STEM CELL LABORATORY (STCL)

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Preparation of CMV-Specific Donor Lymphocytes Infusions Using the Miltenyi CliniMACS

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STCL-PROC-037
PREPARATION OF CMV-SPECIFIC DONOR LYMPHOCYTES INFUSIONS USING THE MILTENYI CliniMACS

1 PURPOSE

1.1 This document describes the procedure to prepare CMV specific donor lymphocyte infusions (DLI).

2 INTRODUCTION

2.1 CMV antigen specific cells are analyzed and enriched using the CliniMACS Cytokine Capture System (CCS) Microbeads. First the cells are restimulated with an internal matrix protein (pp65). Next they are incubated with the CliniMACS IFN-gamma Catch Matrix, which binds to the cell surface of all leukocytes. Incubation at 37° C allows cytokine secretion within the cells to take place. The secreted cytokine (IFN-gamma) is bound to the CliniMACS IFN-gamma Catch Matrix on cells secreting this cytokine. It is then possible to label these cells with IFN-gamma Enrichment Microbeads for magnetic enrichment using the CliniMACS instrument.

3 SCOPE AND RESPONSIBILITIES

3.1 The Adult and Pediatric Blood and Marrow Program Medical Directors, Laboratory Manager, Quality Manager and representatives of the participating laboratory staff are responsible for ensuring that the requirements of this procedure are successfully met.

4 DEFINITIONS/ACRONYMS

4.1	CCS	Cytokine Capture System
4.2	C	Celsius
4.3	IFN	Interferon
4.4	RPMI	Roswell Park Memorial Institute media
4.5	rpm	revolutions per minute
4.6	HSA	human serum albumin
4.7	COA	certificate of analysis
4.8	ENT	enter

5 MATERIALS

5.1 CliniMACS Tubing Set

5.2 600 ml Transfer Packs with Luer Adapter

5.3 120 ml AB Serum

5.4 1 liter of RPMI

5.5 1.2 liter of RPMI Supplemented with 10% AB Serum

5.6 600 ml of RPMI Supplemented with 2% AB Serum

5.7 3 liters of CliniMACS PBS/EDTA Buffer

5.8 Human Serum Albumin

5.9 300 ml Transfer Pack

5.10 Transfer Set Coupler/Needle

5.11 Transfer Set with 2 Spikes (1 box)

5.12 Luer/Spike Interconnectors

5.13 Sampling Site Couplers

5.14 100 ml Gas Permeable Culture Bag

5.15 Pre-System Filter

5.16 Syringes

5.17 Needles

5.18 Pre-Separation Filters-

5.19 CliniMACS CMV pp65 (1 vial = 1 ml)

5.20 CliniMACS CCS (1 Kit)

5.21 Nalgene Filtration Product (Memb 0.80 CN, catalog# 450-0080)

5.22 4 "Pink Basins"

5.23 15ml sterile conicals

5.24 50ml sterile conicals

5.25 BacT Culture Bottles

6 EQUIPMENT

6.1 CliniMACS Instrument

6.2 Plasma Expressor

6.3 Sterile Tubing Welder

6.4 Orbital Rotator (optional)

6.5 Centrifuge at 19°- 25° C

6.6 Centrifuge at 2°- 8° C

6.7 Scale

6.8 Tubing Heat Sealer

6.9 Tubing Stripper

6.10 Laboratory Timers

6.11 Automated Cell Counter

6.12 Biological Safety Cabinet

6.13 Water Bath

6.14 37° Celsius Incubator

7 SAFETY

7.1 Wear appropriate personnel protective equipment when handling any/all potentially hazardous blood and body fluids to include, but not limited to, gloves, lab coat, etc.

8 PROCEDURE

NOTE: Complete the Miltenyi Large Scale CCS (IFN-gamma) Worksheet as each step is completed.

8.1 PRIOR TO BEGINNING THE PROCEDURE, PERFORM THE FOLLOWING.

8.1.1 Turn off the centrifuge to allow it to warm to room temperature.

8.1.2 Bring the RPMI to room temperature.

8.1.3 Increase the temperature of the water bath in the processing area to 58° C so it can be used to "heat inactive" the AB Serum.

8.1.4 Thaw the AB serum for 30 minutes at 58° C.

8.1.5 Filter the AB serum using the 0.8 membrane filter. Repeat using second filter.

8.1.6 Supplement 600mls RPMI with 2% AB serum.

8.1.7 Supplement 1200mls RPMI with 10% AB serum.

8.1.8 Aliquot the RPMI into bags as follows. Label the bags with the volume and % AB serum.

8.1.8.1 Transfer 600mls supplemented with 2% AB serum into a 600 ml transfer pack and refrigerate at 2°- 8° C.

8.1.8.2 Transfer 600mls supplemented with 10% AB serum into a 600 ml transfer pack and incubate at 37° C.

8.1.8.3 Transfer 600mls supplemented with 10% AB serum into a 600 ml transfer pack and incubate at 37° C.

8.1.8.4 Transfer 1000mls to a 1000 ml transfer pack and hold at room temperature.

8.1.9 Prepare working buffer by adding 20mls of 25% HSA to each liter of buffer. Place the working buffer in the refrigerator to chill.

