DOI: 10.46765/2675-374X.2022v3n1p158

PERIPHERAL BLOOD MONONUCLEAR CELLS CRYOPRESERVATION AND TRANSPORTATION

Karen de Lima Prata,[1,2] Andrea Tiemi Kondo[3]

1 Centro de Tecidos Biológicos de Minas Gerais – Fundação Hemominas.
2 Agência Transfusional – Hospital das Clínicas da Universidade Federal de Minas Gerais/EBSERH
3 Departamento de Hemoterapia e Terapia Celular – Hospital Israelita Albert Einstein

Correspondence to: karen.prata@hemominas.mg.gov.br

ABSTRACT

Chimeric antigen receptor-T (CAR-T) cells have emerged as a treatment option for patients with hematologic malignancies. Usually, their manufacture has been carried out by specialized and centralized laboratories. A useful strategy, which allows a better logistical organization, is the peripheral blood mononuclear cells (PBMC) cryopreservation and transportation. This article aims to discuss the most relevant points related to the PBMC cryopreservation and transportation process. In addition, it proposes a kind of standard operational protocol (SOP) that can be very helpful for the cell processing labs.

Keywords: Immunotherapy, Adoptive. Leukocytes, Mononuclear. Cryopreservation. Transportation.

OBJECTIVES:

Describe the steps for the peripheral blood mononuclear cells cryopreservation procedure to obtain the maximum recovery of viable cells.

Describe the steps of the cryopreserved product transportation and shipping.

INTRODUCTION

Chimeric antigen receptor-T (CAR-T) cells are an option for patients with advanced hematological neoplasms.[1] Born from academic research, the manufacture of CAR-T cells is currently carried out chiefly by pharmaceutical industries. Because the production of CAR-T cells is complex and expensive, companies centralize patients' cell manufacture in a few specialized laboratories, allowing cell harvesting in different countries. One strategy to reduce viability loss in patients' leukapheresis products is cryopreservation prior to transportation.[1]

Logistics is the main advantage of cryopreservation. It allows the collection of peripheral blood mononuclear cells (PBMC) in patients' best clinical condition, better planning of transportation, and manufacture into CAR-T cells.[1, 2] Therefore, some companies chose to cryopreserve cell products over fresh products to manufacture CAR-T cells.[2] In this sense, several cell processing labs have been organized to be part of this chain of custody, maintaining product quality and traceability at all stages of the process.[3-5]

Few studies address PBMC cryopreservation.[6-8] Most services use protocols similar to peripheral blood hematopoietic stem cells (PBSC) cryopreservation. This recommendation paper will address the most relevant aspects of the PBMC cryopreservation and their preparation for transportation to centralized CAR-T cell manufacturing facilities. For a comprehensive review on cryopreservation, we suggest De Santis *et al.* and Meneghel *et al.* [9, 10]

FACTORS THAT INFLUENCE THE CELL PRESERVATION

Product volume:

The product volume collected is usually estimated by the apheresis equipment, but it must always be measured in the laboratory by weighing the product's bag. Some services convert from grams to milliliters considering a 1:1 ratio. Some prefer to use the monocytes (1.062 g/mL) or lymphocytes (1.07 g/mL) specific density to perform this calculation.[11, 12]

Volume reduction:

Some products require plasma excess removal to adjust the nucleated cells (NC) concentration. The product is centrifuged and the plasma excess extracted into a transfer bag. Each service must validate the centrifugation parameters: gravitational force (g), time, and temperature. These parameters usually vary between 400 and 1000 g, for 10 to 15 minutes, at refrigerated temperature (2 to 8º C), respectively.[13, 14] Some services use the 1:1 ratio for converting grams to milliliters of the plasma amount to be removed. Others prefer to use the specific plasma density (1.026 g/mL) in this calculation.[12]

To minimize the rupture risk during centrifugation, it is crucial to transfer the product from the original collection bag to a transfer bag that can be centrifuged. Each service must define and validate its maximum volume (usually less than 2/3 of the bag's nominal volume).

