CHAPTER 4

PROCESSING PROCEDURES
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4.1 SAMPLE RECEIPT AND LOG-IN

Principle

Correct assignment of a particular cord blood unit (CBU) and its corresponding paperwork and samples requires a carefully maintained data tracking pathway. One key point in this pathway is at the time of receipt of a CBU by the CBB processing facility. This SOP outlines the steps performed at this time to maintain data tracking and quality assurance.

Specimen

Cord blood aseptically collected after delivery into an CPD anticoagulant collection bag according to the Collection SOP, bar code labels and accompanying paperwork, transported to the Cord Blood Bank according to the SOP for Shipping CBUs from Collection Centers to CBB.

Equipment

Electronic Bar Code Scanner
Computer

Reagents

None

Supplies

Shipping Log From Platelet Shipper
CBU Collection and Receipt Form Cord Blood Bank Stationary
Manila Folder Cord Blood Bank Stationary
COBLT Study Bar Code Labels From collection kit(s) in platelet shipper

Procedure

1. Upon receipt of the CBU from the collection center, open the platelet shipper (if applicable), and record the date and time the shipper was opened, the total number of CBUs received, and the min/max temperature (if applicable) on the Shipping Log. Sign the Shipping Log and record the study ID number. Exclude CBU(s) if pre-processing storage temperature is < 4 \degree C or > 37 \degree C.

2. For each CBU, locate the sheet of adhesive bar code labels and any paperwork included in the collection kit zipper-locked bag.
If more than one CBU is contained in a shipper, Steps 3 through 9 should be completed for one CBU at a time.

3. a. If the bar code labels are absent, discontinue log-in until they are located.
   
   b. Scan the bar code label on the collection bag and on the bar code label set in the collection kit to confirm identity.
   
   c. If identity is not confirmed, discard sample according to the Discarding CBUs SOP. Otherwise continue processing.
   
   d. Place an adhesive bar code label on a new manila folder to create a Cord Blood Unit Paperwork folder. Place the CBU Collection and Receipt Form and any other paperwork in this folder.
   
   e. If the Confidential Information manila envelope is included in the shipper, **DO NOT OPEN IT**. Give the unopened package to the COBLT staff member designated to process confidential information.

4. Record the identity of the Receipt Technologist and the date and the time of receipt on the CBU Collection and Receipt Form and/or CBU receipt database.

5. Check collection bag for leaks and label integrity. Record result on the CBU Collection and Receipt Form and in the database.

6. a. Open the CBU receipt database and enter the collection and receipt data recorded on the CBU Collection and Receipt Form.
   
   b. Calculate the volume of the cord blood without anticoagulant (see Procedure Note 1). If this volume is less than 40 ml, discard the CBU according to the Discarding CBUs SOP.
   
   c. Calculate elapsed time from collection. If more than 48 hours have elapsed, discard the sample according to the Discarding CBUs SOP. Otherwise continue processing.
   
   d. If the bag contains a leak or the label is unreadable discard the CBU according to the Discarding CBUs SOP.

7. Place the CBU, the adhesive labels, and the Paperwork Folder in the Processing in-box or institutional equivalent.

**Quality Control**

1. Quality control is maintained by electronic entry and confirmation of the CBU ID number through use of the adhesive bar code label and scanner.
2. All records are to be maintained both electronically (in the database) and in hard copy form (in the Paperwork Folder).

3. Only one CBU will be processed at a time by a trained staff member.

Procedure Notes

1. Calculation of Cord Blood Volume

\[
\text{Volume of Cord Blood} = \text{Volume of CBU} - 25 \text{ ml Anticoagulant}
\]

\[
\text{(Volume of CBU} = \text{[Post Collection Weight + 7.5 gm]} - \text{Pre-collection Weight})
\]

NOTE: The exact weight (in the example, 7.5 gm) to be added must be determined and validated at each bank.
4.2 CBU SEPARATION AND SAMPLE PREPARATION

Umbilical Cord Blood Volume Reduction and Red Cell Depletion Using Hydroxy Ethyl Starch (Hespan)

Principle

Umbilical cord blood unit (CBU) grafts indicate that umbilical cord blood is a useful source of hematopoietic stem cells for routine bone marrow reconstitution in both the unrelated and related donor setting.

In order to efficiently store a large number of CBUs most of the plasma and red blood cells are removed by differential centrifugation using the ability of Hespan to induce red blood cell agglutination. The final product is then brought to a standard volume in preparation for cryopreservation.

Specimen

Umbilical cord blood properly logged into the bank according to Sample Receipt and Log-In SOP.

Equipment

Laminar Flow Hood
Centrifuge
Heat Sealer e.g. Sebra Model 2100
Plasma Expresor
Bar code scanner
Balance
Tube stripper
Sterile Docking Device
Plastic centrifuge inserts (optional)
RBC sensor (optional) e.g. Melcor

Reagents

Hespan DuPont Pharmaceuticals, Catalog #NDC 0056-0037-44/NSN 6505-01-281-1247

Supplies

Stem Cell Processing Kit (Set #2) Pall Medical Corporation (Figure 4.2.2)
Hemostats Hospital Store Room
18 Gauge Luer Lock Needles Hospital Store Room
(or higher gauge, i.e., a smaller needle may be used)
Alcohol Swabs Hospital Store Room
Sterility Testing Tubes
Procedure

1. Locate the sheet of adhesive COBLT Study bar code labels included in the collection kit. Place a bar code label on the two sterility testing tubes, the cryovials, microscope slides, sample tubes and other forms as required by institutional procedures.

