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Guidelines

Guidelines for Cord Blood Unit Thaw and Infusion

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BACKGROUND

Standard operating procedures (SOPs) for receipt and thaw of cord blood units (CBUs) as well as guidelines for graft infusion are a critical component of cord blood (CB) transplantation [1]. Accepted transplant center (TC) thaw procedures, post-thaw graft evaluation, patient care during infusion, and suggestions for management of associated problems are outlined in these guidelines.

FAQ1: What is the required preparation before receipt of a CBU?

Cell therapy laboratory staff should be aware of the detailed product information, including the type of container, number of bags, cryopreservation volume and bag type, access port type, and whether the unit is RBC depleted or RBC replete. The TC should request arrival within routine hours of operation but also have a SOP in place for CBU handling if the shipment arrives after hours. This is critically important to prevent inappropriate opening of the portable liquid nitrogen shipping container (dry shipper) and/or mishandling of the CBU.

FAQ2: How should CBUs be handled upon arrival at the TC?

To ensure appropriate handling, the TC needs SOPs for the receipt of cryopreserved CBU, verification of product identity and integrity, and liquid nitrogen (LN) storage until the day of transplant [2]. Additionally, review of the temperature curve is essential to ensure that the CBU remained in appropriate condition during transit [3]. Steps in this process are the following:

a) Inspect the integrity of the dry shipper.
b) Inspect the temperature monitoring device and record the displayed temperature; if no display, measure the temperature inside the dry shipper.
c) Open the canister and inspect the CBU (while keeping it in the vapor phase) to verify product identity and integrity; promptly close it and transfer to the LN freezer (storage temperature <-150°C).
d) Review accompanying documentation, labels, and temperature log.
e) Complete the CBU Receipt Form documenting all pertinent information.
f) Document storage condition at the TC.

FAQ3: How do TCs manage the most common CBU shipping problems?

CBUs are shipped frozen in dry shippers validated to maintain a controlled environment and low temperature (typically -196°C) for the entire transit time [3]. The CBU temperature is continuously monitored during transportation and must remain at or below -150°C [3]. If upon arrival the temperature monitoring device is in alarm, the temperature must be measured and documented. Examples of acceptable or nonreassuring temperature curves are shown in Figure 1. In the case of an unacceptable or not reassuring temperature curve, the bank must be notified. The TC must decide to either reject the CBU or proceed with caution. If the TC chooses to proceed, rapid post-thaw $CD34^+$ viability testing or another rapid measure of CBU potency should be performed, and a backup unit must be available for immediate shipment in the event the post-thaw testing is not acceptable.

Another problem can be that the accompanying documentation and/or labels do not match the information previously provided by the bank/registry [2,3]. The discrepancies must be resolved immediately, with emphasis on ensuring unit identity and excluding the unlikely possibility that the wrong unit was shipped.