Nucleated cell concentration

There is no consensus on the ideal nucleated cells concentration (density) for PBMC cryopreservation. Many centers cryopreserve PBMC in vials, with the final concentration varying between 2 and 100 x 10^6 cells/mL.[6, 7, 13] Stroncek *et al.* described PBMC cryopreservation in bags, with concentrations varying from 20 to 300 x 10^6 cells/mL.[7] The main advantages of concentrated products are reducing dimethyl sulfoxide (DMSO) dose (less DMSO toxicity), cost, and storage space utilization[15]. However, too high cell concentration can increase the risk of clumping during the cryopreservation and/or during the thawing and infusion period.[13]

Cryopreservation solution

Cryoprotective agents reduce cell dehydration and mechanical injury induced by the cryopreservation process, maintaining cell viability.[9] The most widespread PBMC cryopreservation solution consists of a protein solution (autologous plasma or human albumin) with 20% DMSO. When combined with an equal volume of cell suspension, it results in a final concentration of 10% DMSO.[6] Reported alternative solutions are 10% DMSO in Plasmalyte®,[16] and 5% DMSO, 6% hydroxyethyl starch (HES) derivatives, 4% human albumin final concentrations.[7] For PBSC cryopreservation, the EBMT (European Society for Blood and Marrow Transplantation) recommends the ACD-A association with the cryopreservation solution at a dose of 0.05 to 0.25 mL per product mL in order to reduce the risk of clumps.[17]

Cryopreservation solutions are hypertonic, and the osmotic stress caused by its introduction into the recipient might induce cell death. Moreover, DMSO is toxic (biochemical toxicity), and DMSO exposure time at room temperature further contributes to cell loss.[18] To reduce the osmotic stress, the cryopreservation solution must be added into the cells' recipient, never the other way around. This process must be refrigerated, systematic, and standardized. Furthermore, it is mandatory to have strict control over the cryopreservation solution addition time and the interval between the completion of it and the initial freezing time.[3] Each service must validate these parameters. Specific data concerning these intervals are lacking, but the ones used in the PBSC cryopreservation can be used as a reference in the validation.[14]

Cryopreservation velocity

The gold standard for PBMC freezing is using equipment programmed to freeze bags at 1º C per minute decay,[3, 6, 18] similar to PBSC.[17] However, passive freezing in mechanical freezers (minus 80º C) is an option.[8] In both cases, the service must validate the process. Physical factors, such as bag configuration, product volume, cell concentration, storage cassette, bag's direct contact with the freezer or on polystyrene layer, may interfere with the cryopreservation product speed and must be considered in the process validation.[9, 19]

To carry out the programmed freezing, one must configure the equipment beforehand, following literature data,[20] service validation, or protocol provided by the CAR-T cell manufacturing service. **Table 1** provides data that can be used as an initial reference for process validation.

When placing the bags in the programmed freezing equipment, it is crucial to observe the placement of the temperature probe. The probe must be in contact with the central region of one among the product bags to be cryopreserved or inside a bag containing cryopreservation solution (periodically changed). The probe must not be placed in the label pocket or in contact with the air.[20]

TABLE 1: PBSC freezing curve example[20]

Step	Rate	Target temperature	Location
1	Wait	0º C	Chamber
2	1º C/minute	- 4º C	Sample
3	25º C/minute	- 55º C	Chamber
4	15º C/minute	- 24º C	Chamber
5	1º C/minute	- 45º C	Chamber
6	2º C/minute	- 80º C	Chamber
7	End		

STORAGE FOR CLINICAL USE

Ideally, PBMC should be stored at temperatures below minus 150º C, in tanks containing liquid or, preferably, steam nitrogen,[3, 7, 18, 21, 22] similar to the EBMT recommendations for PBSC storage.[17] Tanks containing liquid nitrogen (LN_2) seem to be safer in terms of temperature stability.[19] However, additional care is required to avoid cross-contamination between products.[23, 24] For this reason, some industries have demanded the storage of PBMC intended for CAR-T cells in nitrogen steam.

The mandatory criteria to be observed in the product cryopreservation process must be defined following the CAR-T cells manufacture's protocol, together with additional criteria established by each cell processing lab. For example, a) the maximum cell concentration for product overnight storage between the completion of the procurement and the initial of the cryopreservation process; b) the maximum interval between the completion of the procurement and the initial of the cryopreservation process; c) the minimum dose of CD3+ cells to be cryopreserved; d) the maximum concentration of nucleated cells/mL in the final cryopreserved product; e) the cryopreservation solution to be used; f) the freezing method; f) the storage freezer type until transport; g) pre and post-cryopreservation quality control tests and their respective reference values.

