STEM CELL LABORATORY (STCL)

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CliniMACS TCR alpha beta+ T-Cell / CD19+ B-Cell Reduction Procedure

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STCL-PROC-047
CliniMACS TCRαβ+ T-Cell / CD19+ B-Cell Reduction Procedure

1 PURPOSE:

1.1 This document describes the procedure to reduce both TCRα/β+ T cells and CD19+ B cells contained in a HPC from G-CSF mobilized peripheral blood progenitor cells (PBPCs), utilizing the CliniMACS magnetic cell reduction system.

2 INTRODUCTION

2.1 The CliniMACS™ system, can be used to selectively enrich or reduce specific cell populations based on the magnetic cell selection (MACS) technology developed by Miltenyi Biotec. Cell mixtures can be separated in a magnetic field using one or more immunomagnetic-labeled antibodies specific for the cell types of interest (e.g. TCRαβ+ T cells and CD19+ B cells from HPC (A) products). The TCRα/β reagent is conjugated to biotin, unlike other CliniMACS systems in use in the laboratory, and requires two labeling steps.

- One wash in CliniMACS phosphate buffered saline with EDTA and 0.5% human serum albumin (PEH) buffer for platelet removal
- Labeling with TCRαβ – Biotin
- Two washes in PBS/EDTA buffer to remove excess antibody
- Filtration through a blood filter to remove aggregates
- Addition of anti-Biotin reagent together with CD19 reagent
- One wash to remove excess reagents
- Application onto the CliniMACS device for the negative selection
- Preparation of the TCRαβ / CD19-reduced fraction for infusion

2.2 Target cells are specifically labeled with super-paramagnetic particles. After magnetic labeling, the cells are separated using a high-gradient magnetic separation column. The magnetically labeled cells are retained in the magnetized column and separated from the unlabeled cells (the population to be infused). The selected cells are removed from the magnetic field and eluted from the column into a collection receptacle using a buffered saline solution. Flow analysis is performed at product receipt and at the end of processing.

2.3 When processing cellular products for transplantation, sterile technique should be used whenever feasible. Every precaution should be taken to minimize the possibility of contamination.

2.4 **LIMITATIONS**

Material	Unit	TNC Max	Target Max
TCRαβ – Biotin	Vial @ 7.5 mL	60×10^9	24×10^9
Anti – Biotin	Vial @ 7.5 mL	40×10^9	16×10^9 *
CD19 Reagent	Vial @ 7.5 mL	40×10^9	50×10^8
Depletion Tubing Set	1 set	80×10^9	40×10^9

**** Target limit based on recommendation by Miltenyi that two bottles of anti-Biotin be used for 60 x 10^9 TNC treated with TCRα/β – Biotin***

3 SCOPE AND RESPONSIBILITIES

3.1 The Medical Directors, 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	BSC	Biological Safety Cabinet
4.2	COA	Certificate of Analysis
4.3	HPCA	Hematopoietic Progenitor Cell Assay
4.4	HSA	Human Serum Albumin
4.5	ISBT	International Society for Blood Transfusion
4.6	mL	Milliliters
4.7	NTCB	Non-Target Cell Bag
4.8	PBPC	Peripheral Blood Progenitor Cells
4.9	PBS	Phosphate Buffered Saline
4.10	PEH	PBS/EDTA and 25% human serum albumin
4.11	PPE	Personal Protective Equipment
4.12	QSU	Quality Systems Unit
4.13	RAB	Reapplication bag
4.14	RPM	Revolutions per Minute
4.15	Sample A	Pre-Processing sample
4.16	Sample B	Post Wash / Post Incubation / Pre-Selection sample
4.17	Sample C	Post selection (final) sample
4.18	Sample D	Non-Target Cell fraction - TCRαβ/CD19-enriched bag
4.19	SOP	Standard Operating Procedure
4.20	STCL	Stem Cell Laboratory

