STEM CELL LABORATORY (STCL)

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DOCUMENT TITLE:

Dextran Albumin Thawing Vials and Small Cryobags JA2

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STCL-SOP-028 JA2

Dextran Albumin Thawing Vials and Small Cryobags

1 Prepare Dextran 40/Albumin Solution (Thawing Solution)

1.1 Transfer 75 grams of pre-cooled Dextran 40 solution to the 150 mL transfer bag.

1.2 Working in the biological safety cabinet, insert a sampling site coupler into one of the ports of the 150 mL transfer bag containing Dextran 40 solution.

1.3 Clean the rubber stopper of the human albumin bottle with alcohol prep pads.

1.4 Withdraw 15 mL of 25% human albumin from the bottle.

1.5 Clean coupler port with alcohol prep pad and inject 15 mL of human albumin into the transfer bag containing Dextran 40 solution.

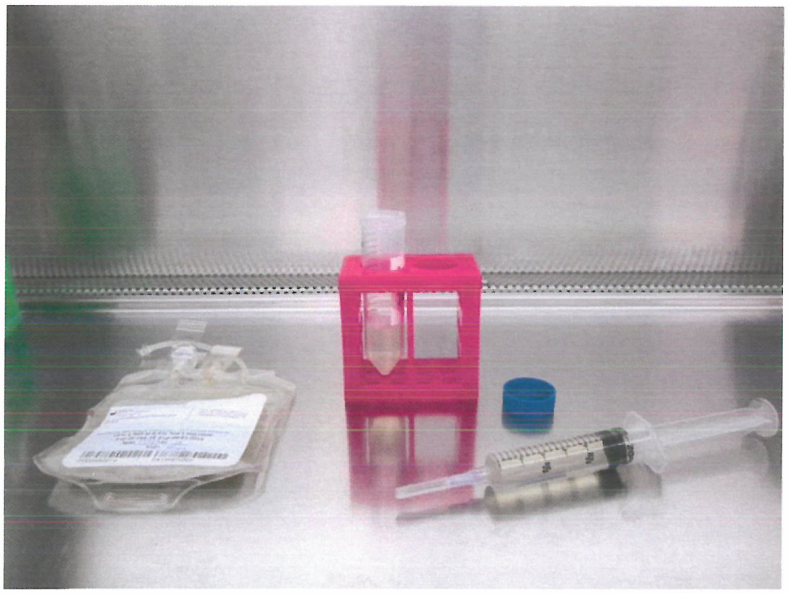
NOTE: The above preparation of thawing solution will accommodate up to two 4.5 mL nunc vials or a 20% fraction of a dual compartment cryobag.

2 Obtain a 50 mL conical and place in BSC.

NOTE: One 50 mL conical can accommodate up to two 4.5 mL nunc vials or a 20% fraction (from a dual compartment cryobag). If more than two 4.5 mL nuncs need to be thawed, obtain a second 50 mL conical.

3 Using a syringe and needle, remove 15 mL of thawing solution and transfer to the 50 mL conical.

4 Prepare a second syringe containing 5 mL of thawing solution to dilute the sample 1:1, process, and add to the syringe to provide final volume of 25 mL.



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5 Working in the vapor phase of the LN2 tank, remove the unit from storage location and metal canister.

5.1 Remove plastic overwrap, if present and visually inspect the cryobag or nunc vial for damage or cracks.

5.2 If applicable, using sharp scissors cut the 2 seals separating the 20 and 80 percent fractions. Restore the 80% fraction if applicable.

6 Spray the outside surface of the cryobag or nunc vial with alcohol and place inside a sterilized zip-lock bag; remove the air and then seal the bag.

7 Confirm and document, with a second technologist, that the label on the cryobag/nunc(s) matches the label on the accompanying paperwork.

8 Thaw the unit in a 37˚C water bath until the product reaches a slushy/liquid consistency. This generally takes approximately 1-3 minutes. Note: Treat the thawed cell suspension very gently. The cell membranes are fragile and the cells are lysed easily.

9 Dry the outside of the zip-lock bag containing the slushy cryobag/nunc vial(s) and wipe with alcohol before placing in the BSC.