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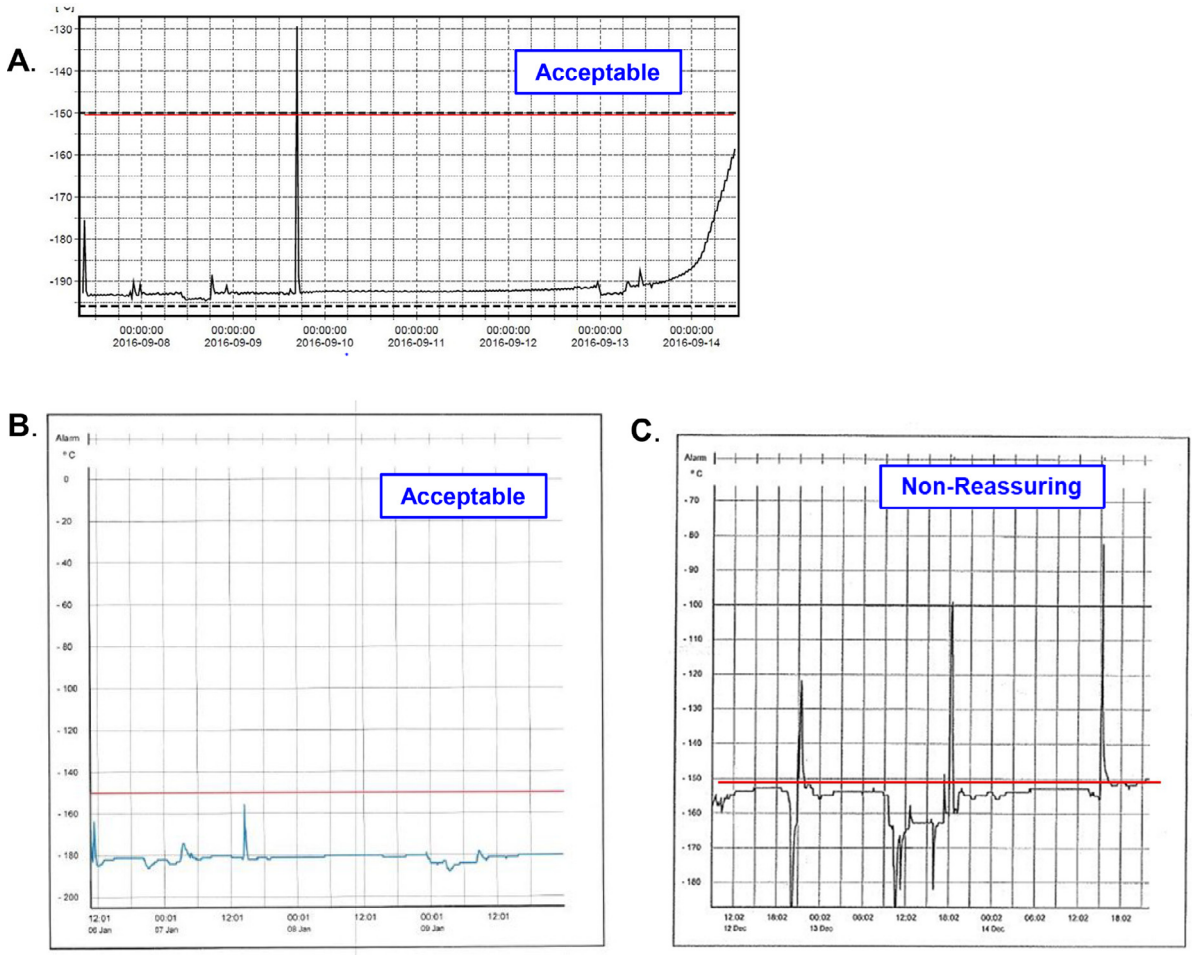

Figure 1. Temperature must remain at or below −150°C (FACT Standard). Examples of acceptable or nonreassuring temperature graphs are shown (−150°C cutoff is shown in red line). (A) Acceptable temperature graph: temperature maintained below −150°C, except a "peak" (acute rise in temperature) at the opening of the dry shipper upon arrival at the TC. (B) Acceptable temperature graph. (C) Graph shows a rise in temperature above −150°C, and the report indicates that the average temperature during transit was −154.8°C. Since this is borderline, the TC needs to proceed with caution, perform immediate post-thaw testing of $CD34^+$ cell viability, and have a backup CBU ready for shipment.

FAQ4: What SOPs must be in place for CBU thaw?

Units must be prepared for infusion with procedures that reproducibly achieve high cell recovery, maintain high cellular viability, and avoid product contamination [2-4]. The TC should use validated SOPs for thaw and preparation for infusion in alignment with the CB bank's recommendations. Additionally, banks may provide training CBUs so that a validated procedure can be developed. This is necessary if special manipulations are to be performed, for example, separation of the 2 compartments of the bag.

The following information is needed prior to the thaw:

- Number of cryopreservation bags per product: 1 or 2 (rarely more than 2)
- Type of cryopreservation bag: 1 or 2 compartments and the types of ports
- Cryopreservation volume: standard 25 mL or different
- Whether the CBU is RBC depleted or not: if not, the total volume of RBCs in the unit
- Final infusion volume ordered
- What samples are needed for post-thaw testing and their specifications

FAQ5: What are the different methods of thawing CBUs?

Bedside thaw is not recommended [1].

Frozen CBUs should be thawed in the cell therapy laboratory at 37°C using either a validated water bath or a heat bath such as a dry plasma thawer.

FAQ6: How should thawed CBUs be prepared for infusion?

The 2 accepted practices are dilution and wash or dilution only. When choosing which to use, TCs should consider age/weight of the patient to adjust the final infusion volume and DMSO amount, distance of the cell therapy laboratory from the patient in order to estimate the time from thaw to infusion, CBU RBC content, and the number of bags (products) to calculate the final total volume [1].

Note: Prior to both practices, in order to avoid osmotic cell damage, it is important to perform an initial 1:1 dilution with the thaw solution and allow up to 5 minutes for equilibration prior to further dilution. Performing the procedure in the cell therapy laboratory biosafety cabinet is critical.