2. a. Place a bar code label on the CBU Processing Form. Open processing database and enter the processing record for the CBU using the bar code scanner.

b. Record the identity of the processing technologist, the date and the time in the database and on the CBU Processing Form. Data may be keyed directly into the database. Hereafter, reference to the CBU Processing Form or processing database will be generically termed “the worksheet”.

3. Strip any tubing attached to the bag with blood in it back into the bag and mix very thoroughly. Seal off the tubing close to the bag after stripping the blood back into the bag.

4. Obtain 0.5 ml of whole blood from the syringe port of the collection bag (Bag #1) using sterile technique as described below. See Figure 4.2.1, CPD Placental/Umbilical Cord Blood Stem Cell Collection Unit.

a. After disinfecting the septum of injection port, use a 1 ml syringe and needle to mix the blood in the tubing between the injection site and the bag back into the bag.

b. After mixing, remove 0.5 ml of whole blood from the collection bag (Bag #1) and transfer to a pre-labeled tube. Scan bag and tube to confirm label match.

c. Perform automated nucleated cell count and manual viability count (using trypan blue according to institutional SOPs). Enter data on the worksheet.

d. If the volume of CBU (cord blood plus anticoagulant) is between 65 and 85 ml, and the total nucleated cell count is less than $6 \times 10^8$ cells, discard the CBU according to the Discarding CBUs SOP. Calculate the volume of CBU using Procedure Note 1 if the
database is not available.

e. Prepare three slides for a manual differential count.

f. Place remainder of sample in “Unit Sample Testing In-Box” or institutional equivalent for ABO and Rh testing.

5. Add the Hespan to the collection bag (Bag #1) as follows.

a. Calculate the volume of Hespan to add to give the bag a final ratio of Hespan: CBU (blood+anticoagulant) of 1:5. The database will automatically calculate this volume or it can be done manually by using the formula in Procedure Note 2.

b. Record the lot number and expiration date of the Hespan on the worksheet.

c. Disinfect the outside of the septum of the sterile injection port and use a 30 or 60 ml syringe to add the correct volume of Hespan through the injection port.

d. Mix the blood and Hespan thoroughly by inverting the bag 4-5 times.

6. Sterile connect the Stem Cell Processing Kit (may be done now or after Step 10, according to institutional protocol) as follows. See Figures 4.2.1 and 4.2.2 for bag diagrams.

a. Use a sterile connection device to attach the tubing from the Stem Cell Processing Kit to the tubing of the collection bag (Bag #1).

b. Place a bar coded label on each bag (processing bag [Bag #2], plasma bag [Bag#3], stem cell freezing bag[Bag #4]), scan all labels on the bags, and compare with the bar coded label on the collection bag. Do not proceed if labels are not identical; resolve any discrepancy.

c. If sterile connection device is not available, connect the spike of the processing bag (Bag #2) to the spike entry port of the collection bag (Bag #1).

7. Place the collection bag (Bag #1) (and Stem Cell Processing Kit, if connected) into a centrifuge cup.

a. Add or adjust inserts to maintain the bag(s) in an upright position free of folds or creases.

b. If using customized Plexiglas inserts, make sure that the sealed bottom edge of the bag extends below the Plexiglas inserts, so that the lower level of blood in the bag is even with the bottom of the Plexiglas inserts. Use velcro straps to secure the blood bag assembly.
8. Balance the centrifuge.

9. Centrifuge at g force, rpm’s, and time as calculated and validated by the bank to give a recovery of \( \geq 60\% \) of nucleated cells or \( \geq 80\% \) of mononuclear cells.

10. Carefully remove the entire centrifuge cup and place near the plasma expression. If Stem Cell Processing Kit has not been connected to the collection bag (Bag #1), then connect and label as detailed in Step 6 above.

11. Place collection bag (Bag #1) into plasma expressor and arrange processing bag (Bag #2) on scale so that only the weight of the bag (not the tubing, hemostats, or clamps) is being weighed. If using Plexiglas inserts, they may be left around the bag in the expressor. Tare the scale.

*NOTE: At this point in the procedure, it is recommended that the collection bag hang on the expressor stand for approximately 15-20 minutes to allow time for additional RBC sedimentation. This clarifies the interface between the leukocyte-rich plasma and the red blood cells before expression of the leukocyte-rich plasma into the processing bag.*

12. Using a hemostat or roller clamp to control flow, slowly express supernatant into processing bag (Bag #2) in order to avoid turbulence.
   a. If a red cell sensor is used, manually express a few ml of plasma to clear RBCs from the tubing between the collection bag and the processing bag. Then turn on sensor and express leukocyte rich plasma until the sensor clamps the tubing. If expressing manually, as red cells start to enter connecting tubing, monitor scale to let the desired number of grams of red cells (validated at the bank) pass through to processing bag (Bag #2), then clamp bag closed. Record weight of the processing bag (actually the weight of the leukocyte-rich plasma) on worksheet and in the database.
   b. If mixing occurs, resulting in apparent retention of white cells or excessive transfer of red cells to processing bag (Bag #2), return all material to collection bag (Bag #1) and re-initiate procedure from step 7.
   c. Temporarily close off the collection bag (Bag #1). Do not permanently seal off this bag at this time to allow for poor end recoveries requiring a second spin.