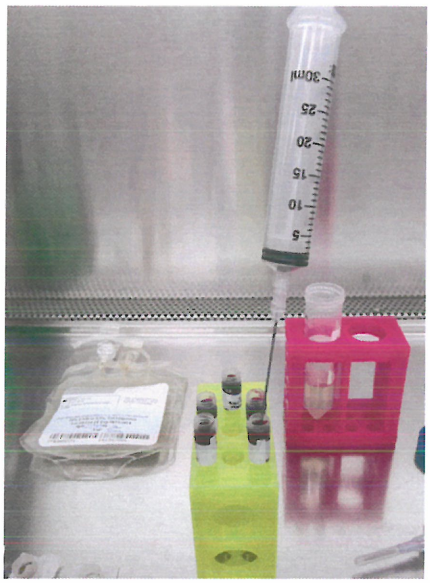
10 Cord Blood Dilution:

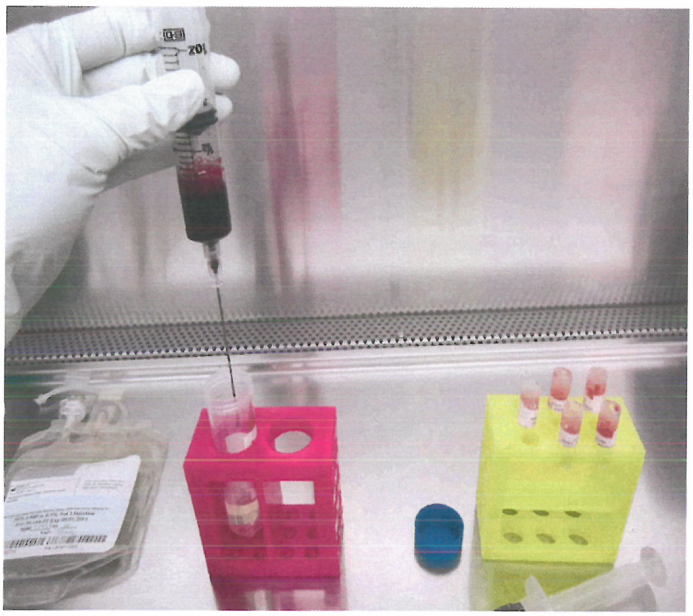
10.1 NUNC VIALS

10.1.1 Remove the nunc vial from the zip-lock bag.

10.1.2 Clean the outside of the nunc vial with alcohol prep pads and using an alcohol prep pad, remove nunc vial cover.

10.1.3 Using a syringe and a spinal needle, withdraw the contents of the nunc vial(s) with syringe that has 5 mL thawing solution and transfer to 50 mL conical.





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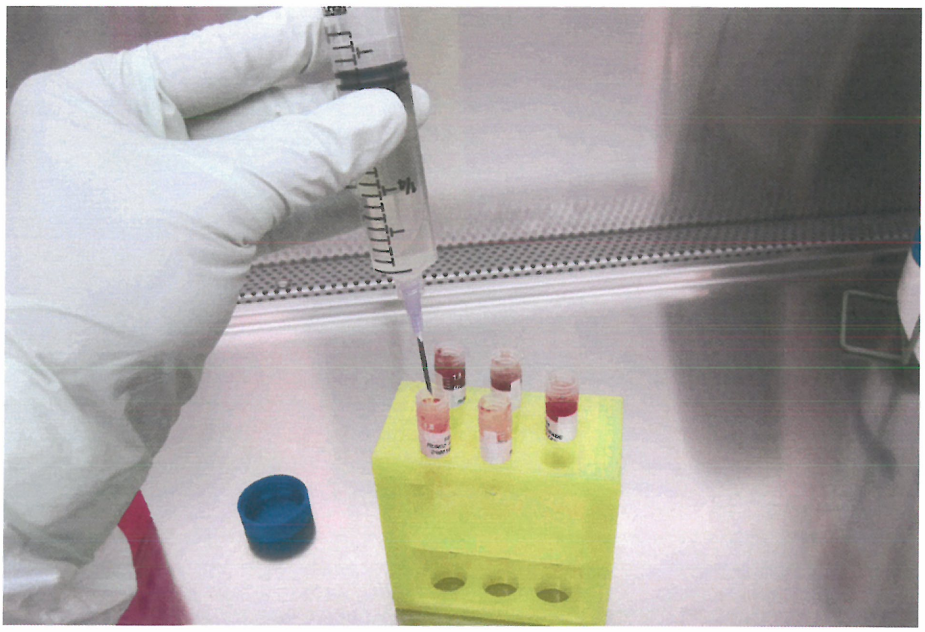
10.1.4 Rinse the nunc vial(s) with thawing solution and transfer to the 50 mL conical. Rinse the nunc vial(s) a second time with thawing solution and transfer to the 50 mL conical. A third rinse may be performed if necessary.