5 MATERIALS

5.1 CliniMACS DT tubing set 261-01

5.2 CliniMACS Anti-Biotin reagent 192-01

5.3 CliniMACS TCRαβ-Biotin reagent 200-02

5.4 CliniMACS CD19 reagent 179-01

5.5 CliniMACS PBS/EDTA Buffer

5.6 Gammagard liquid, 10%; or equivalent

5.7 Pre-system filter

5.8 Standard blood filter

5.9 Luer/Spike inter connector

5.10 600 mL transfer packs

5.11 300 mL transfer pack(s)

5.12 Sampling site couplers

5.13 Plasma transfer sets

5.14 Slide clamps

5.15 Disposable hemostats

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

5.17 50 mL centrifuge printed graduation tubes with flat caps

5.18 Needles (16G, 19G, spinal)

5.19 Alcohol prep pads

5.20 Culture bottles, aerobic and anaerobic

5.21 Pipettor tips

5.22 12 x 75 test tubes

5.23 Snap-cap tubes, sterile and non-sterile

5.24 1.8 mL cryo vials (nuncs)

5.25 25% Human Serum Albumin

5.26 Plasmalyte-A®

5.27 SCD wafers

5.28 Tie tags

5.29 ISBT Demand 128 labels

5.30 Barcode labels

5.31 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 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 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 CliniMACS Worksheet*) and/or on the appreciate lot sheet (*STCL-FORM-049 Processing Lot Numbers – Incoming Cellular Product Processing*). Complete the worksheet as each 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 perform cell count, flow analysis cell count, viability. Adding 3 drops to ABO tubes to perform ABO/Rh testing. Record results in the ABO/Rh log on the processing worksheet.

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 mL of product:

8.3.2.1 Use 0.5 mL of sample to perform cell count, flow analysis cell count, viability. Adding 3 drops to ABO tubes to perform ABO/Rh testing. Record results in the ABO/Rh log on the processing worksheet.

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 Use 0.5 mL of sample to perform cell count, flow analysis, and viability.

8.4.1.2 Add 0.5 mL of sample to the HPCA 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 perform cell count, flow analysis cell count, viability. Adding 3 drops to ABO tubes to perform ABO/Rh testing. Record results in the ABO/Rh log on the processing worksheet.

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

8.4.3.1 Use 0.5 mL of sample to perform cell count, flow analysis cell count, viability. Adding 3 drops to ABO tubes to perform ABO/Rh testing. Record results in the ABO/Rh log on the processing worksheet.

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 (PEH buffer) by adding 20 mL of 25% HSA to each of the three liters of PBS/EDTA buffer. Mix well but gently to avoid foaming.

8.6 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 into this bag and weigh it to obtain the volume.

8.7 The product should be diluted with PEH buffer before labeling with the primary antibody or ligand. Calculation the amount of buffer to be added using the following equation and record it

Weight of buffer to be added (g) = Weight of product (g) x 2

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

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

8.10 When the appreciate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow.

8.11 Heat seal the tubing between the bags; leave enough tubing for several more sterile dockings. Record the weight of the filled "Cell Prep Bag".

8.12 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.

8.13 Centrifuge the cell bag/transfer pack combo at 980 RPM for 15 minutes with **NO** brake.

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

8.15 Express as much supernatant as possible without taking cells.

8.16 Sterile dock the working buffer to the cell bag and fill the bag with buffer to a volume of 95 mL ± 5 mL.

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

8.18 Calculate the desired amount of IgG to add to obtain a product concentration of 1.5 mg/mL. Add calculated amount to target volume.

8.18.1 For example, if target volume is 95 mL, multiple 1.5 mg to 95 mL than divide 100 to determine the amount IgG is needed. (ie. 1.5 mg x 95 mL ÷ 100 = 1.4 ml of IgG would be added to cells).

8.18.2 Add about a 50 mL of air into bag

8.18.3 Mix gently and incubate at room temperature for 5 minutes

8.19 Primary Antibody Labeling:

8.19.1 Withdraw the CliniMACS TCRαβ-Biotin in a 10 mL syringe, be careful not to withdraw the syringe needle if there is pressure in the reagent vial. Inject the reagent into the cell bag following by a slug of air, gently mixing every couple mL to ensure thorough labeling.

