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ON SITE THAWING OF CRYOPRESERVED CORD BLOOD UNIT FOR TRANSPLANTATION

Background

Cord blood transplantation is a reasonable alternative to bone marrow as a source hematopoietic stem cells (1-3). Cells cryopreserved in DMSO have limited viability upon thawing, because DMSO is toxic to cells when warmed to 37°C. Intracellular DMSO creates a hypertonic environment which leads to sudden fluid shifts and cell death upon warming. Lysis of red blood cells leads to accumulation of extracellular free haemoglobin which can be nephrotoxic, if infused intravenously. In addition, DMSO causes adverse effects in vivo after reinfusion, including blood pressure instability, fever, chills, nausea, vomiting, cough, dyspnea, skin flushing and anaphylaxis. To ameliorate these problems several methods are adopted (4-15).

In standard practice, CBUs can be thawed according to the following methods:

1. thawing and direct infusion with all its dimethylsulfoxide (DMSO) content, without further manipulation, as quickly as possible afterwards;
2. thawing, dilution with a dextran/albumin solution and infusion as quickly as possible afterwards;
3. thawing, dilution with a dextran/albumin solution, washing, reconstitution in a dextran/albumin solution and infusion; this method is also reserved to not red cell reduced Cord Blood Unit (CBU) or for administration to patients with allergic reactions to the additives included in the product.

Principle

This procedure is designed to enable the Transplant Center technologist to thaw cryopreserved CBUs manufactured at the Pescara CBB, according to our validated procedure.

The Transplant Center is in charge of doing a risk analysis and then choose which procedure to apply, according to the patient's related variables (diagnosis, comorbidity, age, size), features of the CBU (total volume of the CBU, cell dose to infuse, RBC content, and content of DMSO), single or double CBUs transplantion, and the Processing Lab skill. Furthermore, if the patient is enrolled on a protocol, the Transplant Center should abide by the protocol's rules.

Cord Blood Unit

All CBUs distributed by the Pescara CBB are plasma and RBC reduced with a final volume of approximately 70ml including cryosolution; CBU are cryopreseved by controlled rate freezing down to -150°C in an EVA cryobag containing a final concentration of 10% DMSO, 8% Dextran 40 and 1% of Human Serum Albumin. CBUs are stored inside a metal canister *(dimension: LxWxH - in inches - 9.25x5.51x0.39)* and maintained in the vapour/liquid phase in a liquid nitrogen tank, at a temperature ≥ -150°C. The cryobag containing the CBU may be overwrapped or not to with secondary plastic bag or not, and may have one sealed attached segment or a pilot reference tube, according to year of banking. CBU is shipped to Transplant Center in a dry shipper, at a temperature below -150°C, with temperature recording.

Personnel
The procedure must only be carried out by properly trained staff who are deemed to be competent. Authorization for issue of the CBU is to be performed by Head of Processing Lab.

Equipment
Laminar Flow Hood
Water bath
Balance
Heat Sealer
Refrigerated Blood Bank Centrifuge, *if necessary*
Plasma Extractor, *if necessary*
Sterile Connecting Device, *if necessary*

Reagents for thawing/washing solution, *if necessary*
Human Serum Albumin 5% *(stored at 2-6°C)*
Dextran 40 10% *(stored at 2-6°C)*

Supplies
Protective thermal gloves
Paper towels
Alcohol pads
Refrigerated gel packs
Zip lock bag
Disposables syringes (2, 5, 10, 60 ml)
Sampling site coupler
Needles (16-18 gauge)
Clamp
Hemostat Forceps (autoclaved)
Transfer Bag (150, 300, 600 ml)
Nunc Tube Sterile Gloves
Transfer set for IV infusion
CBU Labels
CBU Thawing Form

Procedure

1. Thawing and Infusion
If you must only thaw the CBU, without any further manipulation, follow the procedure below:

1.1 Prearrange infusion time with patient's nurse and physician
1.2 Review the CBU documentation against the patient's data *(2 staff members)*
1.3 Prepare in advance water bath and wait for temperature reaching 37-38°C
1.4 Prepare labels and the necessary paperwork, completing as much as possible
1.5 Prepare supply
1.6 Remove the cryopreserved CBU from the freezer carefully; handle the frozen cord blood bag with extreme care at every step, including opening the metal containers, inspecting and thawing
1.7 Check the ID number label on the cryo bag against documentation *(2 staff members)*
1.8 Place the cryo CBU in a zip lock bag
1.9 Thaw the CBU, gently mixing the bag; inspect for leakage; *if blood leaks out into the zip-lock bag see note 1*
1.10 As soon as the blood is liquid, remove the bag from the bath, dry off the bag and affix the distribution label.

