

Procedure Title: Bone Marrow Stem Cell Washing Using the Stericell Processor

Procedure #: HSC.B591.01

1. Principle:

After a first processing step, bone marrow or peripheral blood stem cells are prepared for cryopreservation or purging the cells by washing to remove cellular debris and unwanted sample components.

2. Abbreviations:

MDB -marrow displacement bag

3. Procedure:

A. To prepare the Stericell for sedimentation and filtration, refer to procedure HSC.D590. **Make sure all welded seals are open** and do steps A.1.- A.3. while the red cells are sedimenting.

1. Close the Stericell valve to the filters on the BLUE line and open all clamps on the YELLOW line.
2. Open the Stericell YELLOW and GREEN valves. Set the pump to "Fill" and open the clamp on the Stericell wash bag. Pump 250 ml of L15/GENT into the harvest bowl. Stop the pump, set it to "Empty", and reset the speed to not greater than 40 ml /min. Close the Stericell valve on the YELLOW line.
3. Open the Stericell BLUE valve to the filters and open the clamp to the filter wash bag. The clamps to the marrow/HES bags must be closed. Start the pump and gently tap the filters so that air bubbles can be bled off into the filter wash bag. Stop the pump and clamp the line when ~120ml of L15/GENT are in the filter wash bag. The volume is displayed on the front panel of the Stericell.
4. When sedimentation is complete, carefully raise the bags to a vertical position (with the HES/marrow bag ports toward the ceiling) and hang the bags from "S" hooks on the Stericell cross bar using the holes punched in the corners of the MDB.

- a. Make sure that the exit line from the top of the HES/marrow bag to the filter is not crimped; if the line is long it may need to be supported with tape. Let the marrow settle about 5 more minutes.
 - b. Open the clamps to the MDB (marrow displacement bag) to create pressure within the plastic sleeve. Set the pump to "Fill" and the speed to 40-45ml/min.
5. To remove air in the lines from the HES/marrow bag to the Stericell, open the clamp in the exit line of the HES/marrow bag, pump the marrow supernatant to the "Y" joint just above the filters and clamp the line to the bag. Repeat the process for each HES/marrow bag until all lines between the bags and the "Y" joint are filled. Re-clamp all exit lines from the HES/marrow bags.
 6. To remove air from the filter, open the line to the filter wash bag, set the pump to "Empty", and pump L15/GENT from the harvest bowl until all air is purged from the filters and the line to the filter wash bag. The filters must be wet and there should be liquid in the all lines to and from the filters. Re-clamp the line to filter wash bag.

B. Filtration and Concentration

1. Start the centrifuge (3000 rpm), set the pump to "Fill" and the speed to 60ml/min/bag (i.e.120ml/min. for two bags). Open all clamps from the HES/marrow bag and start the pump.
2. Restrict the flow as necessary so that the rise of the red cell pellet is approximately the same for all bags.
3. When the red cell pellet nears the top of a HES/marrow bag, decrease the pump speed. Shut the clamp when the red cell pellet has reached the "Y" on the exit line of the HES/marrow bag. Stop the pump when the red cell pellet from each bag has reached the "Y". Make sure all the lines from the HES/marrow bags are clamped. Open the line to the filter wash bag, and start the pump.
4. Pump all of the L15/GENT out of the filter bag. Do not let filters go dry!

C. Washing Cells

1. Close the Stericell BLUE valve to the filters. 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 - 150ml/min.

2. Press "Agitate" to begin the wash. Continue washing until ~100 ml of L15/GENT remain in the Stericell wash bag. While the marrow is being washed add a minimum 10 ml of HSA to the Stericell collection bag.
3. With approximately 100 ml of L15/GENT still in the Stericell wash bag, press the red "Stop" to turn off the pump and the centrifuge.
4. Open the Stericell valve and all clamps on the RED line, set the pump to "Empty", and the speed to 150ml/min. after checking to see that there is free movement of media and cells. Remove the harvest bowl from the Stericell and swirl the cell suspension in the bowl while pumping into the collection bag until air appears in the line.
5. Open the Stericell YELLOW valve, set the pump to "Fill", and pump the remaining 100ml rinse of L15/GENT into the harvest bowl.
6. When the Stericell wash bag is empty, stop the pump, reset it to "Empty", and reopen the Stericell RED valve. Pump the bowl contents into the Stericell collection bag. Sebra seal the entry tubing to the Stericell 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.
7. Remove the empty MDB bag(s) from the Stericell cross bar and lay it down. Drain the water from the full bag to allow removal of the HES/marrow bag from the plastic sleeve.
8. Return the MDB's to storage. If the cells are to be purged using the **indirect** method (refer to procedure HSC.B532 neuroblastoma purging using the indirect method) Sebra seal and cut the line just below the bottom most filter as in step C.6. Leave enough tubing to use in a sterile tubing weld. Keep the Stericell setup in place and Sebra seal a new collection bag to the system. Otherwise, discard all of the remaining Stericell setup in the biohazard trash.
9. If the marrow is to be cryopreserved, refer to procedure HSC.C101 for washing out the gentamicin and resuspension in media and for cryopreservation.

10. If the cells are to be purged using the direct method refer to procedure HSC.B531 for Neuroblastoma tumor purging. If the cells will be cryopreserved without tumor purging, refer to procedure HSC.C101.