

**Procedure Title: Sedimentation and Filtration of Bone Marrow Using the Stericell Processor**

**Procedure #: HSC.B526.01**

1. Principle:

Red cells and tumor must be removed from harvested bone marrow before it can be infused into the patient. Red cells can be removed by sedimentation with Hetastarch at a final concentration of 1.5%. Red cell sedimentation followed by filtration through both a 40u and 20u filter removes cellular debris and acts as a first step in removing neuroblastoma tumor cells from harvested marrow.

2. Reagents, Supplies, Equipment:

- A. Buret stand with clamp
- B. Cole Parmer masterflex slow speed pump with easy load pump head
- C. Dupont Stericell Processor
- D. Hemocytometer with cover slip
- E. Laminar flow hood
- F. Light microscope
- G. Portable electronic balance
- H. SC IIB sterile large tubing welder
- I. SCD312 sterile small tubing welder
- J. Sebra sealer
- K. Gentamicin (GENT) - Gemini -50 mg/ml / Cat# 400-108
- L. Hetastarch (HES) - Childrens Hospital Pharmacy Hespan (6% hetastarch in 0.9% sodium chloride injection), sterile non pyrogenic

- M. Human Serum Albumin (HSA) - Childrens Hospital pharmacy- Albumin (Human) U.S.P./ 25% solution
  - N. Leibovitz medium (L15) - Irvine Scientific / Cat# 9083- sterile, pyrogen free (less than 0.06 EU/ ml)
3. Abbreviations:
- A. MTBO\_BA - used to transfer marrow from a bottle to a transfer pack
  - B. MDB - marrow displacement bag
  - C. NVMSA\_TA - a non vented male spike adapter welded to a transfer adapter
  - D. NVMSAX2 - a non vented male spike adapter welded to another male spike at the non spike ends

4. Procedure:

#### INITIAL INSPECTION OF BONE MARROW

When the bone marrow arrives at the purging lab, remove the bags or bottles from the transport container and check for:

- A. Leaks in bags or cracks in bottles
- B. Blood on the outside of the marrow container
- C. Evidence of clotting in the marrow 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 AND SAMPLE REMOVAL

- A. Remove two, 500 ml bottles of L15 ( Leibovitz medium ) from the refrigerator and place in the laminar flow hood.. Add tissue culture gentamicin ( 50 mg/ml at 0.1ml/100 ml to the Stericell wash bag.
- B. If the marrow arrives in a bottle, tighten the cap and mix well by inverting it several times. Remove any blood on the rim and neck of the bottle with sterile

- alcohol wipes before removing the samples in step D. with a sterile 5 ml pipet.
- C. If the marrow arrives in a bag, mix the marrow well by inverting the bag several times. Remove the cover from a sterile sampling site coupler and spike into an available port on the marrow bag. Attach a sterile 14-15 gauge needle to a sterile syringe.
- D. Remove samples from the component for the following:
- |   |   |
|---|---|
| 1.0 ml for sterility testing                          | to a sterile 15 ml capped centrifuge tube |
| 0.5 ml ( or $5 \times 10^6$ cells) for CD34 analysis  | to a sterile snap cap tube                |
| 3.0 ml (or $30 \times 10^6$ cells) for tumor analysis | to a sterile 15 ml capped centrifuge tube |
- (Neuroblastoma patients only)
- 0.2 ml for cell counts to a sterile snap cap tube
- E. Weigh the marrow containers and record the weight on the worksheet. Perform white blood cell counts and calculate the total number of bone marrow cells delivered to the lab.

## PROCESSING STEPS

- A. **Preparation of media and Hetastarch (HES) for Marrow Sedimentation and Filtration**
1. Open a sterile package containing a MTBO\_BA. See HSC.D211 for instructions on assembly. Keeping the male needle adapters sterile, insert a sterile 14-15 gauge needle onto each end.
  2. Place the pump in the hood and open the pump head. Sit the tubing portion of the MTBO\_BA snugly into the two slots on the pump head making sure the tubing fits smoothly over the rollers. Close the pump head.
  3. Place the balance in the hood and adjust the bubble level on the balance so

that it is in the center of the red circle by adjusting the feet of the balance. Turn the balance on and wait for the display to clear.

