STEM CELL LABORATORY (STCL)

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CD56+ Selection Using the CliniMACS

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STCL-PROC-019
CD56+ SELECTION USING THE CLINIMACS

1 PURPOSE

1.1 This document describes the procedure for the selection and enrichment of human CD56 positive NK-cells from peripheral blood, 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 simultaneous selection of CD56 positive cells and purging of unwanted cells from a heterogeneous cell population. The CliniMACS system utilizes highly specific CD56 monoclonal antibodies conjugated to super-paramagnetic particles, which are small in size (about 50nm in diameter) and composed of iron oxide and dextran. The magnetic particles form a stable colloidal suspension and do not precipitate nor aggregate in magnetic fields. The CD56 positive cells are specifically labeled for selection during incubation with the CliniMACS CD56 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 selected CD56 positive cells are released from the column by removing the magnetic field and eluting the cells into 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 contamination.

2.3 Limitations

2.3.1 $\leq 10 \times 10^9$ CD56+ cells; $\leq 40.0 \times 10^9$ TNC: 1 vial CliniMACS CD56 reagent

2.3.2 $\leq 5.0 \times 10^9$ CD56+ cells; $\leq 40.0 \times 10^9$ TNC: Tubing Set Ref No. 161-01

2.3.3 $\leq 2.0 \times 10^9$ CD56+ cells

2.3.3.1 2 liters prepared PBS/EDTA buffer

2.3.3.2 300 mL cell collection bag

2.3.4 $2 - 5.0 \times 10^9$ CD56+ cells

2.3.4.1 3 liters prepared PBS/EDTA buffer

2.3.4.2 600 mL cell collection bag

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3 SCOPE AND RESPONSIBILITIES

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

4 DEFINITIONS/ACRONYMS

4.1	STCL	Stem Cell Laboratory
4.2	QSU	Quality Systems Unit
4.3	ISBT	International Society for Blood Transfusion
4.4	RPM	Revolutions per Minute
4.5	BSC	Biological Safety Cabinet
4.6	PBS	Phosphate Buffered Saline
4.7	SOP	Standard Operating Procedure
4.8	PPE	Personal Protective Equipment
4.9	HSA	Human Serum Albumin
4.10	HPCA	Hematopoietic Progenitor Cell Assay
4.11	Sample A	Pre-Processing sample
4.12	Sample B	Post Wash / Post Incubation / Pre-Selection sample
4.13	Sample C	Post selection (final) sample
4.14	mLs	Milliliters
4.15	COA	Certificate of Analysis

5 MATERIALS

5.1 CliniMACS Tubing Set, Ref. No. 161-01

5.2 CliniMACS CD56 Reagent, Ref. No. 271-01

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

5.4 Pre-system filter

5.5 Luer/Spike inter connector

5.6 600 mL transfer packs

5.7 300 mL transfer pack(s)

5.8 Sampling site couplers

5.9 Plasma transfer sets

5.10 Slide clamps

5.11 Disposable hemostats

5.12 Syringes (3 mL, 10 mL, 20 mL, 60 mL)

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5.13 Needles (16G, 19G, spinal)

5.14 Alcohol prep pads

5.15 Culture bottles, aerobic and anaerobic

5.16 Pipettor tips

5.17 12 x 75 test tubes

5.18 Snap-cap tubes, sterile and non-sterile

5.19 1.8 mL cryo vials (nuncs)

5.20 25% Human Serum Albumin

5.21 Plasmalyte-A®

5.22 SCD wafers

5.23 Tie tags

5.24 ISBT Demand 128 labels

5.25 Barcode labels

5.26 Personal Protective Equipment

6 EQUIPMENT

6.1 CliniMACS System

6.2 Sysmex XS-1000i Hematology Analyzer (or equivalent)

6.3 Biological Safety Cabinet (BSC)

6.4 Temperature controlled centrifuge

6.5 Terumo SCD

6.6 Microscope

6.7 Plasma Extractor

6.8 Scale (0-2000g)

6.9 Sebra heat sealer

6.10 Tubing stripper

6.11 Hemostats

6.12 Pipettors

6.13 Timer

6.14 Calculator

7 SAFETY

7.1 Use appropriate PPE when handling any/all potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coat, goggles, etc. Tech should only handle one cellular product at any given time.

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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 the 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-019 (FRM1) CD56+ CliniMACS Worksheet*. Complete the worksheet as the procedure is performed.

8.3 If time permits, perform cell count, viability, ABO, cultures, nuncs, and flow analysis on the product per SOPs, recording results on the appropriate worksheets **the night before** the selection. Label this sample as ***Pre-Overnight***.