TRANSPORTATION AND SHIPPING

The transport of cryopreserved products must be carried out in dry shippers. These containers must comply with local, sender, and receiver regulations.[25]

Dry shippers must be supplied following the manufacturer's guidelines. This process may take longer than a day, as more than one filling cycle may be required according to the type of dry shipper. When properly loaded, they are capable of maintaining the appropriate temperature for transport (below minus 150º C) for 5 to 15 days.[5, 25]

The dry shipper temperature must be monitored continuously throughout the transport. For this purpose, previously validated and calibrated temperature monitors (data loggers) that allow data monitoring printing should be used.[5, 25]

Each service must validate the transport procedure. Dry shippers must be requalified and inspected at regular intervals, at least a visual check, at each procedure.[25]

INDICATION AND CONTRAINDICATIONS

- Patients presented at a multidisciplinary meeting and approved for CAR-T cells treatment, with a medical prescription for PBMC cryopreservation.
- Suitability and eligibility documented duly signed by the responsible medical team.
- No contraindication for this protocol
- Signed informed consent.

MINIMAL REQUIREMENTS

- Medical prescription for the PBMC cryopreservation.
- Initial training of all employees involved in the PBMC cryopreservation and transportation. The annual competence of all staff is required.
- Product cryopreservation forms and freezing

curves approved by the Cell Processing Lab Director and Quality Manager.

- Product release report approved by the Cell Processing Lab Director and Quality Manager.
- In case of exceptional release – report and release approved by Cell Processing Lab Medical Director.
- Cryopreservation and transportation process validated.
- Qualified, calibrated and cleaned equipment's.
- Critical material inspected and approved for use.

PROCEDURE

- Equipment's
- Balance
- Biological safety cabin
- Cryogloves
- Data logger for temperature monitoring
- Dry shipper
- LN_2 source
- Nitrogen tank for storage
- Plasma extractor
- Programmable controlled-rate freezer with a probe
- Refrigerated centrifuge
- Refrigerator
- Sterile connection device
- Tubing sealer

SUPPLIES AND REAGENTS

- Assay sample tubes
- Blood culture medium
- Cryogenic tubes
- Disposable needles
- Cryobags
- Cryopreservation solution with DMSO
- Labels
- Reusable refrigerated ice brick
- Sampling-site couplers
- Sterile syringes
- Storage cassettes
- Transfer bags
- Tube stripper

CRYOPRESERVATION PROCEDURE:

- Programmable controlled-rate freezer (CRF) equipment preparation:

  - Confirm that the CRF equipment is clean.
  - Check the CRF LN_2 source for sufficient LN_2 to perform the entire freezing procedure.
  - Turn on the equipment and verify if it is working correctly.
  - Ensure the dry shipper is loaded without nitrogen residue inside.

- Start filling in the forms to maintain the traceability of the entire process, which includes equipment, supplies, and reagents used, critical calculations, and verification of product identification by two people or one person and one computerized system. Where available, reference values of critical data and formulas for calculations should be included in the forms to minimize the risk of errors.

- Ensure that the ice brick that will be used to maintain the product temperature during the cryopreservation solution addition is in the refrigerator as previously validated.

- Collect samples for pre-processing quality control testing:
  - Properly homogenize the product, as previously systematized.
  - Proceed with collecting the minimum volume necessary to perform tests (relevant cell counts, cell viability, and other tests according to the service routine or manufacturer's protocol), inside the biological safety cabinet (BSC).

- Determine the initial product volume:

  - Properly tare the balance and then weigh the product.
  - Calculate the initial product volume by dividing its weight by its density or use the 1:1 ratio, as previously defined in the institutional or manufacturer's protocol.

- Determine the cell concentration:

  - Check the NC count on the complete cell blood count (CBC). Remember $10^3/\mu L = 10^6/mL$.
  - Calculate the total nucleated cells (TNC) number of the product using the following formula:

TNC = initial product volume (mL) x NC x 10^6/mL.

  - Check the desired NC/mL concentration, as pre-

viously defined in the institutional or manufacturer's protocol.

- Calculate the cell concentrate final volume using the following formula:

$$\text{Cell concentrate volume (mL)} = \frac{\text{total nucleated cells}}{\text{NC concentration/mL}}$$

- Check the maximum product volume of each cryobag.

- Calculate the number of cryobags to be used.

- Prepare the cryopreservation solution:

- The solution volume to be prepared will be the same as the cell concentrate to be cryopreserved.

- Check the previously validated cryopreservation solution.