8.1.10 Label the 300 ml transfer pack **"Cell Collection Bag"**. Aseptically remove the tubing and insert a Luer/Spike connector into one of the ports. Weigh the bag to get the tare weight and record the weight on the bag.

8.1.11 Label 2 600 ml transfer packs as **"Cell Prep"**.

8.1.12 Label 1 600 ml transfer pack as **"Plasma Waste"**.

8.1.13 Label 3 600 ml transfer packs as **"Wash Waste"**.

8.2 PRODUCT HANDLING – PRE-SELECTION

8.2.1 Following standard operating procedure, prepare the appropriate paperwork, tubes and supplies. Accept the product, verifying product labeling, and record the receipt on the ISBT Label Release Log.

8.2.2 Weigh the product to obtain the approximate volume.

8.2.3 Thoroughly mix the product and withdraw appropriate samples for cell count, viability, flow, HPCA, ABO, nuncs and cultures. Label these samples as ***"Sample A"***.

8.2.4 Calculate the volume containing $1x10^9$ total cells and aseptically transfer this volume to one of the bags labeled **"Cell Prep"**.

8.3 CELL PROCESSING – WASHING

8.3.1 Wash the cells by adding the correct volume of room temperature RPMI to the Cell Prep Bag containing the $1x10^9$ total cells to make a total volume of 500mls.

8.3.2 Using the sterile docker, attach one of the 600 ml transfer bags labeled **"Wash Waste"** to this Cell Prep Bag.

8.3.3 Centrifuge the cells at 200xg (800 rpms on Sorval centrifuge) or 1,020 rpms on the Allegra Centrifuge) for 10 minutes (**without brake**) at room temperature (19°C to 25°C).

8.3.4 Express the supernatant without losing too many cells.

8.3.5 Repeat the washing and centrifugation steps once more.

8.4 CELL PROCESSING – RE-STIMULATION

8.4.1 Adjust the volume of the "Cell Prep" bag to exactly 100mls by adding RPMI supplemented with 10% AB serum which has been heated to 37° C.

8.4.2 Using the sterile docker, connect the "Cell Prep" bag to a 100 ml gas permeable culture bag and transfer the entire contents.

8.4.3 Remove a 1 ml sample to serve as an unstimulated control sample. Refer to the procedure for processing the small scale sample.

8.4.4 **At this point, the cells can be placed in the incubator overnight, to begin with step 5 the next morning, or they can be re-stimulated immediately.**

8.4.5 Add 1 ml of pp65. Mix thoroughly but gently. Incubate for 4 hours at 37° C and 5-7.5% CO_2.

8.5 DURING INCUBATION

8.5.1 Fill four (4) 600 ml transfer packs with 125mls 37° C RPMI with 10% AB serum.

8.5.2 Label the bags as #1, #2, #3, and #4; return them to the incubator.

8.5.3 Ensure that a chilled centrifuge is available.

8.6 CELL PROCESSING – LABELING CELLS WITH IFN-GAMMA CATCHMATRIX

8.6.1 Remove the product from the incubator.

8.6.2 Transfer the product completely to the remaining 600 ml transfer pack labeled **"Cell Prep"**.

8.6.3 Fill the bag with cold (2°- 8° C) RPMI supplemented with 2% AB serum.

8.6.4 Using the sterile docker, connect a 600 ml transfer pack labeled **"Wash Waste"** to the Cell Prep bag.

8.6.5 Centrifuge the cells at 300xg (1,000 rpms on the Sorval centrifuge or 1,250 rpms on the Allegra centrifuge) for 10 minutes **without brake** at 2°- 8° C.

8.6.6 Remove as much of the supernatant as possible, being careful not to lose cells.

8.6.7 Re-suspend the cells in the remaining volume. The volume will be the remainder from Step # 6 above (~ 50-100 mls).

8.6.8 Using a 10 ml syringe and a 20 gauge needle, aseptically inject the IFN-gamma Catch Matrix into the Cell Prep bag.

8.6.9 Mix the cells gently and thoroughly and incubate them for 5 minutes on ice.

8.6.10 Add 500mls of 37° C RPMI supplemented with 10% AB serum to the cell sample in the Cell Prep bag.

8.6.11 **Immediately set a timer for 45 minutes.**

8.6.12 Working quickly and carefully (using the sterile docker and balance), distribute 125mls of the cell suspension into the previously prepared bags labeled #1,#2, #3, and #4. Each of these 4 bags now contains 250mls.