8.3.1 If plasma was collected, remove only 1.5 mL of product:

8.3.1.1 Use 0.5 mL of sample to add 2 drops to the viability and ABO tubes and to perform a cell count and flow analysis.

8.3.1.2 Add 0.5 mL of sample to each nunc vial.

8.3.1.3 Pull up 1.5 mL of plasma in the same syringe and add 1.0 mL to each culture bottle.

8.3.2 If NO plasma was collected, remove 2.5 mLs of product:

8.3.2.1 Use 0.5 mL of sample to add 2 drops to the viability and ABO tubes and to perform a cell count and flow analysis.

8.3.2.2 Add 0.5 mL of sample to each nunc vial.

8.3.2.3 Add 0.5 mL of sample to each culture bottle.

8.4 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***.

8.4.1 If ***Pre-Overnight*** testing was done, remove 1.0 mL of sample:

8.4.1.1 Add 2 drops of sample to the viability tube.

8.4.1.2 Add 0.5 mL of sample to the HPCA tube.

8.4.1.3 Add 0.5 mL of sample to the flow analysis tube.

8.4.2 If NO ***Pre-Overnight*** testing was done and plasma was collected, remove 2.0 mL of product:

8.4.2.1 Use 0.5 mL of sample to add 2 drops to the viability and ABO tubes and to perform a cell count and flow analysis.

8.4.2.2 Add 0.5 mL of sample to the HPCA tube.

8.4.2.3 Add 0.5 mL of sample to each nunc vial.

8.4.2.4 Pull up 1.5 mL of plasma in the same syringe, and add 1.0 mL to each culture bottle.

8.4.3 If NO ***Pre-Overnight*** testing was done and NO plasma was collected, remove 3.0 mL of product

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8.4.3.1 Use 0.5 mL of sample to add 2 drops to the viability and ABO tubes and to perform a cell count and flow analysis.

8.4.3.2 Add 0.5 mL of sample to the HPCA tube.

8.4.3.3 Add 0.5 mL of sample to each nunc vial.

8.4.3.4 Add 0.5 mL of sample to each culture bottle.

8.5 Prepare working buffer reagent by adding 20mls of 25% HSA to each liter of PBS/EDTA buffer. Mix well but gently to avoid foaming.

NOTE: Use 2 liters of prepared buffer if $\leq 2.0 \times 10^9$ CD56+ cells. Use 3 liters of prepared buffer if $2 - 5.0 \times 10^9$ CD56+ cells.

8.6 A maximum number of 5×10^9 CD56+ cells out of a total cell number not to exceed 40×10^9 total cells can be processed.

8.7 **WASH 1**: Wash the product with the prepared buffer prior to magnetic labeling.

8.7.1 Label a 600 mL transfer pack, "WASH I" and place it on the scale.

8.7.2 Tare the scale with the empty bag.

8.7.3 Transfer the well mixed product to this bag and weigh it to obtain the volume.

8.7.4 Subtract the weight of the leukapheresis product from 600. The result will be the number of mL of buffer to add to the product for the wash.

8.7.5 **Close** the roller clamp on a plasma transfer set and spike into one of the working buffer bags.

8.7.6 Sterile dock the working buffer to the cell bag, and fill the bag with buffer.

8.7.7 Heat seal the tubing between the bags, leaving enough tubing for several more sterile dockings.

8.7.8 Centrifuge the cell bag at room temperature at 1250 RPM for 15 minutes with **NO** brake.

8.7.9 Before removing the product bag from the centrifuge container, sterile dock it to an empty 600 mL transfer pack labeled "WASH WASTE I." **DO NOT** open the weld.

8.7.10 Place the centrifuged product on a plasma expresser and allow it to sit for 10 minutes before expressing the supernatant.

8.7.11 Express as much supernatant as possible without taking cells.

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

8.7.13 Sterile dock the working buffer to the cell bag and fill the bag with buffer to a volume of 95 mL, +/- 5mLs.

8.7.14 Disconnect the working buffer and mix the cells gently and thoroughly.

8.8 Withdraw the CliniMACS CD56 reagent in a 10 mL syringe, being careful not to withdraw the syringe needle if there is pressure in the reagent vial. Inject the

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reagent into the cell bag followed by a slug of air, gently mixing every couple mLs to ensure thorough labeling.

8.9 Immediately set a timer for 30 minutes to reflect the total antibody incubation time.

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

8.11 At the end of the incubation period, wash the cells.

8.12 **WASH 2**: Wash the product with the prepared buffer at the end of the antibody incubation period.