1.11 Weigh the bag and record
1.12 Insert a sampling site coupler into the port of the bag and withdraw the QC under hood laminar flow
1.13 Deliver the CBU to patient's nurse and physician (at bedside 2 staff members must verify recipient's identification - patient's full name and DOB- and CBU identification, directly from distribution label)
1.11 Infuse the CBU to the patient, within approximately 15 minutes with standard 170µ blood filter.
1.12 Perform all quality controls
1.13 Fill out all paperwork.

The complete process takes 30 minutes, generally. According to the literature (5) a median TNC recovery and viability of 99% and 95% respectively can be obtained.

Note:

(1) If a leak occurs during thawing, find the site of the break in the freezing bag, quickly clump the bag with hemostat forceps, position the bag so as to prevent escape of the blood inside the zipper lock; insert a spike of a transfer bag into the freezing bag's port and let the entire contents drain into the transfer bag and seal the tubing.

(2) This thaw practice does not reduce the load of potentially toxic components (dextran, DMSO), including hypersensitivity.

(3) If infusion must be delayed for any problem occurring to the patient after CBU thawing, loss of potency can occur over time due to DMSO toxicity; after 2 hours loss of potency become significant. In this cases the unit should be returned to the laboratory and diluted or washed to remove the DMSO as soon as possible.

2. Thawing and dilution protocol

2.1 Preparation of thawing solution

Prepare thawing solution at least one hour and no more than 24 hours before thawing the CBU.

2.1.1 Obtain two gel packs; place in refrigerator to cool.

2.1.2 Label a 300-mL or larger transfer pack with "Working Thaw/Wash Solution" and the date/time prepared.

2.1.3 Perform the following steps under a biological safety cabinet to prepare the thaw/wash kit:

a. Clean ports with alcohol, and attach a sampling site coupler to one port and a needle-free luer lock to the other port; attach hemostat to tubing .

b. Add at least 112.5-mL Dextran 40 10% to the 300-mL transfer pack labeled Working Thaw/Wash Solution using 60-mL sterile syringes with needles (Albumin to Dextran ratio must be 1:1).

c. Add at least 112.5-mL 5% albumin (human) solution to the same the 300-mL transfer pack labeled "Working Thaw/Wash Solution" using 60-mL sterile syringes with needles.

d. Mix by inverting the pack at least 10 times.

e. Label one transfer pack with CBU Donation Identification Number (DIN) and "Thaw/Wash." Clean ports with alcohol, and attach a sampling site coupler to one port and a needle-free luer lock to the other port .

f. Label the second transfer pack with CBU DIN and "Expressed"

g. Attach a hemostat to the tubing approximately one inch above the sampling site coupler on the CBU DIN and Thaw/Wash transfer pack .

h. Using a sterile docker, connect the two 300-mL transfer packs.

i. Add 100 mL of thaw/wash solution to the CBU DIN and Thaw/Wash transfer pack.

j. Label two 60-mL sterile syringes and two 30-mL sterile syringes as "Thaw/Wash Solution."

i. Draw 20 mL of thaw/wash solution into each of the two 60-mL syringes; cap and set aside.

ii. Draw 5 mL of thaw/wash solution into each of the two 30-mL syringes syringes; cap and set aside.

k. Label a 60-mL sterile syringe "Resuspension Solution"; fill with 50 mL of pre-chilled thaw solution (1:1 ratio of Dextran/HSA solution) for final end-product resuspension via needle-free luer lock. Cap and place in refrigerator to use after centrifugation.

l. Place the transfer packs and syringes in the refrigerator for at least one hour and no longer than 24 hours.