13. Place Stem Cell Processing Kit in the centrifuge cup. Add inserts to make bag stand upright with no folds or creases. If using customized Plexiglas inserts, place the processing bag (Bag #2) between the Plexiglas inserts. Use velcro straps to secure the blood bag assembly.

14. Balance the centrifuge.

15. Centrifuge at g force, rpm’s, and time as calculated and validated by each bank to give a recovery of \( \geq 60\% \) of viable nucleated cells or \( \geq 80\% \) of viable mononuclear cells.
16. Carefully remove the Stem Cell Processing Kit from the centrifuge cup and place in the plasma expressor.

   a. If using Plexiglas inserts, they may be kept attached to the processing bag (Bag #2) in the plasma expressor.

   b. Arrange plasma bag on scale with tubing and other bags stabilized and not interfering with weight of the plasma.

   c. Tare the plasma bag.

17. Subtract institutional adjustment factor (e.g. 21.5 grams, see Note) from the actual weight of the leukocyte-rich plasma in the processing bag (Bag #2) from Step 12a and express this amount from processing bag (Bag #2) to plasma bag (Bag #3).

   NOTE: The exact weight (in this example, 21.5 grams) to be subtracted must be determined and validated at each bank. The volume retained in the processing bag must be 21.5 ml.

   a. If mixing occurs, resulting in excessive retention of plasma or excessive transfer of white cells to plasma bag, return all material to processing bag (Bag #2) and re-initiate procedure from step 13.

   b. Temporarily close off the plasma bag (Bag #3). Do not permanently seal off this bag at this time to allow for poor end recoveries requiring a second spin.

18. Remove a specimen for sampling.

   a. Under sterile conditions, use a 1 ml syringe to remove a test aliquot of leukocyte-rich plasma from the processing bag (Bag #2) and put into a sterile labeled tube. The test aliquot can be either a standard volume ranging from 450-500 µL or a calculated volume with a minimum cell count of 3x10⁶ nucleated cells.

   b. Remove enough sample from the sterile aliquot to perform an automated cell count and manual viability (using trypan blue according to institutional SOPs).

   c. Remove enough sample from the sterile aliquot to prepare three slides for manual differential count. An optional hematocrit may also be performed.

   d. Record the overall viability on the worksheet and in the database. If viability is < 90%, discard according to SOP.

   e. Record the total nucleated cell count and percent viability on the worksheet and in the database and calculate the recovery of viable nucleated cells either by computer database or manually.
f. If recovery is < 60% of viable nucleated cells and < 80% of viable mononuclear cells (if performed), and the total viable nucleated cell count is < 6 x 10^8 cells, reconstitute plasma and cells back to original product and re-process from Step 7. Do not spin the bag more than twice. If recovery is still not adequate after a second spin, discard CBU according to the SOP.

g. If recovery is adequate, seal off and remove the collection (Bag #1) and plasma (Bag #3) bags and proceed with completion of processing.

19. Remove 400 µL from the test aliquot for flow cytometry. Confirm label by scanning and place the aliquot in the flow cytometry in-box or institutional equivalent.

20. Use the viable nucleated cell count to calculate the volume required to obtain 0.5x10^5 cells from the testing aliquot and remove this volume for colony assays (see Procedure Note 3). Confirm label by scanning and place the aliquot in the colony assay in-box or institutional equivalent.

21. Swab the sterile sampling site injection port of collection bag (Bag #1) with an alcohol wipe, and remove ≥ 7 ml of granulocyte/packed red cells. Add seven ≥ 1 ml aliquots to seven pre-labeled Cryovials. Scan to confirm labels and place vials in Granulocyte/Red Cell Storage freezer (≤ -20°C). Record location on worksheet.

22. Retrieve leukocyte-poor plasma bag (Bag #3), swab the sterile sampling site injection port with an alcohol wipe, and remove 15ml. Add 7.5ml of plasma to each of two pre-labeled sterility testing tubes for sterility testing (Aerobic and Anaerobic). Confirm labels by scanning.

23. Retrieve collection bag (Bag #1), swab the sterile sampling site injection port with an alcohol wipe, and remove 5ml of granulocyte/packed red cells. Add 2.5ml of red cells to each of the two sterility testing tubes referred to in Step 22.

24. Mix sterility testing tubes and place in Unit Sample Testing In-Box or institutional equivalent.

25. Swab the sterile sampling site injection port of plasma bag (Bag #3) with an alcohol wipe, and remove 7 ml of plasma. Add one 1ml aliquot to seven pre-labeled Cryovials. Scan to confirm labels and place vials in Plasma Storage freezer (≤ -20°C). Record location on worksheet.