- Calculate the amount of each reagent, maintaining the ratio of the standard solution to the target volume to be prepared.

- Add the reagents to a previously identified transfer bag using a sampling-site coupler, syringe, and needle. This process must be carried out inside the BSC.

- Place the bag containing the cryopreservation solution in the refrigerator for temperature stabilization as previously defined in the institutional or manufacturer's protocol.

- When relevant, proceed with the excess plasma removal from the product for cell concentration.

- Calculate the plasma volume to be removed using the following formula:

Plasma volume to be removed = initial product volume – cell concentrate volume

- Calculate the plasma bag weight to be removed by multiplying its volume by the plasma density or using the 1:1 ratio, as previously defined in the institutional or manufacturer's protocol.

- Make a sterile connection between the collection bag and a previously identified transfer bag.

- Drain the product to the bag where it will be centrifuged, respecting its maximum volume, as previously defined in the institutional or manufacturer's protocol. If necessary, use more than one transfer bag.

- Centrifuge the product as previously defined in the institutional or manufacturer's protocol.

- Carefully place the product in the plasma extractor. Place the empty bag on the balance (previously positioned next to the extractor) and tare it.

- Remove the predetermined plasma volume with care. Do not lose cells.

- Heat-seal and separate the bags.

- Weigh the product and the plasma bags and register these data.

- Calculate the product volume by dividing its weight by its density or use the 1:1 ratio, as previously defined in the institutional or manufacturer's protocol.

- Carefully homogenize the product.

- Inside the BSC, collect the minimum necessary product to perform the quality control tests, as previously defined in the institutional or manufacturer's protocol.

- Place the product bag into the refrigerator to stabilize the temperature as previously defined in the institutional or manufacturer's protocol.

According to the service routine, calculate the cell recovery after the plasma extraction.

- Freezing

- Identify and double check all labels used to identify bags, segments, cryovials, and storage cassettes.

- Confirm that the CRF equipment is operating, and its internal temperature is stable at 0º C.

- If necessary, resolve all intercurrences with the equipment before proceeding with the addition of the cryopreservation solution.

- Remove the ice brick from the refrigerator and place it on the local where the cryopreservation solution will be added to the cells.

- Remove the product containing the bag and the cryopreservation solution from the refrigerator.

- Make a sterile connection between the cell concentrate and the cryopreservation solution bags.

- Place the bag containing the product over the ice brick and add the cryopreservation solution to the cell concentrate as previously validated by the service. Register the addition of cryopreservation solution start and endpoint times.

- Inside a CBS, proceed with collecting the minimum product necessary to perform the quality control tests (e.g., sterility) and manufacture cryovials as previously defined in the institutional or manufacturer's protocol.

- Proceed with the division of the cell concentrate in the freezing bags.

- Remove air bubbles according to the institutional protocol.
- Seal the product segments and the cryobags.
- Place the cryobags in an overwrap bag to prevent cross-contamination.
- Proceed with positive identification of bags and cassettes.
- Place the cryobags into a labeled storage cassette.
- Place the cryobags and the cryovials into their specific place in CRF.
- Ensure the cryobags are covered by the top plate and that the nitrogen port is free.
- Correctly position the probe. The flat end needs to be against the bag and centered over the central portion of cryobags (not label pocket).
- Run freezing at programmed temperature.
- Register freezing start and endpoint times.
- Calculate the cryopreservation solution addition duration and register it.
- Calculate the time between the completion of the cryopreservation solution addition and the initial freezing time. Register it.
- These data are critical and must comply with the maximum period previously validated.
- Monitor the freezing, following the curve generated by the CRF equipment.

- Storage

  - Identify the location where the bags and cryovials will be stored.
  - Open the CRF door at the completion of freezing.
  - Remove the bags and cryovials using cryogenic gloves. Transport them in a dry shipper to the tank where they will be stored.
  - Carefully store the cells in predetermined locations.
  - Evaluate the cryopreservation curve, verify if it conforms to previously approved models.

- Final check

  - Complete the organization of the forms and forward the folder containing all the procedure data for verification by the responsible professionals

- Post-cryopreservation quality control tests

Perform post-cryopreservation product quality control tests according to the service protocol.

The minimum parameters to be evaluated are the CD3+ viability, the product identification, and the visual check of the bag.

PRODUCT TRANSPORT AND SHIPPING PROCEDURE:

- Proceed with a new verification of all forms generated in the process, including the request to send the product.