8.6.13 Add 125mls of **warm** RPMI supplemented with 10% AB serum to the Cell Prep bag. It will now contain 250mls.

8.6.14 Mix the 4 bags gently and place them in the 37° C incubator for the remainder of the 45 minutes with gentle mixing occurring every 5 minutes.

8.6.15 Fill 4 pink basins with ice.

8.7 CELL PROCESSING – POST CATCHMATRIX LABELING

8.7.1 At the end of the incubation period, remove the bags from the incubator and add 250mls of **cold** working CliniMACS buffer to two of the four bags.

8.7.2 As the buffer is added to a bag, place the bag in one of the pink basins of ice and set a timer for 10 minutes.

8.7.3 Repeat for bags # 3 and #4; place in ice basin. Set timer for 10 minutes.

8.7.4 During the 10 minute incubation, attach waste bags to each of the four bags (while leaving them in the ice basins).

8.7.5 After the incubation, centrifuge the bags at 300xg (1,000 rpms on Sorval centrifuge and 1,250 rpms on the Allegra centrifuge) with **no brake** for 10 minutes at 2°- 8° C.

8.7.6 Express the supernatants completely.

8.7.7 Using the sterile docker and the balance, add 200mls of **cold** working CliniMACS buffer to one of the bags.

8.7.8 Mix the bag thoroughly.

8.7.9 Using the sterile docker, transfer the cells from the other 3 bags into this final bag; rinse each bag thoroughly to recover all available cells.

8.7.10 Mix final bag thoroughly.

8.7.11 Centrifuge the bags at 300xg (1,000 rpms on Sorval centrifuge and 1,250 rpms on the Allegra centrifuge) with **no brake** for 10 minutes at 2°- 8° C.

8.8 CELL PROCESSING – MAGNETIC LABELING

8.8.1 After centrifugation, carefully remove the supernatant.

8.8.2 Resuspend the cells in the residual volume.

8.8.3 Inject the CliniMACS (IFN-gamma) Enrichment Microbeads into the cell suspension using a 10 cc syringe and a 20 gauge needle.

8.8.4 Gently and thoroughly mix the cell suspension.

8.8.5 Immediately set a timer for 15 minutes and place the cells on ice.

8.8.6 After the 15 minute incubation, fill the bag with 500mls of cold buffer and centrifuge at 300xg (1,000 rpms on Sorval centrifuge and 1,250 rpms on the Allegra centrifuge) with **no brake** for 10 minutes at 2°-8°C.

8.8.7 Carefully remove the supernatant. Resuspend the cells in cold buffer, resulting in a final volume of 100mls.

8.8.8 Thoroughly mix the product and withdraw appropriate samples for cell count, viability, flow and HPCA. Label these samples as "***Sample B***".

8.8.9 Calculate and record the cell recovery.

8.9 CELL PROCESSING – MAGNETIC SEPARATION

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

8.9.2 Choose ENRICHMENT 3.2 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".

8.9.3 Select the appropriate tubing set (Ref. No. 161-01) then 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.

8.9.4 To confirm the program and tubing set chosen, press "ENT".

8.9.5 The screen requests as a security check the Ref. No. of the selected CliniMACS Tubing Set. When the number is entered, press "ENT". To confirm, press "ENT".

8.9.6 Unpack the tubing set under the hood. 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.

8.9.7 Press "ENT"; the program automatically continues with the instructions to install the tubing set.

8.9.8 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. To proceed, press "ENT".

8.9.9 Insert the Selection Column into the Selection Column holder, make 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".

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

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

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

8.9.13 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.

8.9.14 To proceed, press "ENT".

8.9.15 Load the pump tubing.

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

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

8.9.15.3 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.

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

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

8.9.15.6 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.

8.9.16 To proceed, press "ENT".

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

8.9.18 The screen prompts to load valves 6,9,10 and 11. Load the tubing into these valves.

8.9.19 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.

8.9.20 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.

8.9.21 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".

8.9.22 Attach the Selection Buffer Bag.

8.9.22.1 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.

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

8.9.23 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.

8.9.24 To proceed, press "ENT".

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

8.9.26 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 you cannot resolve the problem, start over using new tubing set.

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

8.9.27 Perform a final check of all tubing and attachments to include:

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

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

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

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

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

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

8.9.28 To proceed, press "ENT".

8.9.29 Connect the Cell Preparation Bag.

8.9.29.1 Connect the cell preparation bag to the pre-system filter.

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

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

8.9.29.4 Spike the cell preparation bag. See *(JA1) Correct Connection of Pre-System Filter and Tubing Set.*

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

8.9.29.6 Hang the cell preparation bag on the bag hanger.

8.9.29.7 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.