Quality Control

The primary quality assurance tests are recovery of viable nucleated and/or mononuclear cells and sterility testing (post-processing). All samples in the Unit Sample Testing In-Box may be released for testing only after confirmation of receipt of informed consent.

Procedure Notes

1. Calculation of Volume of CBU
Volume of CBU = [Post Collection Weight + 7.5 gm] - Pre-collection Weight

NOTE: The exact weight (in the example, 7.5 gm) to be added must be determined and validated at each bank.

2. Calculation of Volume of Hespan to Add

\[ \text{Volume of CBU} \times 0.2 \]

Volume of CBU = (Post Collection Weight + 7.5 gm) - pre-collection weight

NOTE: The exact weight (in the example, 7.5 gm) to be added must be determined and validated at each bank.

3. Calculation of Volume for Colony Assays

\[ \frac{0.05 \times 10^6 \text{ cells}}{\text{viable nucleated cell count (x 10}^6/\text{ml)}} \]
Appendices

Figure 4.2.1
CPD Placental/Umbilical Cord Blood Stem Cell Collection Unit (Set #1)
Figure 4.2.2
Stem Cell Processing Kit (Set #2)
References


4.3 CRYOPRESERVATION OF CBU

Cryopreservation of Umbilical Cord Blood Cells Using DMSO and Dextran 40 as Cryoprotectants

Principle

Results of umbilical cord blood unit (CBU) grafts suggest that umbilical cord blood is a useful source of hematopoietic stem cells for routine bone marrow reconstitution in both the unrelated and related HLA matched donor setting.

Maintenance of the transplantable hematopoietic cells in umbilical cord blood is achieved by storage in liquid nitrogen (temperature <-185°C). This protocol details steps involved in preparing cord blood for storage at this temperature with minimal loss of cell viability.

Specimen

Cord blood leukocyte-rich plasma (Bag #2) (Figure 4.2.2, SOP 4.2) in a volume of 21 ml following processing according to the SOP entitled "CBU Separation and Sample Preparation"

Equipment

Cryopreservation System
- BioArchive™ System (ThermoGenesis Corp.) which includes:
  - Robotic storage freezer
  - Two controlled rate freezer modules
  - Graphical User Interface (G.U.I.)
  - Bar code scanner and printer
  - Overwrap sealer
  - Magnetic stainless steel canisters
  - Insulated canister sleeves
  OR
- Conventional System
  - Quarantine liquid nitrogen freezer
  - Controlled rate freezer
  - Liquid nitrogen storage freezer
  - Freezer gloves
  - Bar code scanner
  - Vacuum pump
  - Storage cassettes (i.e. Custom Biogenics)

Laminar Flow Hood
Controlled Rate Freezer
Bar code scanner
Heat Sealer e.g. Sebra Model 2100
Syringe pump OR
   Pall Medical positioning jig
Orbital Rotator or rocker
Cool Packs (0-4°C)
Freezer Gloves
Black marker pen
Balance
Ring stand with clamps (optional)
Large rubber band (optional)
Vacuum pump

Reagents

Cryoprotectant ambot containing Pall Medical Corporation
50% DMSO 5% Dextran 40
OR
DMSO and Must be approved for human use.
Dextran 40
10% bleach (or equivalent non-corrosive disinfectant)

Supplies

Stem Cell Freezing Bag Pall Medical Corporation, Stem Cell Processing Set (Set #2)
(Figure 4.2.2, SOP 4.2)
Cryovials (2ml) e.g. Nunc Plastics
1 ml Syringe (2) Hospital Store Room
5 ml Syringe Hospital Store Room
10 ml Syringe (2) Hospital Store Room
30 ml Syringe Hospital Store Room
CBU Cryopreservation Form Cord Blood Bank Stationary
Alcohol Swabs Hospital Store Room
Sterile Gauze Pads Hospital Store Room
Cryopreservation Labels Cord Blood Bank Stationary
Large rubber band Hospital Store Room
Overwrap material e.g. Dupont FEP-L overwrap bags (Thermogenesis Corp.)
COBLT Study Bar Code Labels

Procedure

1. a. Open cryopreservation database and create new cryopreservation record. Scan bar code label into new record.

   b. Record the identity of the responsible technologist, the date and the time in the database and on the CBU Cryopreservation Form (referred to hereafter as "the worksheet").
2. Apply adhesive bar code labels to Cryovials and storage cassette. Scan specimen and tubes to ensure match. Apply product labels to the stem cell freezing bag (Bag #4) (Figure 4.2.2, SOP 4.2). See Figure 4.3.1 for positioning COBLT label set bar code labels and product labels on the stem cell freezing bag (Bag #4). Labels produced by the BioArchive label printer should be attached as indicated in the freezer documentation.

3. If cryoprotectant ambot containing 50% DMSO and 5% Dextran 40 is available, then record the lot number and expiration date of the cryoprotectant on the worksheet and in the database. If cryoprotectant ambot is not available, then proceed with Steps 3a and 3b.
   a. Load a 10ml syringe with 5.25ml pre-cooled cryoprotectant (see Procedure Notes below on Preparation of Cryoprotectant).
   b. Record the lot number and expiration date of the DMSO and the Dextran on the worksheet and in the database.