8.12.1 Weigh the cell bag to obtain the volume.

8.12.2 Subtract the weight of the leukapheresis product from 600. The result will be the number of mL of buffer to add to the product for the wash.

8.12.3 Sterile dock the working buffer to the cell bag, and fill the bag with buffer.

8.12.4 Heat seal the tubing between the bags, leaving enough tubing for several more sterile dockings.

8.12.5 Centrifuge the cell bag at room temperature at 1250 RPM for 15 minutes with **NO** brake.

8.12.6 Before removing the product bag from the centrifuge container, sterile dock it to an empty 600 mL transfer pack labeled "WASH WASTE II." **DO NOT** open the weld.

8.12.7 Place the centrifuged product on a plasma expresser and allow it to sit for 10 minutes before expressing the supernatant.

8.12.8 Express as much supernatant as possible without taking cells.

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

8.12.10 Sterile dock the working buffer to the cell bag and fill the bag with buffer to a volume of 150 mL, +/- 5mLs.

8.12.11 Disconnect the working buffer and mix the cells gently and thoroughly.

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

8.13.1 Remove 1.0 mL of product:

8.13.1.1 Add 2 drops of sample to the viability tube.

8.13.1.2 Add 0.5 mL of sample to the HPCA tube.

8.13.1.3 Add 0.5 mL of sample to the flow analysis tube.

8.14 In the BSC, inject 60 mL of air in the cell bag prior to connecting it to the tubing set.

8.15 Heat seal the tubing on a 300 mL transfer pack (for $\leq 2.0 \times 10^9$ CD56+ cells) or a 600 mL transfer pack (for $2 - 5.0 \times 10^9$ CD56+ cells) and remove the tubing.

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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.

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.***

8.16 Unpack the tubing set under the hood. Use disposable hemostats to clamp off the lines ending in luer adapters. This is where we will eventually connect the buffer bag(s) and cell bag.

8.17 Check luer lock connections throughout the tubing set to ensure that they are tightly closed.

8.18 Keeping the caps sterile, attach the cell collection bag to the luer connector on the tubing set.

8.19 Connect the pre-system filter to the tubing set.

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

8.19.2 Remove the cap from the lower opening (non-spike) of the pre-system filter and firmly insert the spike into the pre-system filter.

NOTE: Be extremely careful; refer to *STCL-PROC-018 Correct Connection of Pre-system Filter and Tubing Set-CliniMACS* for further connection instructions.

8.20 Connect the cell bag to the pre-system filter.

8.20.1 Clamp the tubing set below the bubble trap.

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

8.21 Connect the remaining buffer bag to the tubing set.

8.21.1 Clamp the tubing below the spike on the tubing set.

8.21.2 Remove the cap from the tubing set spike and spike the bag of buffer.

NOTE: If $2 - 5.0 \times 10^9$ CD56+ cells, the CliniMACS will require 2 liters of prepared working buffer. Connect these using a plasma transfer set, sampling site coupler, and 16G needle. Be sure to close the roller clamp on the plasma transfer set **before** spiking into the buffer bag.

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 Select ENRICHMENT 1.1 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 back arrow. To confirm choice and proceed, press "ENT."

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8.22.3 Select CliniMACS TS and press "ENT." To confirm the program, press "ENT".

8.22.4 Enter the reference number for the tubing set (Ref. No. 161-01) and press "ENT." 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.

8.22.5 When prompted, enter the WBC concentration (Sample B), the percentage of labeled cells (% viable CD56+ [Total] on Sample A flow printout), and the sample loading volume (Sample B), pressing "ENT" after each entry.

8.22.6 The analyzer will calculate the total number of labeled cells. Verify that this calculation is correct and press "ENT".

8.22.7 Ensure that the following amount of buffer and bags are available:

8.22.7.1	Selection Buffer	1 Liter
8.22.7.2	Negative Fraction Bag	500 mL
8.22.7.3	Buffer Waste Bag	500 mL
8.22.7.4	Cell Collection Bag	300 mL

NOTE: If $2 - 5 \times 10^9$ CD56+ cells, the CliniMACS will require 2 liters of prepared working buffer and a 600 mL cell collection bag. Connect the 2 liters of working buffer using a plasma transfer set, sampling site coupler, and 16G needle. Be sure to close the roller clamp on the plasma transfer set **before** spiking into the buffer bag.

8.22.8 On the instrument, hang the working buffer bag(s) on the left hand hanger, the cell bag on the middle hanger, and the priming waste bag on the right hand hanger. Adjust the heights of the hangers, if needed, but be sure to position them high enough to prevent severe bending of the tubing that could restrict flow and low enough to avoid the tubing connections being stretched.