(A) Dilute and wash: CBUs are diluted with a solution of 10% Dextran 40 and 25% human albumin followed by centrifugation to remove the supernatant plasma and DMSO [5]. A

1:8 volume/volume dilution should be targeted. Automated devices or manual washing can be used [1]. Wash offers advantages of longer product stability, removes DMSO and RBC debris, and permits adjustment of the final volume based on patient weight. Disadvantages are graft manipulation that can result in cell loss and increased technologist time. TCs that perform automated wash usually infuse 50 mL final volume in adults and smaller volumes in children [1]. Most centers recommend washing for patients with body weight <20 kg [1].

(B) Dilution only (reconstitution): Thawed CBUs are diluted with a dextran-albumin solution to a final 1:7-1:8 dilution. The 1:8 dilution for a 25-mL cryopreservation bag is frequently used as it results in a final 200-mL volume [6]. Many centers have adopted this method as it is faster and requires less manipulation, minimizing cell loss. However, as DMSO is not removed, the product must be infused promptly (within 4 to a maximum of 6 hours) to ensure viability is maintained. The product can be kept at 4°C to 6°C until infusion. The patient must be premedicated and monitored more closely given the DMSO content and the higher total infusion volume, especially for double-unit graft recipients.

FAQ7: How should RBC-replete CBUs be handled?

As life-threatening infusion reactions have been reported with RBC-replete CBUs [7,8], most centers recommend against their use. If such CBUs are chosen, wash is recommended [2,8], following the CB bank's instructions. However, the wash requires considerable expertise as it involves a high-volume product, separation of the supernatant is difficult, and there are high risks of cell loss and clumping. Further, if unit and recipient are ABO incompatible, the volume of incompatible RBCs to be infused should not exceed 2 mL/kg patient body weight.

FAQ8: What post-thaw tests are recommended on thawed CBUs?

- Complete blood counts (including total nucleated cell count and hematocrit)
- Flow cytometric evaluation of $CD34^+$ and $CD45^+$ content and viability
- Calculation of Total Nucleated Cell (TNC) and $CD34^+$ cell recoveries
- Sterility cultures
- Colony forming unit assays and ABO/Rh typing (optional at some TCs)

TCs should have specifications for acceptable post-thaw TNC and $CD34^+$ cell doses and recovery, as well as $CD34^+$ viability [1]. If the post-thaw values do not meet the predetermined specifications, the laboratory must immediately alert the clinical team to further evaluate the results, estimate the unit engraftment potential, and decide whether a replacement (backup) unit should be shipped [9].

FAQ9: What are the potential problems during CBU thaw?

Cryopreserved bags are brittle and should be handled with care. If a break, crack, or leak is observed, the CBU should be thawed in a sterile overwrap bag, the area at risk clamped with a sterilized hemostat, and the CBU placed in an orientation to minimize leaking. The most common sites of leaks are at the junctions where the segment connects to the bag and at the bridges. Another possible area of leak is at the bag entry ports if the spikes used are not appropriate. In these cases, a backup technologist should be called to assist with product handling, the estimated volume of leaked product should be documented, and a bacterial culture obtained. Photographs of the thawed bag should be obtained, and the bag should be sent to the bank for further evaluation. If the CB product cannot be retrieved, detailed documentation and shipment of a backup CBU are necessary [9].

FAQ10: How should CB recipients be managed before and during CBU infusion?

- For thaw-dilute products, hydrate intravenously (at approximately twice maintenance 6 hours before and 12 hours after the infusion) with close monitoring of fluid balance [1]. Shorter posthydration may be considered with washed products.
- Premedicate (diphenhydramine and acetaminophen) 15 to 30 minutes before infusion [1]. Many centers also give hydrocortisone to further minimize allergic reactions and some lorazepam to reduce nausea. Emergency medications for infusion reactions must be available [10].
- Verify patient and product identity before infusion [2].
- Spiking the product should be done carefully to avoid inadvertent puncture of the bag. The CBU should be infused through a central venous catheter by gravity (adults) or by a pump (smaller children). The unit should *not* be irradiated. The unit should *not* be administered through a filter designed to remove leukocytes. A filter of 170 to 260 microns can be used to remove clots, if needed [4]. Only 0.9% sodium chloride (normal saline) should be infused through the same line [4].
- Units are typically infused over 30 to 45 minutes. The initial infusion rate should be slow and can be increased as tolerated [4]. Trained nursing staff should be at the bedside continuously during the first 15 minutes of the infusion [6]. The patient should be monitored frequently thereafter and vital signs documented.
- Infusion adverse reactions must be treated *promptly* (see FAQ11) [6]. In the event of a moderate/severe infusion reaction, infusion should be stopped. Once the reaction is treated, the infusion can be restarted slowly and advanced as tolerated.
- Units of a double-CBU graft should be thawed and infused one at a time [4]. Thaw of the second CBU should not commence until infusion of the first unit has started and the patient has been stable for *at least* 15 minutes. Some centers prefer to separate the infusion of the 2 CBUs by 2 hours.