- Preparation of the dry transport container:

  - Select a dry shipper that is suitable for use and fill it with antecedence according manufacturer's guidelines.
  - Prepare the labels, including those affixed to the dry shipper cover.
  - Prepare the necessary documentation.
  - Confirm the dry shipper has been correctly loaded and LN2 excess has been removed.
  - Check the bags' and cryovials' location in the tanks.
  - Remove the bags and cryovials from the tanks and carefully place them in the dry shipper using cryogenic gloves.
  - Place polystyrene boards inside the dry shipper to stabilize the products to not move during transport.
  - Place the data logger probe inside the container, wait for the temperature stabilization, and, after that, activate the data logger.
  - Weigh the dry shipper (inside the cover) and register this data.
  - Ask the receiving service to return the transport data (the arrival temperature and the dry shipper's weight at minimum) as soon as they receive the cells.

- Dry shipper return

  - Perform visual check for external damage.
  - Keep monitoring the temperature until it reaches positive parameters.
  - Download the data logger information and print the graph.
  - Verify if the data complies with validation, including time to maintain the proper temperature for transportation.

CRITICAL POINTS AND RISKS:

- Checking of product identification by two people or one person and a computerized system upon receipt of the product, identification of documentation, at each bag exchange, making segments or cryovials, and cassette insert time. Risk: product identification and exchange error.

- Start of cryopreservation processing after 24 hours of collection. Risk: loss of cell viability with compromised quality of CAR-T cells.

- Bag breakage during centrifugation. Risk: product loss with the need for a new collection.

- Cell loss in plasma extraction. Risk: product loss with the need for a new collection.

- Quick addition of cryopreservation solution. Risk: cells heating with consequent loss of viability due to toxicity to DMSO.

- Delay in starting the freezing after cryopreservation solution addition. Risk: loss of cell viability due to DMSO toxicity.

- Liquid nitrogen supply interruption. Risk: interruption of the cryopreservation process with cell viability loss and CAR-T cells compromised quality.

- Inadequate dry shipper filling. Risks: inadvertent product heating, with loss of cell viability and CAR-T cells' quality; skin injury of people involved in transportation due to contact with LN_2; fracture of the product bag, which becomes unsuitable for CAR-T cell manufacture.

STANDARD OF PRACTICE

- Reference values for each critical process step must be established according to the validation of each service and agreement with the CAR-T cells manufacturer's protocol.

- Literature data that can guide these criteria:
  - TNC recovery after plasma extraction greater than 90%[11]
  - Cryopreserved TNC recovery: 92 ± 17%[7]
  - CD3+ cells recovery: 79 ± 19%[7]
  - Total cell viability (flow cytometry, 7AAD): 84 ± 6%[7]

QUALITY INDICATORS

- Cell loss in plasma extraction

- Cryopreserved CD3+ cell viability – test performed on segment or cryovial sample.

- Sterility test negative for aerobic, anaerobic bacteria, and fungi

ACKNOWLEDGMENTS

The authors thank Pedro Henrique de Lima Prata for his review of the manuscript.

CONFLICT OF INTEREST

The authors have disclosed no conflict of interest.

REFERENCES

1. Tyagarajan S, Schmitt D, Acker C, et al. Autologous cryopreserved leukapheresis cellular material for chimeric antigen receptor–T cell manufacture. Cytotherapy. 2019;21(12):1198-205.
2. Tyagarajan S, Spencer T, Smith J. Optimizing CAR-T Cell Manufacturing Processes during Pivotal Clinical Trials. Mol Ther Methods Clin Dev. 2019;16:136-144.
3. Mukherjee S, Reddy O, Panch S, et al. Establishment of a cell processing laboratory to support hematopoietic stem cell transplantation and chimeric antigen receptor (CAR)-T cell therapy. Transfus Apher Sci. 2021;60(1):103066.
4. Lindgren C, Leinbach A, Annis J, et al. Processing laboratory considerations for multi-center cellular therapy clinical trials: a report from the Consortium for Pediatric Cellular Immunotherapy. Cytotherapy. 2021;23(2):157-64.
5. Chen LN, Collins-Johnson N, Sapp N, et al. How do I structure logistic processes in preparation for outsourcing of cellular therapy manufacturing? Transfusion. 2019;59(8):2506-18.
6. Glassman A, Bennett C. Cryopreservation of human lymphocytes: a brief review and evaluation of an automated liquid nitrogen freezer. Transfusion. 1979;19(2):178-81.
7. Stroncek DF, Xing L, Chau Q, et al. Stability of

cryopreserved white blood cells (WBCs) prepared for donor WBC infusions. Transfusion. 2011;51(12):2647-55.