4. When the electronics are stable a small circle (stable circle) will appear in the top left of the display. Make sure that the weighing pan is clean and empty. Press the zero button to tare the balance.
5. Place a HES and patient ID label on each one liter viaflex bag to be filled. Insert one needle into the empty viaflex bag and the other needle into the Hetastarch bag.
6. Place the empty bag on the balance and when the stable circle shows, press the zero button to tare the bag.
7. Determine the appropriate number of transfer pack bags to use for subsequent steps based on the total number of marrow cells using the following guidelines: Sedimentation of bone marrow should take place at a cell concentration of no more than  $15 \times 10^6$ / ml in a total volume of 850 ml per HES bag. Pump 212 ml ( grams ) of the HES into each bag. Remove the tubing from the pump.
8. Open the package of a sterile MTBO\_BA ( See HSC.D211 for instructions on assembly) and, keeping it sterile, place the pipet end into a 500 ml bottle of L15/GENT. Attach a sterile 14-15 gauge needle to the male luer adapter. Fit the tubing portion into the pump head as in step A.2.
9. Place the L15 - GENT label on an empty 600 ml transfer pack (Stericell wash bag ) with drainage tubing attached. Clamp off the tubing with an aluminum sealing ring and open a port on the transfer pack. Remove the cover from a sampling site coupler spike it into an available port.
10. Insert the needle end of the MTBO\_BA into the septum of the empty bag. Using the balance as in step A.6.- A.7., pump in approximately 600 ml of L15/GENT. Take the bag to the Stericell and leave the MTBO\_BA in place.

**B. Preparation of marrow for sedimentation and filtration with Stericell**

1. See HSC.D211 for instructions on the assembly bags ( MDB's ) . Making sure the clamps on the MDB's are closed lay the bag containing 212 ml of HES on top of the empty MDB so that the entry ports of the HES bag and the MDB bag are at opposite ends from one another.

2. Slide the HES and empty MDB bag into a plastic sleeve (see HSC.D211 for instructions on preparing the plastic sleeve).

**C. If the marrow comes to the lab in a bottle, steps C1 - C6 must be done in a laminar flow hood.**

1. With the clamp closed, remove the spike cover from a non-vented male spike adapter welded to a transfer adapter ( NVMSA\_TA - see HSC.D211 for instructions on assembly ) and spike it into the HES bag.
2. Remove the plunger from a 60 ml luer-lok syringe and place in the sterile packaging to keep it sterile. Tighten the syringe body in a clamp attached to a buret stand.
3. Remove the protective caps from the NVMSA\_TA and the syringe. Screw the NVMSA\_TA onto the syringe.
4. Using the syringe body as a funnel, pour an appropriate amount of marrow into the HES bag. Sedimentation of bone marrow should take place at a cell concentration of no more than  $15 \times 10^6$ / ml in a total volume of 850 ml per HES bag
5. Pour enough L15/GENT through the syringe barrel to take the volume of HES bag to approximately 850 ml. The volume can be estimated measuring the weight of the bag while pumping media into the Viaflex bag using the peristaltic pump.
6. Replace the syringe plunger and use it to remove as much air as possible from the bag and close the NVMSA\_TA clamp. Repeat steps C.1. through C.6. until all of the marrow has been dispensed into HES bags.

**D. If the marrow arrives in a bag spike into the bag with an NVMSAX2. See HSC.D211 for assembly instructions.**

1. Close the clamp on the NVMSAX2, remove the cover from one end and insert the spike into an available port on the marrow bag. Remove the cover from the other end and spike it into an available port on a viaflex bag containing 212 ml of HES.
2. Using the balance as in step A.6., open the clamp and squeeze marrow into the HES bag to the appropriate volume (grams) and close the clamp.

Sedimentation of bone marrow should take place at a cell concentration of no more than  $15 \times 10^6$ / ml in a total volume of 850 ml per HES bag.

3. Sebra seal the NVMSAX2 three times overlapping each seal and cut the middle seal. Refer to procedure HSC.D337 for operation of the Sebra Sealer.
4. If marrow needs to be added to another HES bag, weld the non spike end of a sterile non- vented male spike adapter to the sebra sealed end of the NVMSAX2 on the HES/ marrow bag using the SCD IIB. Return the bags to the laminar flow hood.
5. Repeat steps D.1. through D.3. until all of the marrow has been dispensed into HES bags
6. Use MTBO\_BA from step A.8. Use the balance to tare the HES/ marrow as in step A.6., pump L15/ GENT into each bag to take the total volume per bag to 850 ml.
7. Mix the marrow well by inverting the bags 15 - 20 times.
8. Use the SCD IIB sterile tubing welder, weld the non- vented male spike adapter from the marrow bag(s) to the appropriate section of the Y tubing.
9. Lay the HES / marrow bags on their sides (ports vertical to the bench top) for ~20 minutes or until the red cell pellet is below the bottommost port. After sedimentation is complete hang the HES/ marrow bags from the Stericell crossbar.
10. Refer to procedure HSC.B591 to wash the cells before cryopreservation or purging.