

Procedure Title: Carbonyl Iron Treatment and Washing Peripheral Blood Stem Cells Before Tumor Purging

Procedure #: HSC.B522.01

1. Principle:

If granulocytes and monocytes are removed from harvested peripheral blood stem cells before purging, non specific binding to antibody coated beads can be greatly reduced. Incubation with carbonyl iron followed by exposure of the phagocytic cells to samarium cobalt magnets removes unwanted cells without a large reduction of the CD34+ cell population.

2. Reagents, Supplies, Equipment:

- A. Cole Parmer masterflex slow speed pump with easy load pump head
- B. Dupont Stericell Processor
- C. Hemacytometer with cover slip
- D. Laminar flow hood
- E. Light microscope
- F. Portable electronic balance
- G. SC IIB sterile large tubing welder
- H. SCD312 sterile small tubing welder
- I. Sebra sealer
- J. Gentamicin (GENT) - Gemini -50 mg/ml / Cat# 400-108
- K. Human Serum Albumin (HSA) - Childrens Hospital pharmacy- Albumin (Human) U.S.P./ 25% solution
- L. Leibovitz medium (L15) - Irvine Scientific / Cat# 9083- sterile, pyrogen free (less than 0.06 EU/ ml)
- M. Heparin Sodium (Preservative free)

3. Abbreviations:

- A. NVMSA_TA - a non vented male spike adapter welded to a transfer adapter
- B. STSX2- two solution transfer sets welded together at the spike ends
- C. NVMSAX2 - two non vented male spike adapters welded together male spike at the non spike ends

4. Procedure:

INITIAL INSPECTION OF STEM CELLS

When the PBSC apheresis arrives at the purging lab, remove the bags from the transport container and check for:

- A. Leaks in bags
- B. Blood on the outside of the stem cell primary container
- C. Observation of cell clumps or anything unusual that might affect the quality of the final product
- D. **Report unusual observations to the laboratory supervisor and note them on the worksheet.**

PREPARATION STEPS FOR CARBONYL IRON REMOVAL OF PHAGOCYtic CELLS FROM PBSC

- A. Remove the appropriate number of 500 and 100 ml bottles of L15 (Leibovitz medium) from the refrigerator and place in the laminar flow hood. Add tissue culture grade gentamicin (50 mg/ml stock) at 0.1 ml/100 ml to all of the L15 used in this procedure. **Add heparin to 10 units / ml final concentration of heparin only to the to the L15 used in the carbonyl iron treatment of PBSC and to the untreated PBSC's if heparin has not been added at the collection facility.** Add (HA) human serum albumin to L15 to 10% v/v.

Note: **Do not add heparin sodium to solutions prepared in subsequent procedures in the process of Neuroblastoma Tumor Purging:**

- B. Attach a sterile 14-15 gauge needle to a sterile 10 ml syringe and remove samples as necessary (step B.3.) before combining multiple PBSC collections
 - 1. Mix the cells by inverting the bag several times.
 - 2. If there is no site coupler in place, remove the cover from a sampling site coupler and spike it into an available port on the bags containing the cells.
 - 3. Combine the cells collected from multiple days of collection using an NVMSA_TA or STSX2 - see HSC.D211 for instructions on assembly.

Remove samples from the pooled cells for the following:

1.0 ml for sterility testing	to a sterile 15 ml capped centrifuge tube
0.5 ml (or 4×10^6 cells) for CD34 analysis	to a sterile snap cap tube
3.0 ml (or 30×10^6 cells) for tumor analysis	to a sterile 15 ml centrifuge tube
0.2 ml for cell counts	to a sterile snap cap tube

- C. Weigh the bag with the pooled cells and record it on the worksheet. Perform the white blood cell counts and calculate the total number of white blood cells delivered to the lab.

D. USE OF CARBONYL IRON ON HARVEST DAY

1. The average percent recovery of CD34+ cells after carbonyl iron treatment is ~80%. The average recovery of CD34+ cells after carbonyl iron and purging is ~30%. The maximum ratio of CI to PBSC is 37.5 g carbonyl iron (CI) per 12.5×10^9 cells. The minimum acceptable ratio is 6 g CI per 12.5×10^9 cells. The actual ratio may be slightly lower than the minimum or slightly higher than the maximum since carbonyl iron is washed using cell counts submitted to the laboratory.
2. Use the guide below for preparing the CI wash media. Do not add more than 40 gm CI to one 600 ml transfer pack. Attach a needle to a solution transfer set and spike it into transfer pack containing the L15 and additives.

37 - 45 grams - wash with 500 ml of L15:

500 ml L15

10 ml HA

5 ml preservative free heparin (1000 U/ml)

0.5 ml of gentamicin (50 mg) /ml

3. Remove the plunger from a 60 ml syringe and keeping it sterile replace the plunger in its original sterile packaging. Clamp the syringe barrel to a buret stand. Remove the cover from the end of the syringe and attach the luer end of the NVMSA_TA.
4. Spike a sampling site coupler into an available port on an empty 600 ml transfer pack which has had the drainage tubing Sebra sealed and removed from the bag. Remove the spike cover from the NVMSA_TA and insert it into the remaining port.