2.2 Dilution procedure

If you must dilute the CBU after thawing, follow the procedure below:

2.2.1 Prearrange infusion time with patient's nurse and physician

2.2.2 Review the CBU documentation against the patient's data (2 staff members)

2.2.3 Prepare in advance water bath and wait for temperature reaching 37-38 °C

2.2.4 Label a transfer bag for thawed product infusion

2.2.4 Prepare labels and the necessary paperwork, completing as much as possible

2.2.5 Prepare supply

2.2.6 Under the laminar flow hood, prepare a 10%Dextran /5% albumin solution and chill the solution as explained above

2.2.7 Remove the cryopreserved CBU from the freezer carefully

2.2.8 Check the ID number label on the cryo bag against documentation *(2 staff members)*

2.2.9 Place the cryo CBU in a zip lock bag

2.2.10 Thaw the CBU, gently mixing the bag; inspect for leakage; *if blood leaks out into the zip-lock bag see note 1 above*

2.2.11 As soon as the blood is liquid, remove the bag from the bath and dry off the bag

2.2.12 Check the bag for signs of cell clumping

2.2.03 Weigh the bag and record as volume

2.2.14 Insert a sampling site coupler into the port of the bag and withdraw the QC (optional)

2.2.15 Insert the spike of the prelabeled transfer bag and transfer cells suspension into the prelabelled transplant bag

2.2.16 Slowly transfer inside the transplant bag the same volume of the bag of the prechilled Dextran/Albumin solution, rinse the freezing bag, add all the residual cell content into the transfer bag

2.2.17 Allow 3-5 minutes for equilibration

2.2.18 Draw again the QC

2.2.19 Deliver the CBU to patient's nurse and physician (at bedside 2 staff members must verify recipient's identification - patient's full name and DOB- and CBU identification, directly from distribution label)

2.2.20 Infuse the CBU to the patient, within approximately 15 minutes with standard 170µ blood filter.

2.2.21 Perform all quality controls

2.2.22 Fill out all paperwork.

According to our stability testing program we obtained the following results adopting the thawing and dilution method:

	RECOVERY (%)				VIABILITY 7-AAD (%)		
	NC	**MNC**	**CD34 +**	**CFU-GM**	**NC**	**MNC**	**CD34 +**
MEDIA	88,1	98,0	77,5	67,3	58,6	81,9	91,2
±DEV ST	4,6	9,0	18,8	28,0	8,2	7,2	7,7
MEDIANA	88,3	99,4	75,9	72,0	59,8	82,0	93,4
MIN	78,7	81,5	56,9	26,2	42,6	69,2	80,0

MAX	93,9	114,4	110,7	136,2	72,3	91,6	98,9

Storage time of the thawed CBUs ranged from 15 to 226 months.

Note:

(1) This thaw practice does not reduce the load of potentially toxic components (dextran, DMSO), including hypersensitivity; furthermore the method requires a controlled environment (classified biosafety cabinet) for preparation and sampling, and trained staff.

(2) According to the literature (4), the product stability is up to a maximum time of 4 hours.

3. Thawing and washing protocol

If you must wash the CBU after dilution, follow the procedure below:

3.1 Prearrange infusion time with patient's nurse and physician
3.2 Review the CBU documentation against the patient's data *(2 staff members)*
3.3 Prepare in advance water bath and wait for temperature reaching 37-38 °C
3.4 Label a transfer bag for thawed product infusion
3.5 Prepare labels and the necessary paperwork, completing as much as possible
3.6 Prepare supply
3.7 Under the laminar flow hood, prepare a the Dextran /HAS thawing solution and chill the solution
3.8 Remove the cryopreserved CBU from the freezer carefully
3.9 Check the ID number label on the cryo bag against documentation *(2 staff members)*
3.10 Place the cryo CBU in a zip lock bag
3.11 Thaw the CBU, gently mixing the bag; inspect for leak; *if blood leaks out into the zip-lock bag see note 1 above*
3.12 As soon as the blood is liquid, remove the bag from the bath and dry off the bag
3.13 Check the bag for signs of cell clumping
3.14 Weigh the bag and record as volume
3.15 Insert a sampling site coupler into the port of the bag and withdraw the QC
3.16 Insert the spike of the prelabeled transfer bag and transfer cells suspension into the prelabelled transplant bag
3.17 Slowly transfer inside the transplant bag the same volume of the bag of the prechilled Dextran/Albumin solution (70ml±4ml), rinse the freezing bag, add all the residual cell content into the transfer bag
3.18 Centrifuge the CBU at 800xG, for 15 minutes, at 10°C; *if the Processing Lab is experienced with automatic washing it is to be performed under the responsibility of the Head of Processing Lab*
3.19 Place the centrifuged CBU in plasma extractor
3.20 Insert the spike of a new transfer bag set into the exit port of the first bag and remove most of the supernatant into the new transfer bag carefully, to avoid accidental passage of cells with the supernatant. *If you detect passage of cells by accident to the transfer bag see note 2*
3.21 Reconstitute cell pellet with the same initial volume of the bag of dextran/albumin solution through a sampling site coupler, slowly, while mixing it with the cells
3.22 Weigh the bag and remove a small volume of cells for QC
3.23 Deliver the CBU to patient's nurse and physician (at bedside 2 staff members must verify recipient's identification - patient's full name and DOB- and CBU identification, directly from distribution label)
3.24 Infuse the CBU to the patient within 15 minutes with standard 170μ blood filter.
3.25 Perform all QC
3.26 Fill out all paperwork.

The complete process takes 1 hour and 15 minutes generally. According to the literature (5) a median

TNC recovery and viability of 78.6% and 73% can be obtained, respectively.

Notes:

(3) *If you detect passage of cells by accident to the transfer bag close immediately the clamp, seal the transplant bag, perform the cell count, multiply by the total volume and calculate the recovery; then decide whether the escaped cells should be recovered in a second centrifugation to reach the transplant cell dose.*

(4) *In case of delay to infusion after dilution/washing procedure, store the CBU at +4°C±2. Elapse of time decreases cell potency; according to the literature(4),expiration time for diluted/washed products is 24 hours.*

Recommendations

If the patient is enrolled on a protocol, the Transplant Center should abide by the protocol's rules; if the patient is not enrolled on protocol, the Transplant Center must prepare CBU according to manufacturer's instructions or by the Processing Lab validated procedure.

CBU must be inspected for container integrity and unusual appearance immediately before thawing and administration. Aseptic technique must always be employed.

The administration time will be determined by the total volume relative to the patient weight to be infused.

If the thawed CBUs have not been washed to remove DMSO, care should be taken not to exceed 1 gr. (1 ml) of DMSO per kilogram of recipient weight per day administration, particularly when a transplant is performed with a double CBUs [i.e.: 70 mL of a 10% solution contains 7 mL (7 gr) of DMSO].

Washing is recommended if exposure to DMSO, free haemoglobin and volume of the product approaches critical limits for pediatrics recipients. In this case, care should be taken not to adversely affect cell dose.

The time from initiation of thaw to completion of infusion should be minimized.

CBU should be infused at a rate of ≈5 cc/Kg/hr.

CBU must *not* be administered through a filter designed to remove leukocytes.

CBU may be filtered through a 170μ blood filter designed to remove clots.

CBU products must not be irradiated.

Sufficient observation should be undertaken by nursing and medical staff to detect symptoms and/or signs suggestive of acute immunologic or infectious complications. Specifically, vital signs (pulse and respirations, blood pressure) should be measured at baseline, during, and for 2-4 hours post infusion.

When administering double cord blood transplants, a minimum of 2 hours should elapse between the completion of the first infusion and the initiation of the second infusion. Should a reaction occurs, appropriately manage the reaction before second unit is thawed for infusion.

Any severe adverse event must be report to the Competent Authorities and to Pescara CBB.