FAQ11: How should specific infusion reactions be managed?

Infusion of frozen-thawed products can be associated with adverse reactions due to the DMSO and hemolyzed RBCs. These include hypertension, hypotension, nausea, vomiting, fever, chills, dyspnea, chest tightness, and hematuria [4]. Life-threatening reactions, such as anaphylaxis, angioedema, seizures, and severe bronchospasm, are rare but are more likely during the first 15 minutes of the infusion [6]. Emergency medications must be available at the bedside, including hydralazine for hypertension. Adverse reactions should be promptly treated, and if serious, product infusion should be interrupted until the patient recovers. Common reactions and their management are listed in Table 1. The adverse event severity (according to current Common Terminology Criteria

Table 1
Management of Adverse Infusion Reactions

Reaction	Management
Allergic reaction/anaphylaxis	Stop product infusion. Administer corticosteroids, antihistamines, famotidine, and aggressive life support as needed.
Bradycardia	Monitor closely, slow or stop product infusion, and continue hydration.
Bronchospasm	Stop product infusion, administer oxygen, bronchodilator. If severe, add corticosteroids, antihistamines, and famotidine.
Chest tightness	Administer oxygen, slow or stop product infusion.
Chills	Stop product infusion. If severe, administer meperidine ± hydrocortisone. Consider blood cultures and product cultures.
Dyspnea	Administer oxygen, slow or stop product infusion, review fluid balance, and consider diuretic. If bronchospasm, see above.
Erythema, rash	If hives, treat as an allergic reaction. Monitor and slow product infusion if needed.
Fever	Consider blood cultures and product cultures. Administer acetaminophen and slow product infusion.
Flushing	Slow product infusion and monitor; if worsens, treat as an allergic reaction.
Headache	Slow product infusion, administer analgesia and oxygen.
Hematuria (can occur after the infusion)	Continue postinfusion hydration and monitor urine output.
Hypertension	Slow or stop product infusion. Administer antihypertensive (hydralazine) ± diuretics.
Hypotension	Consider medications to support blood pressure. Slow or stop product infusion, administer fluid bolus.
Nausea/vomiting	Administer antiemetics (eg, lorazepam).
Seizures	As this could be a DMSO reaction, the remaining product should be washed. Stop product infusion, administer anticonvulsants and normal saline.

for Adverse Events criteria) [11], the treatment, and response must be documented in the medical record. Additionally, in the United States, product complaints and adverse events should be reported to the Center for International Blood and Marrow Transplant Research.

FAQ12: What are the considerations for CBU infusion for small children?

Most centers recommend the *dilute and wash* thaw technique for pediatric patients who weigh <20 kg [1] to control the final volume and deliver less than 5 cc/kg/h, if possible. CBUs should be infused through a central venous catheter by gravity or by pump (see also FAQ6).

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REFERENCES

1. Barker JN, Kurtzberg J, Ballen K, et al. Optimal practices in unrelated donor cord blood transplantation for hematologic malignancies. *Biol Blood Marrow Transplant*. 2017;23(6):882–896.
2. FACT-JACIE International Standards for Hematopoietic Cellular Therapy, Product Collection, Processing and Administration, seventh edition. March 2018.
3. NetCord-FACT International Standards for Cord Blood Collection, Banking, and Release for Administration, seventh edition. October 2019.
4. US Food and Drug Administration. Package Insert HEMACORD (Hematopoietic Progenitor Cells, Cord Blood), 2011.
5. Rubinstein P, Dobrila L, Rosenfield RE, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. *Proc Natl Acad Sci U S A*. 1995;92:10119–10122.
6. Dahi PB, Ponce DM, Devlin S, et al. "No wash" albumin-dextran dilution for double-unit cord blood transplantation is safe with high rates of sustained donor engraftment. *Biol Blood Marrow Transplant*. 2014;20(4):490–494.
7. Barker JN, Scaradavou A. Response. the controversy of red cell replete cord blood units. *Blood*. 2011;118(2):480.
8. Ballen K, Logan BR, Chitphakdithai P, et al. Unlicensed umbilical cord blood units provide a safe and effective graft source for a diverse population: a study of 2456 umbilical cord blood recipients. *Biol Blood Marrow Transplant*. 2020.
9. Ponce DM, Lubin M, Gonzales AM, et al. The use of back-up units to enhance the safety of unrelated donor cord blood transplantation. *Biol Blood Marrow Transplant*. 2012;18:648–651.
10. Memorial Sloan Kettering Cancer Center Nursing Policies and Procedures (2019).
11. https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5.