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

8.19.3 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.

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

8.20 **WASH 1**: Wash the product with the prepared buffer at the end of the antibody incubation period.

8.20.1 Label a 600 mL transfer pack, "**WASH WASTE I**".

8.20.2 Tare the scale with the empty bag

8.20.3 Weight the cell bag to obtain the volume

8.20.4 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.

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

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

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

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

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

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

8.20.11 Express as much supernatant as possible without taking cells.

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

8.20.13 Sterile dock the working buffer to the cell bag and fill the bag with buffer to a volume 150 mL ± 5 mL.

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

8.21 **WASH 2**: Repeat this procedure a second time with 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.

8.22 Transfer all cell into new product 600 mL transfer pack. Filter clumped of needed with a standard blood filter (not a leuko-reduction filter). Sterile dock the working buffer to the new cell bag, and fill the bag with buffer. Adjust the volume to 175 mL ± 5 mL.

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

8.24 Keep the Wash Waste Bags until processing is complete in case cells were inadvertently lost.

8.25 Secondary Antibody Labeling: Anti-Biotin and CD19 addition.

8.25.1 Withdraw the CliniMACS Anti-Biotin in a 10 mL syringe, be careful not to withdraw the syringe needle if there is pressure in the reagent vial. Inject the reagent into the cell bag following by a slug of air, gently mixing every couple mL to ensure thorough labeling.
Remove 1.0 mL of product:

8.25.1.1 Add one vial of Anti-biotin reagent for **≤ 40 x 10^9 TNC** and additional half vial of antibody (3.75 mL) for up to **60 x 10^9 TNC**. Use a full second vial for **> 60 x10^9 TNC (up to 80 x10^9** which is the tubing set limit) or if the second half of the Anti-Biotin reagent is not needed.

8.25.2 Withdraw the CliniMACS CD19 reagent in a 10 mL syringe, be careful not to withdraw the syringe needle if there is pressure in the reagent

vial. Inject the reagent into the cell bag following by a slug of air, gently mixing every couple mL to ensure thorough labeling.

8.25.2.1 Like the Anti-Biotin reagent, one vial of the CD19 reagent can be used to label up to **40 x 10^9 TNC**, but also has an upper limit of **5.0 x 10^9 B cells**. So as long as B cell limits are not exceeded follow same steps as above.

8.25.3 Immediate set a timer for 30 minutes to reflect the total antibodies incubation time.

8.25.4 Rotate and invert the cell bag every 5 minutes during these antibodies incubation time.

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

8.26 **WASH 3:** Wash the product with the prepared buffer again before proceeding with the testing of ***Sample B***.

8.26.1 Label a 600 mL transfer pack, "**WASH WASTE III**".

8.26.2 Tare the scale with the empty bag

8.26.3 Weigh the cell bag to obtain the volume.

8.26.4 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.

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

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

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

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

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

8.26.10 Express as much supernatant as possible without taking cells.

NOTE: This wash procedure should result in the removal of between 300 and 500 mL of supernatant. If less volume is removed, repeat this wash procedure again.

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

8.26.12 Sterile dock the working buffer to the cell bag and fill the bag with buffer to a volume of 150 mL ± 5 mL.

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

8.26.14 After wash is completed. Adjust the cell concentration to ≤ 400 x 10^6 WBC (based on previous count).

8.27 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***.

8.27.1 Remove 1.0 mL of product:

8.27.1.1 Use 0.5 mL of sample to perform cell count, flow analysis cell count, viability.

8.27.1.2 Add 0.5 mL of sample to the HPCA tube.

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

8.29 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.

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.***

8.30 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.

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

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

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

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

8.33.2 Remove the cap from the **lower opening** of the pre-system filter and firmly insert the spike into the pre-system filter; leaving top cap on the pre-system filter.