4. If using frozen gel packs to pre-chill the CBU, then proceed with Steps 4a and 4b.
   a. Precool the processing bag (Bag #2) containing the CBU, the stem cell freezing bag (Bag #4), and the labeled cryovials for 15-25 minutes and a maximum of 6 hours.
   b. Retrieve the bag set and express the air from the stem cell freezing bag (Bag #4) into the processing bag (Bag #2) by opening the clamp on the tubing connecting the two bags, squeezing Bag #4, and reclamping the tubing.
   c. Surround the processing bag (Bag #2) with pre-cooled CoolPacks. Secure with a rubber band. Place the processing bag (Bag #2) in plastic dish on rocker and CoolPacks on the Orbital rotator and start rotation.

5. Initiate metered flow (0.25 ml/min) of DMSO into processing bag (Bag #2) of the stem cell processing set. Continue rotating the processing bag (Bag #2) to ensure adequate mixing of cells and cryoprotectant until addition of cryoprotectant is complete. A syringe pump or Pall Medical positioning jig may be used to add this solution.

6. After all cryoprotectant is added transfer contents of processing bag (Bag #2) into stem cell freezing bag (Bag #4).

   Note: It is expected that some of the contents of Bag #2 will remain in the tubing connecting Bag #2 and Bag #4. This material will be used to create segments (step 8) and to save cells for future testing (step 13).

7. Seal tubing to create 3 segments, starting from the proximal end of the tubing. Seals should be made at 90° to the coronal axis of the bag to allow tubing to bend parallel to the bag for proper storage (see diagram).
NOTE: Must seal the tubing first at the proximal end closest to the bag. Then seal out to end of the tubing to alleviate pressure buildup in the tubing. Then detach the processing bag (Bag #2) by cutting across the heat seal most distal to the stem cell freezing bag (Bag #4). Put processing bag (Bag #2) with attached tubing back on ice for removal of residual cells and processing of cryovials after completion of step 11. See step 12.

8. Wipe the surface of the stem cell freezing bag (Bag #4) with gauze soaked in 10% bleach or equivalent disinfectant. Wipe off excess. Overwrap the stem cell freezing bag (Bag #4) with overwrap material. Place the overwrapped bag into the storage cassette.

9. Controlled Rate Freezing

BioArchive System: Insert the cassette into the controlled rate freezer (CRF) module and insert the loaded CRF into Port 1 or 2 as available. Use cursor of Graphical User Interface (GUI) to activate recommended CRF program. Each cord blood bank should empirically validate their BioArchive CRFs, beginning with the recommended CRF program 10°C/min to -3°C, 100% fan power to -15°C and 2°C/min to -50°C.

OR

Conventional System: Place the cassette into the controlled rate freezer, insert the temperature probe into the cassette, and initiate the freezing program. The precise program used is determined by the equipment and the number of CBUs being cryopreserved. Each cord blood bank should independently empirically optimize and validate its equipment and controlled rate freezing curves through measurement of recovery of viable colony-forming cells, beginning with a recommended freezing rate of 1°C/min to 2°C/min, starting at 4°C to -40°C and 10°C/min thereafter to -90°C.

10. After the CBU reaches the target temperature (e.g. -50°C in the BioArchive system, -90°C in the conventional system), place the CBU in the quarantine storage location and record the location on the CBU Cryopreservation Form.

In the BioArchive system, the robotic arm will remove the CBU from the CRF and transfer it to a storage address in liquid nitrogen. In the conventional system, the CBU can be placed in a quarantine freezer in the vapor phase of liquid nitrogen.

11. Remove the printout of the freezer trace, apply an adhesive bar code label to the trace and place in the CBU’s processing folder. For digitally recorded traces, record the trace ID number in the cryopreservation database and CBU Cryopreservation Form.

12. To prepare cryovials, remove residual cells from processing bag (Bag #2) and freeze in cryoprotectant as follows.

a. Prepare 2 ml of 1x cryoprotectant by mixing 0.4 ml of residual 5x cryoprotectant with 1.6 ml of dextran in a 3 cc sterile syringe.

b. Inject a total of 2 ml of 1x cryoprotectant formulated in 12a into processing bag (Bag #2).
c. Strip any remaining cells + cryoprotectant left in the tubing that had been attached to the cryo bag back into processing bag (Bag #2). Mix with 1x cryoprotectant.

d. Remove all residual fluid from processing bag (Bag #2). Divide equally into two, 1-2ml labelled cryovials and freeze in liquid nitrogen according to institutional practices.

13. Seal cryovials inside of tubing for safe storage of LN2 (to avoid the possibility of contamination and explosion)(optional). Freeze cryovials to maintain cell viability. Place cryovials in the vapor phase of the quarantine liquid nitrogen storage freezer. Record location on the worksheet.

**Quality Control**

The trace of the controlled rate freezer showing the freezing curve/sample temperature must be within validation tolerances as specified below in Procedure Notes.

