4 600 mL transfer packs

5.5 Sampling site couplers

5.6 Plasma transfer sets

5.7 300 mL transfer pack

5.8 Luer/spike inter connector

5.9 Pre-system filter

5.10 25% Human serum albumin

5.11 Slide clamps

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

5.13 Sterile Needles, various sizes

5.14 Sample tubes

5.15 SCD wafers

6 EQUIPMENT

6.1 Terumo SCD

6.2 Temperature controlled centrifuge

6.3 Plasma Extractor

6.4 Biological safety cabinet (BSC)

6.5 CliniMACS cell separator

6.6 Hematology analyzer

6.7 Balance

6.8 Tubing heat sealer

6.9 Tubing stripper

6.10 Hemostats

6.11 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. Refer to SOP for collection, labeling and handling of the products collected for manipulation in this procedure.

8.2 Record lot numbers and expiration dates for all appropriate reagents/disposables on the worksheet (*STCL-PROC-015 (FRM1) CliniMACS Worksheet)* and/or on the appropriate lot sheet (*STCL-FORM-049 Processing Lot Numbers – Incoming Cellular Product Processing*). Complete the worksheet as each procedure is performed.

8.3 Perform cell count, viability, HPCA and flow analysis on the product per SOPs, recording results on the appropriate worksheets. Label this sample as ***Sample A***.

8.4 Prepare working buffer reagent by adding 20 mL of 25% HSA to each of three liters of PBS/EDTA buffer. Mix well but gently to avoid foaming.

8.5 Label a 600 mL transfer pack "Cell Prep Bag" and place on the scale and tare the scale with the empty bag. Transfer the well mixed product to this bag and weigh it to obtain the volume.

8.6 Close the roller clamp on a plasma transfer set and spike into the PBS bag.

8.7 Sterile dock the PBS to the cell bag and fill the bag with buffer.

8.8 Heat seal the tubing between the bags; leave enough tubing for several more sterile dockings.

8.9 Remove the PBS and sterile dock an empty 600 mL transfer pack labeled "plasma waste bag" to the cell suspension. Do not open the weld. Mix the cells carefully and thoroughly.

8.10 Centrifuge the cell bag/transfer pack combo at room temperature at 200xg for 15 minutes with no brake.

8.11 Remove enough supernatant so that 95 mL, +/- 5mLs (for less than 60 x 10^9 TNCC) or 190 mL, +/- 5mLs (for 60 – 120 x 10^9 TNCC) remain in the bag.

NOTE: ***It is better to remove too much and add the correct amount back than to remove too little and have to re-spin the product.***

8.12 Disconnect the waste bag and mix the cells gently and thoroughly.

8.13 Determine the number of vials of CliniMACS CD34+ reagent to add. One vial is added if the volume is 95 +/- 5 mL; two vials are added if the volume is 190 +/- 5 mL. Withdraw the reagent into a syringe, adding a slug of air and inject into the sample, using the air to push the entire contents of reagent into the bag. Immediately set a timer for 30 minutes. Fill the bag with several more syringes of air and mix gently and thoroughly.

8.14 Rotate and invert the cell bag every 5 minutes during this antibody incubation to ensure that the reagent makes good contact with all cells.

8.15 At the end of the incubation period, sterile dock buffer to the bag and fill the bag with buffer (i.e. add 400 – 500 mL of buffer). Heat-seal the tubing and remove the buffer bag.

8.16 Mix the cell suspension bag well.

8.17 Label a 600 mL transfer pack "wash waste I" and sterile dock it to the cell suspension bag. Centrifuge this combo at room temperature at 200xg for 15 minutes with no brake. Express as much supernatant as possible without taking cells. Re-suspend the cell pellet and mix well.

8.18 Repeat this procedure a second time with a 600 mL transfer pack labeled "wash waste II". This second wash procedure should result in the removal of between 300 and 500 mL of supernatant. If less volume can be removed, repeat the wash procedure a third time.

8.19 Add buffer to the final product to produce a volume of 150 mL (if using 1 vial of reagent) or 275 mL (if using two). Mix the sample gently and thoroughly and remove samples for cell count, viability, HPCA and flow. Label this sample, ***Sample B***.

8.20 Heat seal the tubing on a 300 mL transfer pack and remove the tubing. Insert a plasma transfer set with a female luer adapter into the transfer pack. Label this transfer pack "Cell Collection Bag". Weigh the pack and record the weight. This will be the tare weight for the final product.

8.21 Unpack the tubing set under the hood. Keeping the caps sterile, remove caps and attach the cell collection bag to the luer connector on the tubing set. Check luer lock connections on the columns. Luer locks must be tightly closed.

NOTE: ***CliniMACS tubing sets have been sterilized with ethylene oxide. Prior to opening the tray, inspect the package for any damage, punctures or tears which might indicate that the sterility of the set has been compromised.***

8.22 Set up the CliniMACS

8.22.1 Switch on the instrument and press "ENT" to proceed to the program menu.

8.22.2 Choose CD34+ Selection 1 (150 mL product) or CD34+ Selection 2 (275 mL product) by highlighting the name of the program. Move the

bar up and down by using the "0" and the "8" key. To proceed with the highlighted program, press "ENT". Changes can be made to selections by pressing the "UNDO" key. To confirm choice and proceed, press "ENT".