5. Open a 100 ml bottle of L15 +gent and pour ~20 ml of media into the bottle containing the CI. Make sure the bottle is tightly capped and mix the slurry well to break up any clumps of CI.
6. Remove the cap and pour the slurry into the 60 ml syringe. Massage the exit tubing and the transfer pack to assist in the passage of the CI. Wipe the rim of the CI bottle after each pour with a sterile alcohol wipe.
7. Repeat steps A.5. and A.6. until all of the CI has been added to the transfer pack and the syringe barrel is empty. The plunger may be used to force CI through the syringe barrel and additional media from the CI wash bags may be used if needed. Replace the plunger into the barrel of the syringe before removing the bag from the hood in the next step.
8. When all of the CI is in the transfer packs, replace the plunger, clamp the NVMSA_TA, Sebra seal the tubing three times 1-2 inches below the bag (overlapping each seal), and cut from the bag at the middle seal. Mix each bag well by inverting several times.
9. Place the transfer pack in a book magnet and rotate for 5 min.
10. In a laminar flow hood, attach a 14-15 gauge needle to each end of an STSX2 (see HSC.D211 for instructions on assembly) which has been clamped closed. Insert a needle into the sampling site coupler of the transfer pack in the book magnet. Insert the other needle into the septum of an empty one liter viaflex bag. Open the clamp and drain the media into the viaflex bag.
11. Clamp the STSX2 and remove the needle from the CI bag. Remove the CI bag from book magnet and add wash media to the CI bag using an electronic balance to measure the weight (volume). Mix well. Wash the CI three times repeating steps A.9. through A.10.
12. If the cells are to be processed the following day; place CI transfer packs in a ziploc plastic bag and store at 4⁰ C, otherwise keep the transfer pack with the CI in the laminar flow hood until needed.
13. Add no more than 25×10^{10} PBSC's to a transfer pack of washed CI. Add the appropriate volume of PBSC to each washed CI bag using an NVMSA_TA or NVMSAX2 (see procedure HSC.D211 for instructions on assembly).

14. Incubate the transfer packs for 45 min. to one hour at 37⁰ C.
15. Place each transfer pack in a book magnet, rotate for 5 min., and drain **all of the cells into an empty one liter viaflex bag** (bag weight = 40 gm) using an STSX2 (two solution sets welded together at the spike ends) . Remove an aliquot for cell counts. Weigh the bag and record the weight on the worksheet.
16. Place the bag in a book magnet and Sebra seal the viaflex bag to the Stericell blue line for washing.

E. **WASHING CELLS USING THE STERICELL CELL WASHER**

1. To prepare the Stericell refer to procedure HSC.B590. **Make sure all welded seals are open.**
2. Close the Stericell valve on the BLUE line and open all clamps on the YELLOW line.
3. Open the Stericell YELLOW and GREEN valves. Set the pump to "Fill" and open the clamp on the wash bag. Pump 250 ml of L15/GENT into the harvest bowl. Stop the pump. Close the Stericell valve on the YELLOW line.
4. Start the centrifuge (3000 rpm), set the pump to "Fill" and the speed to 100ml/min. Open all clamps from the PBSC bag and start the pump.
5. Pump all of the PBSC's out of the bag.
6. Close the Stericell BLUE valve to the empty PBSC bag. Make sure the valves and all clamps on the YELLOW (L15/GENT) line are open. Increase the centrifuge bowl speed to 4000 rpm. Set the pump speed at 100 - 150 ml/min.
7. Press "Agitate" to begin the wash. Continue washing until ~100 ml of L15/GENT remain in the wash bag. While the PBSC's are being washed add a minimum 10 ml of HSA to the Stericell collection bag.
8. With approximately 100 ml of L15/GENT still in the wash bag, press the red "Stop" to turn off the pump and the centrifuge.
9. Open the Stericell valve and all clamps on the RED line, set the pump to

"Empty" checking to see that there is free movement of media and cells. Remove the harvest bowl, gently swirl the bowl to mix, and pump the contents of the bowl into the collection bag until air appears in the line.

10. Open the Stericell YELLOW valve, set the pump to "Fill", and pump the remaining 100ml rinse of L15/GENT into the harvest bowl.
11. When the wash bag is empty, stop the pump, reset it to "Empty", and reopen the Stericell RED valve. Pump the bowl contents into the collection bag. Sebra seal the entry tubing to the collection bag three times overlapping each seal. Cut the tubing at the middle seal, remove the bag from the harvest set and take it to the laminar flow hood.
12. Discard the waste bag, PBSC bag, and the plasmapheresis set in the biohazard trash.
13. Refer to procedure HSC.B531 for Neuroblastoma tumor purging.