**Procedure Notes**

**Batch Preparation of Cryoprotectant (If Required)**

1. Cryoprotectant is to be prepared freshly each day with the lot numbers and expiration dates of each reagent noted and recorded on the container (a 150 ml glass bottle with a needle entrance port). Weigh the empty bottle for later calculations. Record the weight onto the bottle.

2. The volume of cryoprotectant prepared each day is dependent upon the expected workload. The basic CBU is approximately 105ml.

3. Using a syringe remove 55ml of DMSO and inject into the 150 ml glass bottle.

4. Weigh the bottle, subtract the weight of the empty bottle and divide the answer by 1.1 to determine the volume to dextran to add.

5. Using a second syringe remove the calculated volume of dextran from the container and add to the DMSO in the bottle.

6. Mix the cryoprotectant by inverting the bottle 4-5 times and place in the refrigerator to pre-cool.

**Controlled Rate Freezer Program**

The precise program used is determined by the equipment and the number of CBUs being cryopreserved. Each Cord Blood Bank will independently validate their equipment and program to provide freezing from 10°C/min starting at 4°C to -3°C, 100% fan power to -15°C and 2°C/min to -50°C for the BioArchive system, or 1°C/min to 2°C/min, starting at 4°C to -40°C and 10°C/minute thereafter to -90°C for the conventional system.
References


Figure 4.3.1

POSITION OF COBLT BAR CODE LABELS AND PRODUCT LABEL ON THE FRONT OF THE STEM CELL FREEZING BAG

POSITION OF COBLT PRODUCT LABEL ON THE BACK OF THE STEM CELL FREEZING BAG
4.4 RELEASING CBU FROM QUARANTINE TO LONG TERM STORAGE

**Principle**

All umbilical cord blood units (CBU) will be kept in a quarantine storage location until the results of all exclusion criteria have been determined and found to be negative. All samples of the mother’s peripheral blood or umbilical cord blood stored for future testing will be stored in long term storage freezers at appropriate temperatures. The following guidelines will apply for release from quarantine of the CBU and placement in long term storage.

**Guidelines**

1. No CBU will be released from quarantine until all exclusion criteria have been determined and found to be negative. This determination will be made by a designated CBB staff member AND the Director/PI of the Cord Blood Bank, or his/her designate, using the CBU Exclusion and Quarantine Release Form.

2. If one or more of the exclusion criteria are met at any point, the CBU will be discarded using the discard procedures in CBB SOP - Discarding CBUs. If the infectious disease testing results are positive for HIV-1, HIV-2, Hepatitis B, Hepatitis C or HTLV-I/II, the mother will be notified according to CBB SOP - Notifying Donors of Positive Infectious and Genetic Disease Test Results.

   CBU’s will be excluded from long term storage and discarded if newborn hemoglobinopathy screenings indicate the presence of the following diseases:

   - β Thalassemia (homozygous)
   - Hemoglobin C (homozygous)
   - Hemoglobin E (homozygous)
   - Hemoglobin SS
   - Hemoglobin SC
   - Hemoglobin S/β Thalassemia
   - Hemoglobin S/ and other clinically relevant abnormal β gene

3. When all criteria for release into long term storage have been met, the CBUs will be transferred from the quarantine freezer (as documented in the CBB SOPs) into the long term storage freezer, where they will be stored in the liquid phase of liquid nitrogen. If the BioArchive system is used to store cryopreserved CBUs, the CBU storage address will remain unchanged.

4. Vials of the mother/donor’s peripheral blood, designated for future HLA typing, will be placed in long term storage freezer at ≤ -20°C.

5. The aliquot of the mother/donor’s serum, designated for future infectious disease testing, will be placed in long term storage freezer at ≤ -20°C.
6. The information on the CBU Exclusion and Quarantine Release Form and all other data regarding the CBU will be transmitted to the Medical Coordinating Center for entry into the cord blood bank registry.
4.5 RELEASE FROM LONG TERM STORAGE

Principle

Once a Transplant Center, in collaboration with the MCC, has designated a particular cord blood unit (CBU) for a particular recipient there are several steps which must be followed to ensure that the correct CBU is supplied to the Transplant Center in a timely fashion. Further, Cord Blood Bank Quality Control activities require that the Transplant Center provide feedback regarding the state of the CBU following thawing.

Specimen

Cryopreserved umbilical cord blood that has been designated for transplant into a particular recipient.

Equipment

Database computer

Reagents

None

Supplies

- Release Checklist
- CBB Stationary
- Confirmation of Registration/CBU Release Request
- Transplant Center
-Investigators Brochure
- Medical Coordinating Center

Procedure

1. a. CBUs can be released by the COBLT CBB after receipt of a Confirmation of Registration/CBU Release Request from the Transplant Center and an Investigators Brochure from the MCC.

   b. The Transplant Center will provide the following information on the Confirmation of Registration/CBU Release Request:

      1. the requested CBU ID
      2. the COBLT Recipient ID
      3. name and contact address of Transplant Center staff receiving CBU
      4. proposed CBU shipment and transplant dates
      5. the signature of the responsible physician

   In addition, the Transplant Center must provide the delivery address of the Transplant
Center processing laboratory and, if possible, a map showing the precise location.