8.22.9 Place the pre-column in the holder, ensuring that the plastic projections are facing outward. To proceed, press "ENT."

8.22.10 Insert the Selection Column into the Selection Column Holder, ensuring that the plastic projections are facing outward. 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."

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

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

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

8.22.14 Insert the tubing between valve No. 2 and the bubble trap into the liquid sensor. To assure proper operation, the liquid sensor and the tubing must be dry. Carefully inspect and dry with a lint free cloth if needed. To proceed, press "ENT."

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.

8.22.15 Load the pump tubing.

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

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

8.22.15.3 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.

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

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

8.22.15.6 Close the pump door and press "ENT" to proceed.

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.

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

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

8.22.18 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.

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

8.22.20 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

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firmly two times. Check that none of the tubing is kinked or twisted. To proceed, press "ENT."

8.22.21 The instrument will prompt you to attach the Selection Buffer Bag (i.e. working buffer); however, this was done in the BSC. Press "ENT" to proceed.

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

8.22.23 Start the priming procedure by pressing "RUN," ensuring first that the disposable hemostats are removed from the working buffer line. 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 a NEW tubing set.

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

8.22.24 Perform upper and lower integrity tests, following the instructions on the CliniMACS screen. Then perform a final check of all tubing and attachments.

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

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

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

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

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

8.22.24.6 Verify that the clamp to the cell collection bag is open.

8.22.25 To proceed, press "ENT."

8.22.26 The instrument will prompt you to attach the Cell Preparation Bag (i.e. cell bag); however, this was done in the BSC. Press "ENT" to proceed.

8.22.27 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."

8.22.28 Press "RUN." The instrument automatically performs the selection procedure.

8.23 When the run is complete, record the process code on the CliniMACS screen.

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8.24 Clamp or seal the tubing above the luer lock connecting the cell collection bag to the CliniMACS tubing set and transfer the bag to the BSC. Replace the cap on the luer connector.

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

8.26 Remove the tubing set.

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

8.26.2 Release the columns from the column holders.

8.26.3 Dispose of the tubing set as biohazardous waste.

8.26.4 To proceed, press "ENT."

8.27 Shut down the CliniMACS.

8.28 Weigh the cell collection bag and subtract the tare weight from step 9.16. Use this to calculate the volume.

8.29 Perform cell count, viability, HPCA, flow analysis, Gram stain, and endotoxin on the product per SOPs, recording results on the appropriate worksheets. Label this sample as ***Sample C***.

8.29.1 Remove 2.0 mL of product:

8.29.1.1 Add 2 drops of sample to the viability tube.

8.29.1.2 Add 0.5 mL of sample to the HPCA tube.

8.29.1.3 Add 0.5 mL of sample to the flow analysis tube.

8.29.1.4 Add 0.5 mL of sample to a nunc vial. Send to Microbiology for a STAT Gram stain.

8.29.1.5 Add 0.5 mL of sample to a nunc vial. Send for endotoxin testing.

8.30 Process the selected cells for transplant. If the reinfusion date is in the future, cryopreserve the cells per SOPs. If the cells are to be infused fresh, process them accordingly per physician's orders.

8.30.1 Prior to infusion or cryopreservation, the CD56 enriched fraction must be removed from the CliniMACS PBS/EDTA Buffer and suspended in a medium suitable for clinical use (i.e. Plasma-Lyte A with 10% HSA).

8.30.1.1 Prepare wash media by adding 10 mL of 25% HAS to 100 mL of Plasma-Lyte A

NOTE: Number of conical centrifuge tubes is determined by product volume. **If volume is >100mL due to using two liters of buffer for selection, skip to 9.30.9**

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8.30.2 Transfer cells from the collection bag and divide them equally among sterile 50 mL conical centrifuge tubes with no more than 25 mL added to each tube.

Rinse the collection bag with approximately 50 mL of wash media and add to the tubes.

8.30.3 Fill the tubes to capacity with wash media. Cap the tubes.

8.30.4 Centrifuge at room temperature at 1800 RPM for 15 minutes with **NO** brake.

8.30.5 Using a spinal needle, remove supernatant up to the pellet (leave pellet in ~5-7 mL of solution). Retain supernatant for inoculation of culture bottles.

8.30.5.1 Add 5 mL of supernatant to each culture bottle.

8.30.6 Using a spinal needle, combine the pellets from the tubes and rinse each tube with wash media to ensure recovery of all cells.