8.22.3 Enter the respective reference numbers for the tubing set (Ref. No. 161-01 or 162-01) and reagent (Ref. No. 171-01) used. Press "ENT" after each entry. The system is programmed to recognize reference numbers that do not correlate to the program chosen. The operator will be prompted to re-enter incorrect codes.

8.22.4 Place the pre-column in the holder, ensuring that the plastic projections found at the bottom of the column are facing you. Attach the Priming Waste Bag to the right hand bag hanger on the instrument. Adjust the height of the hanger, if needed. To proceed, press "ENT".

8.22.5 Insert the Selection Column into the Selection Column Holder, making sure the "wings" are to the front. To avoid possible pinch injury, insert the column as follows: Hold the top and the bottom of the column between thumb and index finger, then carefully insert the column into the column holder. Load the tubing into valve No. 5. To proceed, press "ENT".

8.22.6 The screen prompts to load valves 1, 2, 3 and 4. The valves shown on the screen will be opened automatically.

8.22.7 Load the tubing securely into valve No. 4, then into valve No. 1.

8.22.8 Position the 4-way fitting just below Valve No. 2. Insert the tubing into valves No. 2 and No. 3.

8.22.9 Mount the tubing between valve No. 2 and the bubble trap into the liquid sensor. To assure proper operation, both the liquid sensor and the tubing being inserted must be dry. Carefully inspect both and dry with a lint free cloth if needed.

NOTE: Only insert tubing into open valves (i.e. when the button is pushed inward). If tubing needs adjustment after the valve has closed, do not pull the tubing without pressing the valve button to open the valve.

8.22.10 To proceed, press "ENT".

8.22.11 Load the pump tubing.

8.22.11.1 Open the pump door by lifting up at the left hand edge.

8.22.11.2 Insert the upper retaining ring on the pump tubing into the retaining ring groove on the pump housing.

8.22.11.3 Rotate the pump roller clockwise until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins.

8.22.11.4 Insert the lower retaining ring on the pump tubing into the retaining ring groove on the pump housing.

8.22.11.5 Repeat clockwise rotation of the pump roller to be certain that the pump roller moves freely.

8.22.11.6 Close the pump door. Caution: During the cell selection sequence the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 10 minutes, the instrument will abort the run in progress.

8.22.11.7 To proceed, press "ENT".

8.22.12 The screen prompts the operator to load valves 7 and 8. Load the tubing into those valves and press "ENT" to proceed.

8.22.13 The screen prompts the operator to load valves 6, 9, 10 and 11. Load the tubing into these valves and press "ENT" to proceed.

8.22.14 Place the Negative Fraction Bag and the Buffer Waste Bag in the bag compartment. Make sure the tubing is not compressed under the bag compartment lid. Press "ENT" to proceed.

8.22.15 In order to ensure the proper fitting of tubing in the valves, the analyzer will operate all of the valves in sequence, twice. Watch and listen to make sure all valves are working properly.

8.22.16 Double check the placement of all tubing. Be certain that the tubing enters and leaves each valve through the enlargement at the inner end of the slot and is positioned in the center of the jaws of the valve. If tubing has to be readjusted, be sure to open the valve first. Once the tubing has been readjusted, it is absolutely necessary to press the respective valve firmly two times. Check that none of the tubing is kinked or twisted. To proceed, press "ENT".

8.22.17 Attach the Selection Buffer Bag.

8.22.17.1 Remove the cap from the buffer spike on the tubing and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid.

8.22.17.2 Attach the buffer bag to the buffer bag hook on the bag hanger.

8.22.17.3 Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the buffer bag. Position it high enough to prevent severe bending of the tubing that could restrict flow and low enough to avoid the tubing connections being stretched.

8.22.17.4 To proceed, press "ENT".

8.22.18 Recheck all tubing one last time. Press "ENT" to proceed.

8.22.19 Start the priming procedure by pressing "RUN". The priming phase will take approximately 1 minute and the priming status will be updated on the display. During this priming, check all tubing and connections

for leaks or impediments to flow. If problems are found, press "STOP". You will have 10 minutes to resolve the problem. Restart the process by pressing "RUN".

NOTE: After 10 minutes, the selection will be aborted. If the problem can't be resolved, start the process over again using NEW tubing set.

NOTE: Once priming has started, it is not possible to return to the instrument set-up procedure.

8.22.20 Perform a final check of all tubing and attachments and:

8.22.20.1 Verify that there is fluid in all parts of the tubing set.

8.22.20.2 Verify that there is no excess air in the tubing set.

8.22.20.3 Verify that there is fluid in the priming waste bag and the buffer waste bag.

8.22.20.4 Verify that there is no fluid in the negative fraction bag or the cell collection bag.

8.22.20.5 Verify that there is no fluid in the bubble trap or in the pre-system filter.

8.22.20.6 Verify that the clamp to the product collection bag is open.

8.22.21 To proceed, press "ENT".

8.22.22 Connect the Cell Preparation Bag to the pre-system filter

8.22.22.1 Remove the cap from the bubble trap spike of the drip chamber.

8.22.22.2 Remove the cap from the lower opening of the pre-system filter and firmly insert the spike into the pre-system filter.