10.1.5 Add any remaining thawing solution in the syringe to the 50 mL conical.

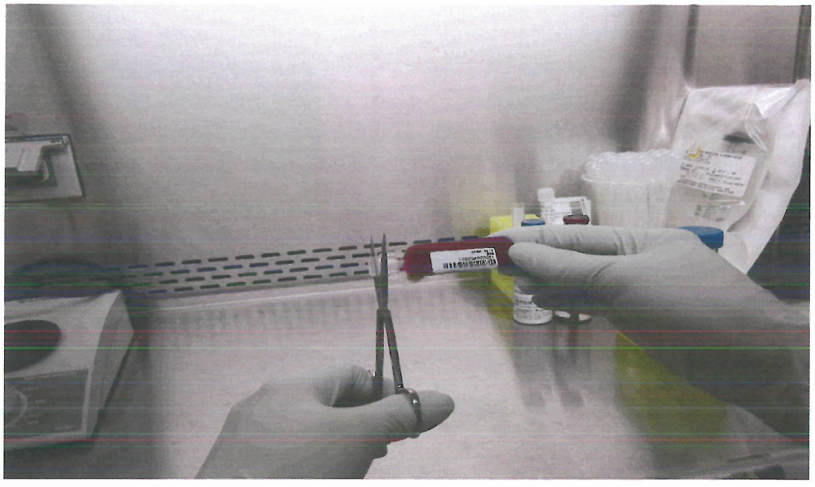
10.1.6 Replace the 50 mL conical cap and mix the contents by inversion.

10.2 20% FRACTION

10.2.1 Remove the cryobag from the zip-lock bag.

10.2.2 Clean outside of the port cover with ChloroPrep SEPP.

10.2.3 Cut port cover with sterile or disinfected scissors.



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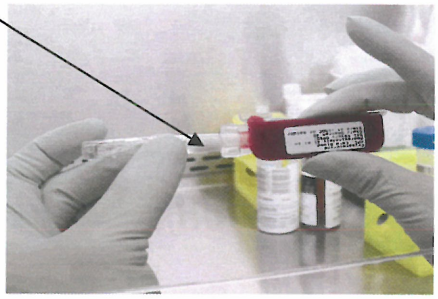
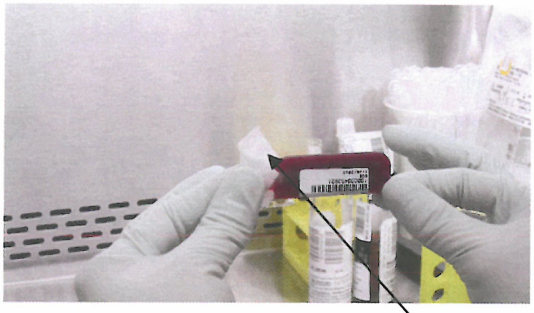
10.2.4 Clean cut surfaces with ChloroPrep SEPP pad, follow with alcohol prep pad, and allow cut surfaces to air dry.

ChloroPrep SEPP

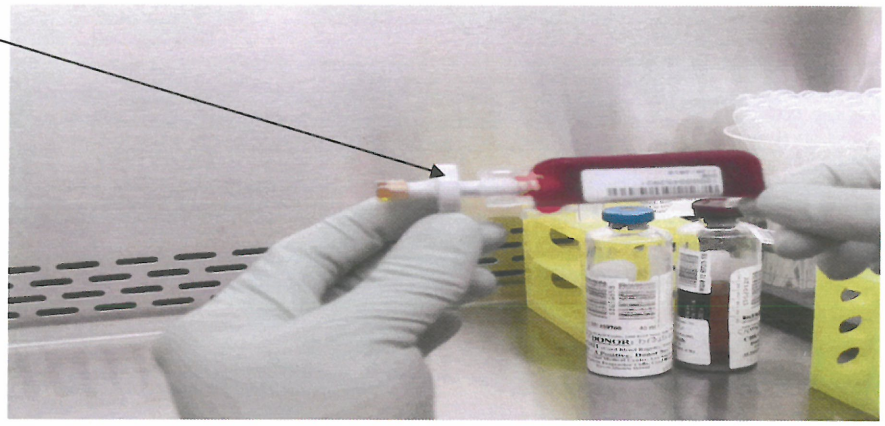




Alcohol Prep Pad

10.2.5 Insert a sampling site coupler into the dry and disinfected port.

Sampling Site Coupler



10.2.6 Using a needle and syringe, withdraw contents of bag into syringe with 5 ml thawing solution to makle a 1:1 dilution.

10.2.7 Rinse the cryobag and transfer to the 50 mL conical. Rinse the cryobag a second time with thawing solution and transfer to the 50 mL conical. A third rinse may be performed if necessary.