8.30.7 Add the final cells to a tared 300 mL transfer bag via a sampling site coupler.

8.30.8 Suspend the cells in ≥ 50 mL of wash media.

8.30.9 **If volume is >100 mL**, add equal amount of Plasma-Lyte A with 10% HSA (*example 30ml of 25% HSA to 300mL of Plasma-Lyte-A*) to cell collection bag. Mix well. Centrifuge the cell collection bag at room temperature at 1800 RPM for 15 minutes with **NO** brake.

8.30.10 Before removing the product bag from the centrifuge container, sterile dock it to an empty 300 or 600 mL transfer pack labeled "*CELL COLLECTION BAG WASTE*." **DO NOT** open the weld.

8.30.11 Place the centrifuged product on a plasma expresser and allow it to sit for 10 minutes before expressing the supernatant.

8.30.12 Express as much supernatant as possible without taking cells. Retain supernatant for inoculation of culture bottles.

8.30.12.1 Add 5 mL of supernatant to each culture bottle.

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

8.30.14 Using a sampling site coupler, remove cells with 60mL syringe. Transfer to 300mL bag if >60mL. If < 50mL add wash media to achieve ≥ 50 mL of cells.

8.30.15 Weigh the tared bag containing the final product to determine volume.

8.30.16 Perform cell count and viability on the product per SOPs, recording results on the appropriate worksheets. Label this sample as ***Sample to Infuse***.

8.30.16.1 Remove 0.2 mL of product.

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

8.31 If selected cells are being infused fresh immediately, complete the Cellular Therapy Infusion Request Form to accompany the product to the infusion location along with the Summary of Donor Eligibility Infectious Testing. Also complete the CD56+ Certificate of Analysis (COA) and have the physician and Quality Manager or Laboratory Supervisor sign it after confirming that the infusion product has met product specifications.

8.32 If selected cells are being held overnight for fresh infusion on the following day, store cells in an approved blood bank refrigerator as soon as possible. Complete the Cellular Therapy Infusion Request Form to accompany the product to the infusion location along with the Summary of Donor Eligibility Infectious Testing. Also complete the CD56+ COA and have the physician and Quality Manager or Laboratory Supervisor sign it after confirming that the infusion product has met product specifications.

8.32.1 Before infusion on the following day, withdraw 0.1 mL of product to perform a viability test, and add the results of this testing to the COA.

8.33 If selected cells are being cryopreserved for future use, freeze cells in aliquots according to the physician's orders.

9 RELATED DOCUMENTS / FORMS

9.1 STCL-PROC-019 (FRM1) CD56+ CliniMACS Worksheet

9.2 STCL-PROC-019 (FRM2) CD56+ Certificate of Analysis

9.3 STCL-PROC-015 (JA1) Selection Cheat Sheet

9.4 STCL-PROC-018 Correct Connection of Pre-system Filter and Tubing Set-CliniMACS

9.5 STCL-PROC-022 (FRM1) Stem Cell Laboratory Clinical HPCA Worksheet

9.6 STCL-FORM-040 Peripheral Blood Progenitor Cell Worksheet Adult

9.7 STCL-FORM-041 Doctor's Orders Adult Stem Cell Transplant Program

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

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

9.10 STCL-FORM-056 Cellular Therapy Infusion Request Form

9.11 STCL-FORM-062 Stem Cell Laboratory Processing Order Form Lab Billing Log

9.12 STCL-FORM-064 Manual Differential Worksheet- Clinical Products

9.13 STCL-GEN-009 (FRM1) Cellular Product Chain of Custody Form

9.14 STCL-DIST-001 (JA1) Incoming NMDP Products- STCL Checklist

9.15 FLOW-GEN-012 (FRM5) Stem Cell Laboratory Flow Cytometry Worksheet

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9.16 COMM-PAS-003 Labeling Cellular Therapy Products

9.17 M0226 Form

10 REFERRENCES

10.1 CliniMACS System User Manual, Miltenyi Biotec, Presidential Way, Woburn, Massachusetts, 01801; current version

10.2 Adult Bone Marrow Transplant Program Protocol Notebooks – internal protocols, Duke University Medical Center, Durham, NC.

10.3 FACT, Standards for Hematopoietic Progenitor Cell Collection, Processing, & Transplantation, Current edition

11 REVISION HISTORY

Revision No.	Author	Description of Change(s)
06	B. Waters-Pick	• Moved Limitations to Section 2.3 • Added C of A to Section 5.15 • Section 9 Entire section was modified and reorganized to provide more detail and provide better flow of information to make procedure easier to follow • Section 10 organized documents in numerical order • Section 11 – Added current version for CliniMACS User's manual and FACT standards

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