8.33.3 Remove the top cap from Pre-system Filter and connect to the blunt end of Spike Connect (included with set)
NOTE: Be extremely careful; refer to *STCL-PROC-018 Correct Connection of Pre-system Filter and Tubing Set-CliniMACS* for further connection instructions.

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

8.34.1 Clamp the tubing set below the bubble trap.

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

8.35 Connect the buffer bag to the tubing set.

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

8.35.2 Remove the cap from the tubing set spike and spike the bag of buffer. Gently squeeze the bag to ensure the septum has been punctured.

8.36 Set up the CliniMACS.

8.36.1 Power on the instrument

8.36.2 Press ENT to enter the Program Menu and select Depletion Protocol 3.1

8.36.3 Follow the on-screen prompts and directions to perform the following steps:

8.36.3.1 Use the "Separation Planner" for the CliniMACS software Version 2.4 to calculate the estimated processing time, volume of buffer required, and volumes of the final product (TCRαβ/CD19-reduced/Cell Collection Bag), Waste, and TCRαβ/CD19-enriched fraction (Non-Target Cell Bag). Use these estimates to determine the size bags to use for the various.

8.36.3.2 If volumes of bags supplied with set are acceptable, no additions are needed.

8.36.3.3 Enter the separation parameters including:
1. Expected the cell concentration/mL
2. Frequency of labeled cells (%TCRαβ + %CD19 from starting product flow assay + correction factor)
The formula ((Absolute TCRαβ+ Absolute CD19+ 3.6 x 10^9) ÷ TNC at receipt).
3. Sample loading volume.

8.36.3.4 Confirm the choice of program **DEPLETION 3.1** and enter the volume, corrected frequency of labeled cells, and cell count as calculated by worksheet.

8.36.3.5 Press ENT to confirm the program selection.

8.36.4 Perform material check following the onscreen prompts as described in the CliniMACS user manual. If using bags other than those provided with the DT tubing set, weigh and appropriately sized Non-Target Cell bag (TCRαβ/CD19-enriched cells) and Collection Bag (TCRαβ/CD19-reduced). Record weight on the worksheet.

8.36.5 On the instrument, hang the working buffer bag(s) on the left hand, the cell bag on the middle hanger and reapplication bag (RAB) and Non-Target Cell Bag (NTCB) on the right 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.

8.36.6 Cell collection bag should be in the lowest position of the three bags, following by the RAB and NTCB at mid height, and finally the PEH bag at the highest level.

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

8.36.8 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 by holding the top and bottom between the thumb and index finger. Press “ENT”.

8.36.9 Load valves 1, 2, 3, 4, and 5, making sure tubing is placed correctly into the sensor fitting and that valves is opened. Press and hold on the valve top to open valves.

8.36.10 Load the Liquid Sensor. Ensure that tubing and sensor are dry. Carefully inspect and dry with a lint free cloth if needed. To processed, 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, to not pull the tubing without pressing the valve button to open the valve.

8.36.11 Load the pump tubing.

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

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

8.36.11.3 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.

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

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

8.36.11.6 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.

8.36.12 Load valves 6, 7, 8, 9, and 10. Load the tubing into those valves and press “ENT” to proceed.

8.36.13 Place the Buffer Waste Bag (BWB) in the bag compartment. Avoid catching tubing in compartment lid. Press “ENT” to proceed.

8.36.14 Check the valves using the window display screen 4.3-5. Ensure that all valves open and close properly. Press “ENT” to proceed.

8.36.15 Confirm that tubing is properly placed in the valves at Screen 4.3-6. Look for twists or kinks and be sure tubing is positioned in the center of the valve jaws. If tubing needs to be adjusted, open valve before trying to reposition. Once tubing is adjusted, press the valve top firmly twice before processing. Press “ENT”

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

8.36.17 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.

8.36.18 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.

8.36.19 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."

8.36.20 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.

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

8.36.22 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. Check for any system leaks or blockage of buffer flow during this priming. Before beginning run, confirm the following:

1. Fluid in all parts of tubing set.
2. No excess air in tubing set.
3. Fluid in Priming Waste Bag and Buffer Waste Bag
4. No fluid in Negative Fraction or Cell Collection Bag(s)
5. No fluid in bubble trap or Pre-system Filter.
6. If there are concerns regarding the integrity of the tubing set, refer to the User Manual for additional tests that can be performed before proceeding.