8.9.30 To proceed, press "ENT".

8.9.31 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.

8.9.32 Press "RUN"; as the buffer passes through the filter into the Cell Prep Bag, saturate the Pall filter by tapping vigorously on the filter using metal hemostat. The instrument will then automatically perform the selection procedure.

8.9.33 When the run has finished, 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.

8.10 CELL PROCESSING – POST MAGNETIC SEPARATION

8.10.1 Calculate /measure the volume of the final product and remove a well mixed sample (~1.5 mls) for cell count, viability, HPCA and flow. Label this sample "***Sample C – Pre-Concentration***". Keep the flow sample on ice.

8.10.2 To proceed, press "ENT".

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

8.10.4 Remove a well mixed sample from both the negative fraction bag and the buffer waste bag for cell count and flow. Keep the flow samples on ice.

8.11 SHUT DOWN THE CLINIMACS

8.11.1 **Record the process code.**

8.11.2 Remove the tubing set.

8.11.3 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.

8.11.4 Release the columns from the column holders.

8.11.5 Dispose of the tubing set as biohazardous waste.

8.11.6 To proceed, press "ENT".

8.11.7 Turn off the CliniMACS.

8.12 PROCESSING THE SELECTED CELLS

8.12.1 Transfer the well mixed selected cells to sterile 50ml conicals.

8.12.2 Centrifuge the conicals at 300xg (1,250 rmps for the Allegra centrifuge) for 10 minutes at 2°- 8° C.

8.12.3 Remove supernatant from conical tube using a sterile spinal needle.

8.12.4 Send the following for QC:

8.12.4.1 10 mls **of the supernatant** (place in a sterile 15 ml conical tube for stat gram stain and 14 day culture to the Microbiology Lab).

8.12.4.2 5 mls of the supernatant for Endotoxin testing

8.12.4.3 2 mls of the supernatant for culture in the Stem Cell Laboratory

8.12.5 Re-suspend the cells in Plasmalyte containing 5% HSA to a volume appropriate for infusion (~50 mls).

8.12.6 Remove 1 ml of sample for cell count to evaluate cell count and flow cyometry. Calculate the cell recovery post concentration. Label this sample as "***Sample C – Post-Concentration***".

8.12.7 Prepare the selected cells to be infused fresh, refrigerated overnight, or frozen for future use based on the doctor's orders.

8.12.8 If selected cells are being infused fresh immediately, do so according to *Packing and Transporting Non-Frozen Cellular Products Locally.* Complete the *Hematopoietic Progenitor Cell Infusion Request Form* to accompany the product to the infusion location along with the *Summary of Donor Eligibility Infectious Testing.* Also complete the appropriate *Certificate of Analysis (COA)* and have the physician, at the infusion location, sign it after confirming that the infusion product has met product specifications. The Quality Manager or Laboratory Manager will sign the COA within 48 business hours following the selection procedure.

8.12.9 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 appropriate Certificate of Analysis (COA). Confirm that the product meets the criterion on the COA. Notify the responsible physician if any parameters fail. Before infusion on the following day, withdraw an aliquot to perform viability. Add the results of this testing to the COA. When cells are to be delivered for infusion, do so according to *Packing and Transporting Non-Frozen Cellular Products Locally.* Complete the *Hematopoietic Progenitor Cell Infusion Request* to accompany the product to the infusion location along with the *Summary of Donor Eligibility Infectious Testing* and COA. Have the physician, at the infusion location, sign the COA after confirming that the infusion product has met product specifications. The Quality Manager or Laboratory Supervisor will sign the COA within 48 business hours following the selection procedure.

8.12.10 If selected cells are being cryopreserved for future use, freeze cells in aliquots according to doctor's orders *Doctor's Orders, Adult Stem Cell Transplant Program.*

9 RELATED DOCUMENTS/FORMS

9.1 Miltenyi Large Scale CCS (IFN – gamma) Worksheet

9.2 Certificate of Analysis

9.3 Correct Connection of Pre-System Filter and Tubing Set

9.4 Summary of Donor Eligibility Infectious Testing

9.5 Hematopoietic Progenitor Cell Infusion Request Form

9.6 Cellular Product Chain of Custody Form

10 REFERENCES

10.1 FACT, Standards for Hematopoietic Progenitor Cell Collection, Processing, & Transplantation, Second Edition, 2002

10.2 CliniMACS System, Operator's Manual, August 2004

11 REVISION HISTORY

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Barbara Waters-Pick (WATE02)		21 Dec 2012, 04:45:30 PM	Approved

Manager Approval

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Barbara Waters-Pick (WATE02)		21 Dec 2012, 04:45:48 PM	Approved

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