IMPORTANT: See *STCL-PROC-018 Correct Connection of Pre-System Filter and Tubing Set (CliniMACS)* for proper filter insertion instructions.

8.22.22.3 Remove the cap from the pre-system filter spike and spike the cell preparation bag.

8.22.22.4 Check the connection between the pre-system filter and the CliniMACS tubing set to confirm that the connection is secure.

8.22.22.5 Hang the cell preparation bag on the bag hanger.

8.22.22.6 Adjust the pre-system filter support and the bag hanger for the cell preparation bag to hold the pre-system filter and the cell preparation bag in an upright position.

8.22.22.7 To proceed, press "ENT".

8.22.22.8 Check the liquid sensor tubing to ensure that it has been properly inserted, that it is free of any external liquid and that it has not been dislodged during the loading procedure. To proceed, press "ENT".

8.23 Press "RUN"; the instrument automatically starts the selection procedure.

8.24 When the run is complete, clamp or seal the tubing above the luer lock connecting the cell collection bag to the CliniMACS tubing set and transfer the bag to a hood. Replace the cap on the luer connector.

8.25 Calculate /measure the volume of the product and remove a well mixed sample for cell count, viability, HPCA and flow. Label this sample, ***Sample C***.

8.26 To proceed, press "ENT".

8.27 Heat seal the tubing above the luer lock of the negative fraction bag and the buffer waste bag and remove these bags.

8.28 Remove the tubing set.

8.28.1 Beginning with valves 6, 9, 10 and 11 and working upwards, release the tubing from the valves and from the liquid sensor by pressing the valves.

8.28.2 Release the columns from the column holders.

8.28.3 Dispose of the tubing set as biohazardous waste.

8.28.4 To proceed, press "ENT".

8.29 Record the process code.

8.30 Shut down the CliniMACS.

8.31 Process the selected cells for transplant. If the reinfusion date is in the future, cryopreserve the cells as per standard operating procedures. If the cells are to be infused fresh, process them accordingly as per doctor's orders.

8.31.1 Prior to infusion or cryopreservation, the CD34 enriched fraction must be removed from the CliniMACS PBS/EDTA Buffer and suspended in a medium suitable for clinical use (ie. Plasma-Lyte A with 10% HSA).

8.31.2 Transfer cells from the collection bag and divide equally between two sterile 50 mL conical centrifuge tubes with not more than 25 mL added to each tube.

8.31.3 Rinse the collection bag with approximately 50 mL of infusion/cryopreservation medium and add to the tubes.

8.31.4 Fill the tubes to capacity with infusion/cryopreservation medium. Cap the tubes.

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

8.31.6 Remove supernatant up to the pellet (leave pellet in ~5-7 mL), retain supernatant for inoculation of culture bottles for sterility.

8.31.7 Combine the pellets from the tubes and rinse each tube with infusion/cryopreservation medium to ensure recovery of all cells.

8.31.8 Count the washed cells. Perform viability.

8.31.9 Suspend cells in ≥ 50 mL infusion/cryopreservation medium.

8.31.10 Store cells during release testing and prior to infusion or cryopreservation at refrigerator temperatures (1-10°C).

9 RELATED FORMS

9.1 STCL-PROC-015 (FRM1) CliniMACS Worksheet

9.2 STCL-PROC-015 (FRM2) CD34+ Certificate of Analysis (COA)

9.3 STCL-PROC-015 (FRM3) CD34+ Certificate of Analysis (COA) for CliniMACS Allogeneic Donors

9.4 STCL-PROC-015 (JA1) Auto CD34+ Selected PBPC Treatment of Severe Chron's Disease General Practices for Miltenyi CliniMACS

9.5 STCL-PROC-018 Correct Connection of Pre-system Filter and Tubing Set (CliniMACS)

9.6 COMM-PAS-003 Labeling Cellular Therapy Products

9.7 STCL-FORM-045 Processing Lot Numbers – Bone Marrow Processing *(Use if bone marrow is being processed)*.

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

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

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)
05	B. Waters-Pick	• Changed ml to mL throughout document Added Sections 8.31.1 thru 8.31.9

Signature Manifest

Document Number: STCL-PROC-015 **Revision:** 05

Title: CD 34 Positive Selection Using Miltenyi CliniMACS

All dates and times are in Eastern Time.

STCL-PROC-015 CD34 Positive Selection Using Miltenyi CliniMACS

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Quality

Name/Signature	Title	Date	Meaning/Reason
John Carpenter (JPC27)		08 Oct 2015, 10:00:02 AM	Approved

Document Release

Name/Signature	Title	Date	Meaning/Reason
Sandy Mulligan (MULLI026)		21 Oct 2015, 04:16:08 PM	Approved