On receipt of the Confirmation of Registration/CBU Release Request, the CBB Distribution Coordinator (or authorized representative) will check the information and fax the completed request to the MCC.

c. The MCC will provide the Investigators Brochure with the following information:

- a packing information sheet
- a Transplant Center Feedback Sheet with the CBU ID and COBLT Recipient ID
- CBU receipt procedures
- COBLT CBU thawing procedures
- COBLT CBU infusion procedures
- product information

2. The CBB Distribution Coordinator (or authorized representative) will confirm that the CBU ID and Recipient ID from the Transplant Center and from the MCC match. Any discrepancy must be corrected before continuing. The CBB Coordinator will then confirm, using an internal checklist, the location of the CBU and of saved specimens, and recheck that all paperwork in the CBU folder is complete.

3. The Cord Blood Distribution Coordinator (or authorized representative) will contact the Transplant Center Coordinator to confirm receipt of the request and the proposed shipping date.

4. The Cord Blood Distribution Coordinator (or authorized representative) will contact Federal Express to schedule the pick-up and delivery of the CBU ‘dry shipper’ to the Transplant Center.

5. Once a preliminary schedule is obtained, the Cord Blood Distribution Coordinator (or authorized representative) will contact the Transplant Center Coordinator to confirm delivery. The Cord Blood Distribution Coordinator records the confirmed date of delivery on the Transplant Release Checklist, then signs and dates. The completed checklist must be sent to the Transplant Center Coordinator and CBB Laboratory Supervisor for review within 24 hours.

6. On the day of release of a CBU, the Laboratory Supervisor ensures that:

a. The Dry Shipper is loaded with sufficient liquid nitrogen for maintenance of the CBU at < -150°C for at least 48 hours following the scheduled time of arrival at the Transplant Center.

b. The Investigators Brochure and return shipping information to accompany the Dry Shipper are completed and packed with the shipper (see SOP 5.2).

7. The CBB Cord Blood Unit Paperwork Folder is located and a minimum of 8 bar code labels are removed and packed with the Investigators Brochure.
8. The cryopreserved CBU is removed from long term storage as documented in the CBB SOPs. The CBU identity is re-confirmed after retrieval from long term storage by the Laboratory Supervisor and at least one other staff person. Confirmation is obtained by one person reading the number aloud while the other confirms with the written request.

9. The CBU is then placed in the Dry Shipper, packaged, labeled, and handed to the courier for transport according to CBB SOP Section 5.2, Shipping Cryopreserved CBUs to Transplant Centers.

10. Following shipment, data on the CBU Disposition Form indicating the CBU was shipped for transplant are entered into the COBLT data system. The CBU Disposition Form is filed in the CBU Paperwork Folder.

**Quality Control**

1. All CBU paperwork, information, and infectious disease data are checked prior to release.

2. The identity of the CBU is confirmed by the Laboratory Supervisor and at least one other staff person. Confirmation is obtained by one person reading the number aloud while the other confirms with the written request.

3. The Transplant Center is provided with a feedback sheet to provide information on the following Quality Control variables:
   - Date and time of receipt
   - Weight of unpacked ‘dry shipper’
   - Condition of CBU on receipt
   - Condition of shipper on receipt
   - Shipper temperature

4. Only one CBU will be released at a time if more than one CBU is scheduled to be shipped on the same day.
4.6 DISCARDING CBUS

Principle

A number of tests and standards are used to determine the potential suitability of a cord blood unit (CBU) for transplant. When a particular CBU fails to meet any one of these standards (as defined by the exclusion criteria on the CBU Exclusion and Quarantine Release Form) that CBU must be discarded. CBUs returned to the Cord Blood Bank (CBB) from a transplant center will also be discarded following the procedures below. Records associated with discarded CBUs must be updated accordingly.

Specimen

Umbilical cord blood at any stage of processing and storage that has failed to meet all tests and standards; CBUs returned to the CBB from a transplant center.

Equipment

Electronic Bar Code Scanner
Database computer

Reagents

None

Supplies

Cord Blood Unit Paperwork Folder From Cord Blood Unit Records
Adhesive Bar Code Label Sheets From Collection Kit
CBU Disposition Form Cord Blood Bank Stationary

Procedure

1. Locate the paperwork folder for the CBU and the associated adhesive bar code labels. Scan labels, folder, and CBU to confirm identity.

2. a. Review and confirm the test result(s) that triggered the exclusion criterion.

   b. If the result is not confirmed take the CBU and all paperwork to the Laboratory Supervisor for second confirmation. If the CBU is found to be within acceptable standards it can be returned to the processing path. A Corrective Action form outlining the confirmation process must be filed in the CBU Paperwork Folder.

   c. If the result is confirmed, the laboratory supervisor or director/PI must be brought in to repeat confirmation. If the supervisor or director/PI confirms the result the CBU must be discarded according to the following procedures.

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This is a working research document and may be revised.
3. If any additional tests on the CBU are pending (e.g.: Flow Cytometry, Colony Assays, Infectious Disease Screening etc.) inform the laboratory supervisor or designee, who will then take appropriate steps to halt further testing.