10.2.8 Add any remaining thawing solution in the syringe to the 50mL conical.

10.2.9 Replace the 50 mL conical cap and mix the contents by inversion.

11 Cord Blood Unit Wash

11.1 Centrifugation of thawed/diluted product

11.1.1 Place the 50 mL conical inside the centrifuge bucket adapter.

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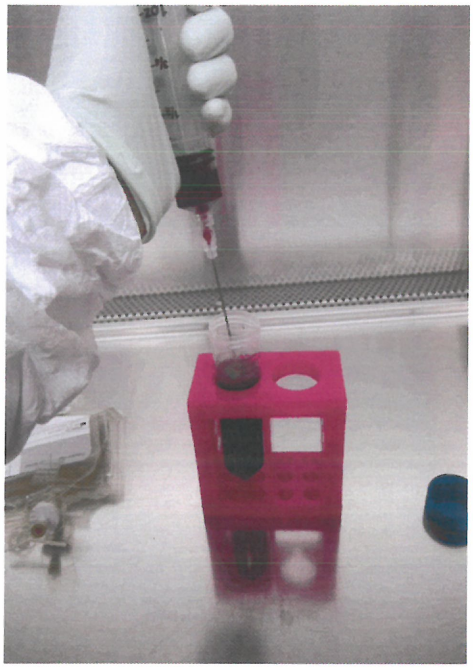
11.1.2 Balance the centrifuge bucket by placing a second 50 mL (filled to appropriate volume with water) conical into the opposite centrifuge bucket adapter.

11.1.3 Pellet the cells via centrifugation at 3000 rpms for 20 minutes at 2-10˚C.

12 Removal of the Supernatant and Removal of Cells from Conical

12.1 Post-centrifugation, place the 50 mL conical in the BSC.

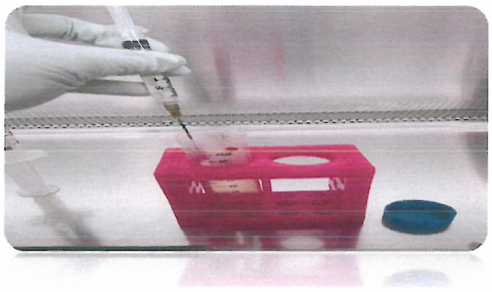
12.2 Remove the cap and using a syringe and spinal needle, remove as much supernatant as possible without disrupting the cell pellet or compromising recovery.



12.3 Reserve the supernatant for inoculation of culture bottles.

12.4 Resuspend the cell pellet with fresh dextran albumin (DA) solution leaving a volume appropriate for infusion (based on the infusion orders) and place cells into 150 mL transfer bag.

12.5 Filter the cells using a syringe filter (or equivalent) and prime the tubing for infusion.



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12.6 Measure the combined volume and record on paperwork.

13 Preparation of Cells for Transplantation

13.1 Remove 0.7 mL aliquot for QC testing from syringe. Mix sample adequately before testing.

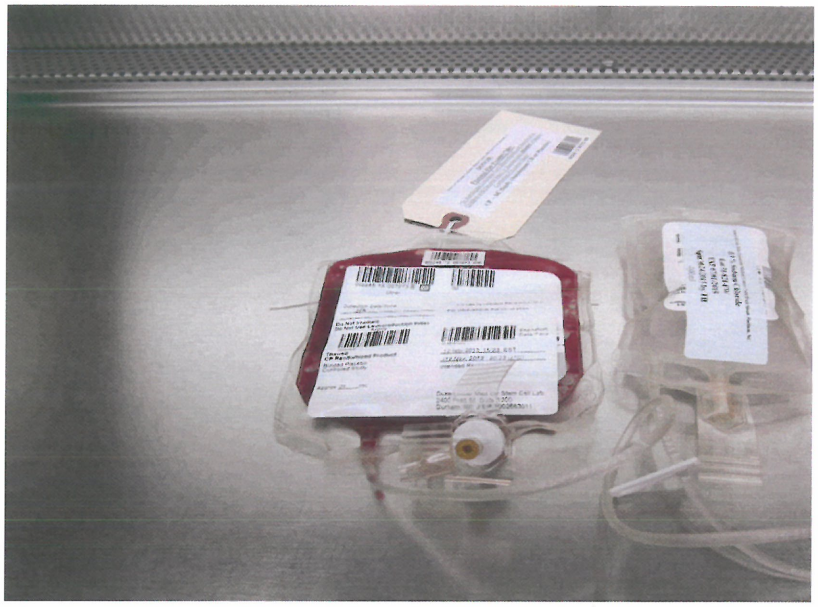
NOTE: This QC sample is used to perform viability, total nucleated cell count, WBC differential, colony assay for CFU-GM, GEMM and BFU-E, viable CD34+ determination by flow analysis (CD3/4/8 will also be performed if product is allogeneic).

13.2 Calculate the viable cell recovery.

13.3 Record all values on the designated laboratory thawing form.

13.4 Label the infusion bag per institutional labeling procedures.

13.5 Attach a tie tag containing the recipient and donor demographic information to the infusion bag.



13.6 Perform sterility testing using 10 mL of reserved supernatant from step 12.5 (5 mL for aerobic and 5 mL for anaerobic culture bottles).

13.7 Record all lot numbers and expiration dates of all reagents and disposables used during processing.

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Signature Manifest

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