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.

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

8.36.24 To proceed, press "ENT."

8.36.25 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.

8.36.26 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."

8.36.27 Press "RUN." The instrument automatically performs the selection procedure**.** Use the hemostat to tap the Pall Filter to ensure that air bubbles rise up into the product bag.

8.36.28 Note that the program may run in stages if the number of magnetically labeled cells calculated by the internal computer exceeds the biding capacity of the Deletion Column. The program automatically loads and separated the cells sample into smaller portions, called staged loading. After the last step (final elution of the enriched cells) has been completed the next portion of sample will be loaded. As a result, depletion run may take more time than enrichment programs.

8.37 When the run is complete, record the process code from the CliniMACS screen.

8.38 Heat seal the tubing above the luer lock attached to the cell collection bag, below Valve 9.

8.39 Remove the TCRαβ/CD19-enriched cells (Cell Collection bag) from the tubing set. Determine the volume of both the enriched and reduced fractions by weight and record final volumes on the worksheet.

8.40 Post selection cleanup and shutdown.

8.40.1 Disconnect the buffer bag and the buffer waste bag. Remove the tubing set beginning with valves 6, 9, 10 and working upwards. Press valves to release tubing.

8.40.2 Release the columns from the column holders.

8.40.3 Dispose of the tubing set as biohazardous waste.

8.41 Shut down the CliniMACS.

8.42 Weigh the Cell Collection bag (target cell fraction - TCRαβ/CD19-reduced bag(s)) and subtract the tare weight from step 9.29. Use this to calculate the volume.

8.43 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***.

8.43.1 Remove 2.0 mL of product:

8.43.1.1 Use 0.5 mL of sample to perform cell count, flow analysis cell count, viability.

8.43.1.2 Add 0.5 mL of sample to the HPCA tube.

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

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

8.43.1.5 Archive sample, ≥ 20 x 10^6 cells split into two vials, volume based on post selection cell count. The archive sample should be prepared for cryopreservation on the day of processing. Controlled-rate freezing is not required. This sample is not required if the product itself is to be frozen prior to infusion.

8.44 Weigh the Non-Target Cell fraction - TCRαβ/CD19-enriched bag(s) and subtract the tare weight from step 9.29. Use this to calculate the volume.

8.45 Perform cell count, viability, flow analysis, on the product per SOPs, recording results on the appropriate worksheets. Label this sample as ***Sample D***.

8.46 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.

8.46.1 Prior to infusion or cryopreservation, the TCRαβ/CD19-reduced fraction should be volume reduced.

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

8.46.1.2 **If volume is > 100 mL**, add equal amount of Plasma-Lyte A with 10% HSA (*example 30 mL of 25% HSA to 300 mL 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.

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

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

8.46.1.5 Express as much supernatant as possible without taking cells. Retain supernatant for inoculation

8.46.1.5.1 Add 5 mL of supernatant to each culture bottle.
Disconnect the waste bag and mix the cells gently and thoroughly.

8.46.1.6 Using a sampling site coupler, remove cells with 60ml syringe, transfer to 300 mL bag if > 60 mL; if < 50 mL add wash media to achieve ≥ 50 mL of cell. ***Can infuse in syringe of maximum 60 mL.***

8.46.1.7 Weight the tared bag containing the final product to determine volume.

8.46.1.8 Perform cell count and viability on the product per SOPs, recoding results on the appropriate worksheets. Label this sample as ***To Infuse***.

8.46.1.8.1 Remove 0.2 mL of product.

8.46.1.9 **If volume is < 100 mL**; 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.

NOTE: Number of conical centrifuge tubes is determined by product volume.