Literature

1 *R. Mitchell, J.E.Wagner, C.G.Brunstein, Q. Cao, D.H. McKenna, T.C. Lund, M.R. Verneris. Impact of long term cryopreservation on single umbilical Cord Blood Transplantation outcomes. Biol Blood Marrow Transplant 21 (2015) 50-54.*

2 *Parmar S., De Lima M., Worth L., et Al. Is there an expiration date for a cord blood unit in storage? Bone Marrow Transplant. 2014; 49:1109-1112.*

3 *Purtill D, Smith K., Meagher R., et Al. Analysis of 402 cord blood units to assess factors influencing infused viable CD34+ cell dose: The critical determinant of engraftment. Biol Blood Marrow Transplant.2014;20:S59.*

4 *Akel S, Regan D, Wall D, Petz L, and McCullough J. Current thawing and infusion practice of cryopreserved cord blood: the impact on graft quality, recipient safety, and transplantation outcomes. Transfusion 2014;54:2997-3009.*

5 *Regan DM, Wofford JD, Wall DA. Comparison of cord blood thawing methods on cell recovery, potency, and infusion. Transfusion. 2010 Dec;50(12):2670-5.*

6 *Ruiz-Delgado GJ, Mancías-Guerra C, Tamez-Gómez EL, Rodríguez-Romo LN, López-Otero A, Hernández-Arizpe A, Gómez-Almaguer D, Ruiz-Argüelles GJ. Dimethyl sulfoxide-induced toxicity in cord blood stem cell transplantation: report of three cases and review of the literature. Acta Haematol. 2009;122(1):1-5.*

7 *Yoo KH, Lee SH, Kim HJ, et Al. The impact of post-thaw colony-forming units-granulocyte/macrophage on engraftment following unrelated cord blood transplantation in pediatric recipients. Bone Marrow Transplant. 2007; 39:515-521.*

8 *Laroche V, McKenna DH, Moroff G, Schierman T, Kadidlo D, McCullough J. Cell loss and recovery in umbilical cord blood processing: a comparison of postthaw and postwash samples. Transfusion. 2005 Dec;45(12):1909-16.*

9 *Kelley L. Commentary on 2003-808: infusion of unwashed umbilical cord blood stem cells after thawing for allogeneic transplantation. Bone Marrow Transplant. 2004 Oct;34(8):741.*

10 *Antonenas V, Shaw PJ, Bradstock KF. Infusion of unwashed umbilical cord blood stem cells after thawing for allogeneic transplantation. Bone Marrow Transplant. 2004 Oct;34(8):739.*

11 *Rodríguez L, Azqueta C, Azzalin S, García J, Querol S. Washing of cord blood grafts after thawing: high cell recovery using an automated and closed system. Vox Sang. 2004 Oct;87(3):165-72.*

12 *Nagamura-Inoue T, Shioya M, Sugo M et al. Wash-out of DMSO does not improve the speed of engraftment of cord blood transplantation: follow-up of 46 adult patients with units shipped from a single cord blood bank. Transfusion 2003; 43:1285-1294.*

13 *Broxmeyer HE, Srour EF, Hangoc G. et Al. High-efficiency recovery of functional hematopoietic progenitor cells and stem cells from human cord blood cryopreserved for 15 years. Proc Natl Acad Sc USA 2003 100 (645-650).*

14 *Hahn T, Bunworasate U, George MC, Bir AS, Chinratanalab W, Alam AR, Bambach B, Baer MR, Slack JL, Wetzler M, Becker JL, McCarthy PL Jr. Use of nonvolume-reduced (unmanipulated after thawing) umbilical cord blood stem cells for allogeneic transplantation results in safe engraftment. Bone Marrow Transplant. 2003 Jul;32(2):145-50.*

15 *Rubinstein P, Dobrila L, Rosenfield RE et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. PNAS 92, 10119-101022-1995.*

16 *Duggleby RC, Querol S, Davy RC, Fry LJ, Gibson DA, Horton RB, Mahmood SN, Gomez SG, Madrigal JA. Flow cytometry assessment of apoptotic CD34+ cells by annexin V labeling may improve prediction of cord blood potency for engraftment. Transfusion 2012;52:549-59.*

17 *Scaradavou A, Smith KM, Hawke R, Schaible A, Abboud M, Kernan NA, Young JW, Barker JN. Cord blood units with low CD34+ cell viability have a low probability of engraftment after double unit transplantation. Biol Blood Marrow Transplant 2010;16:500-8.*