4. Place an adhesive bar code label on the CBU Disposition Form. Open the CBU Disposition database and scan to confirm label identity. The CBU Disposition Form and database should both be completed following this procedure. Hereafter, they will be collectively termed the database.

5. Record on the database the reason for discard and the name of the responsible technologist and the laboratory supervisor or designee who confirmed the results. The Laboratory Technologist should sign the CBU Disposition Form at this time.

6. Open the database, locate the record for this CBU (using the scanner to enter the ID number) and record the date of discard, technologist ID, laboratory supervisor ID, and reason for discard.

7. a. The laboratory supervisor shall then determine whether the CBU can be used for Quality Assurance purposes. Note: If the reason for discard is failure to obtain consent the CBU must be discarded according to Step 9 below and cannot be used for Quality Assurance or research.

   b. If the CBU is deemed suitable for Quality Assurance testing this should be recorded in the database. Quarantined samples shall then be transferred to the QA section of the Quarantine Liquid Nitrogen Storage Freezer and the new location recorded in the database. Unprocessed CBUs will be processed according to the appropriate SOP and stored in the QA section of the Quarantine Liquid Nitrogen Storage Freezer.

8. If the CBU to be discarded is not required for Quality Assurance the Director or Medical Director may release it for ancillary research provided that consent for such use was obtained and that the researcher provides evidence of a currently approved, IRB reviewed, research protocol that allows for use of umbilical cord blood. The identity of the Principal Investigator of the research program, the IRB approval number, and the date of release will be recorded in the database.

9. If the CBU is unsuitable for research or is surplus to requirements, the sample can be discarded as Biohazardous Waste according to the standard practices of the host institution. The means and date of disposal must be recorded in the database.

Quality Control

The Laboratory Supervisor and the Responsible Technologist must both sign the CBU Disposition Form and their identities must be recorded in the database once both have confirmed that a CBU is
unsuitable for banking. The Laboratory Supervisor must also sign for the release of any CBU for Quality Assurance or research.
4.7 MANUAL DIFFERENTIAL CELL COUNTS

Principle

Manual differential cell counts will be required to calculate the leukocyte count and recovery of viable mononuclear cells before Hespan sedimentation and after plasma depletion/pre-cryopreservation.

Specimens

Blood smears on microscope slides will be made in triplicate from the pre-Hespan and post-plasma depletion/pre-cryopreservation product. The amount of materials per slide may be estimated as follows:

a. Pre-Hespan: Three slides with one drop of unprocessed umbilical cord blood per slide.

b. Post-plasma depletion/pre-cryopreservation: Three slides with 20 µl of the leukocyte enriched fraction per slide.

Procedure

1. Proportions of leukocytes and nucleated erythrocytes will be determined from a 200 nucleated cell differential. Mononuclear cells (MNC) will be differentiated from other leukocytes and nucleated erythrocytes. The proportions of mononuclear cells and nucleated erythrocytes in 200 cells counted will be recorded on the CBU Processing Form. The proportion of leukocytes will be calculated by subtracting the nucleated erythrocytes from the total nucleated cell count.

2. Mononuclear (defined as blasts + monocytes + lymphocytes) cell counts, nucleated erythrocytes, and leukocyte (defined as all nucleated cells other than nucleated erythrocytes) cell counts will be determined by performing a manual differential of unprocessed umbilical cord blood (pre-Hespan sedimentation) and 20 µL of leukocyte enriched umbilical cord blood (post-plasma depletion/pre-cryopreservation). One slide of each triplicate will be stained using Wright-Giemsa. The method of staining may be automated or manual using manufacturer’s instructions. The four remaining unstained slides will be stored for future use.

3. Total viable leukocyte count in the umbilical cord blood unit (CBU) before Hespan sedimentation and after plasma depletion/pre-cryopreservation will be determined by calculating the product of the total viable nucleated cell count and the percentage of leukocytes (i.e., excluding nucleated erythrocytes) in the manual differential.

\[
\text{Total viable leukocyte count} = (\text{total viable nucleated cell count}) (100\%-% \text{nucleated erythrocytes})
\]

4. The total viable mononuclear count (MNC) in the CBU before Hespan sedimentation and after plasma depletion/pre-cryopreservation will be determined by the following calculation and recorded on the CBU Processing Form:

\[
\text{Total viable MNC} = (\text{total viable nucleated cell count}) (% \text{ mononuclear cells})
\]
5. Viable mononuclear cell recovery after plasma depletion/pre-cryopreservation will be determined by the following calculation and recorded on the CBU Processing Form:

\[
\% \text{ viable MNC recovered} = \frac{\text{total viable MNC}_{\text{post}}}{\text{total viable MNC}_{\text{pre}}} \times 100
\]

Quality Control

1. Specimens from only one CBU will be made at any one time.

2. Bar code numbers on the slide label will be verified against the CBU before and after Hespan sedimentation.

3. Bar code numbers on CBU Processing Form will be verified against bar code numbers on the microscope slides.

4. Technologist’s ability to perform manual differentials on umbilical cord blood specimens will be documented by in-service training and biannual recertification testing.

5. Filed unstained slides will be available for future analyses.