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

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

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

8.46.1.13 Post centrifugation, place all the conicals in the BSC.

8.46.1.14 Remove the caps and using 60 ml syringe with a spinal needle, remove as much as supernatant as possible without disrupting the cell pellet (leave pellet in ~ 5-7 mL of solution). Transfer supernatant back into Cell Collection Bag - TCRαβ/CD19-reduced fraction bag. Retain the supernatant for inoculation of culture bottles.

8.46.1.14.1 Add 5 mL of supernatant to each culture bottle.

8.46.1.15 Resuspend the cell pellet. Using a 60ml syringe and spinal needle mix the cell pellet.

8.46.1.16 Withdraw the cell suspension and set syringe aside.

8.46.1.17 Using a 20mL or 30mL syringe and needle, withdraw the wash media and rinse the 50mL conicals.

8.46.1.18 Withdraw the rinsed cells into the cell suspension syringe set aside.

8.46.1.19 Measure the combine volume. Add the final volume with wash media to ≥ 50 mL. ***Can infuse in syringe of maximum 60 mL.***

8.46.1.20 Perform cell count and viability on the product per SOPs, recoding results on the appropriate worksheets. Label this sample as ***To Infuse***.

8.46.1.20.1 Remove 0.2 mL of product.

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

8.47 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 TCRα/β / CD19 Cell Reduction 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.

8.48 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 TCRαβ / CD19 Cell Reduction COA and have the physician and Quality Manager or Laboratory Supervisor sign it after confirming that the infusion product has met product specifications.

8.48.1 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.

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

8.50 If the TNCC is >80 x 10^9, it may be necessary to remove cells before starting the selection.

9 RELATED FORMS

9.1 STCL-PROC-047 FRM1 CliniMACS TCRαβ+ T-Cell / CD19+ B-Cell Reduction Worksheet

9.2 STCL-PROC-047 FRM2 CliniMACS TCRαβ+ T-Cell /CD19+ B-Cell Reduction Certificate of Analysis

9.3 STCL-PROC-047 JA1 Installation of CliniMACS DTS Tubing Set

9.4 STCL-PROC-047 JA2 CSIDE Clinical Protocol v2.0_clean

9.5 STCL-PROC-047 JA3 CSIDE MOP v2.0

9.6 STCL-PROC-015 FRM4 Cell Selection - Product at Receipt/Pre-Processing/Pre-Column Form

9.7 STCL-PROC-015 FRM5 Graft Characterization Form for CTN 1301 Protocol

9.8 STCL-PROC-015 FRM6 Certificate of Analysis for CTN 1301 Protocol

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

9.10 STCL-PROC-015 JA3 Thawing of Cryopreserved Cellular Products in Preparation for CD34 Selection Using the Miltenyi CliniMACS

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

9.12 STCL-PROC-022 FRM1 Stem Cell Laboratory Clinical HPCA Worksheet

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

9.14 STCL-FORM-041 Doctors Orders Adult Stem Cell Transplant Program

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

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

9.17 STCL-FORM-056 Cellular Therapy Infusion Request Form

9.18 STCL-FORM-062 Stem Cell Laboratory Processing Order Form

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

9.20 STCL-GEN-009 FRM1 Cellular Product Chain of Custody FRM1

9.21 STCL-DIST-001 JA1 Incoming NMDP Products- STCL Checklist

9.22 FLOW-GEN-012 FRM5 Stem Cell Laboratory Flow Cytometry Worksheet

9.23 COMM-PAS-003 Labeling Cellular Therapy Products

9.24 M0226 Form

10 REFERENCES

10.1 CliniMACS User Manual, Reference Document 37000/02, CD3-depletion. Miltenyi Biotec, Feb 2005.

10.2 CliniMACS TCRα/β – Biotin for research Use, Reference Document 140-003-13401, Miltenyi Biotec, 2010.

10.3 TCRα/β/CD19 depletion from leukapheresis products. Preliminary worksheet, CliniMACS system Short Instructions. Miltenyi Biotec, 2011.

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

10.5 FACT, Standards for Hematopoietic Progenitor Cell Collection, Processing, & Transplantation, Current Edition

11 REVISION HISTORY

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