8. Zeng G, Hu Y, Hu X, et al. Cryopreservation of peripheral blood mononuclear cells using uncontrolled rate freezing. Cell and Tissue Banking. 2020;21(4):631-41.

9. Santis GC, Prata KL. Criopreservação de células-progenitoras hematopoéticas. Medicina (Ribeirao Preto Online). 2009;42(1):36.

10. Meneghel J, Kilbride P, Morris GJ. Cryopreservation as a Key Element in the Successful Delivery of Cell-Based Therapies - a review. Front Med (Lausanne). 2020;7:592242

11. McKenna DH. Basic Cellular Therapy Manufacturing Procedures. In: Areman; E, Loper. K, editors. Cellular Therapy: Principles, Methods, and Regulations. 2nd ed. Bethesda: AABB; 2016. p. 361-67.

12. European Committee on Blood Transfusion. Processing, storage and distribution of blood components. Guide to the preparation, use and quality assurance of blood components. 20th ed. Strasbourg: EDQM; 2020. p. 169-94.

13. Martin-Henao GA, Torrico C, Azqueta C, et al. Cryopreservation of hematopoietic progenitor cells from apheresis at high cell concentrations does not impair the hematopoietic recovery after transplantation. Transfusion. 2005;45(12):1917-24.

14. Belisario AR, da Costa Funes AP, Luz JR, et al. Influence of laboratory procedures on postthawing cell viability and hematopoietic engraftment after autologous peripheral blood stem cell transplantation. Transfusion. 2021;61(4):1202-14.

15. Lin DM, Hiskey M, Huang J, et al. Effect of high nucleated cell concentration on product viability and hematopoietic recovery in autologous transplantation. Transfusion. 2020;60(3):575-81.

16. Best A, Hidalgo G, Mitchell K, et al. Issues concerning the large scale cryopreservation of peripheral blood mononuclear cells (PBMC) for immunotherapy trials. Cryobiology. 2007;54(3):294-7.

17. Wuchter P. Processing, Cryopreserving and Controlling the Quality of HSCs. In: Carreras; E, Dufour; C, Mohty; M, Kröger N, editors. The EBMT Handbook: Hematopoietic Stem Cell Transplantation and Cellular Therapies. Cham: EBMT; 2019. p. 127-30.

18. Li R, Johnson R, Yu G, et al. Preservation of cell-based immunotherapies for clinical trials. Cytotherapy. 2019;21(9):943-57.

19. Rowley SD. Cryopreservation of Hematopoietic Cells. In: Forman; SJ, Negrin; RS, Antin; JH, Appelbaum FR, editors. Thomas' Hematopoietic Cell Transplantation: stem cell transplantation. 5th ed. Chichester: Wiley-Blackwell; 2016. p. 631-44.

20. Hubel A, Mathew AJ. Cryopreservation of Cellular Therapy Products. In: Areman; E, Loper. K, editors. Cellular Therapy: Principles, Methods, and Regulations. 2nd ed. Bethesda: AABB; 2016. p. 444-52.

21. Panch SR, Srivastava SK, Elavia N, et al. Effect of Cryopreservation on Autologous Chimeric Antigen Receptor T Cell Characteristics. Molecular Therapy. 2019;27(7):1275-85.

22. Reddy OL, Stroncek DF, Panch SR. Improving CAR T cell therapy by optimizing critical quality attributes. Semin Hematol. 2020;57(2):33-8.

23. Tedder RS, Zuckerman MA, Goldstone AH, et al. Hepatitis B transmission from contaminated cryopreservation tank. Lancet. 1995;346(8968):137-40.

24. Leemhuis T, Padley D, Keever-Taylor C, et al. Essential requirements for setting up a stem cell processing laboratory. Bone Marrow Transplantation. 2014;49(8):1098-105.

25. Regan D, Yost A. Transportation and Shipping of Cellular Therapy Products In: Areman; E, Loper K, editors. Cellular Therapy: Principles, Methods, and Regulations. 2nd ed. Bethesda: AABB; 2016. p. 483-94.

ANNEX

Receipt form of incoming cells

Patient data	Name: Date of birth: Age: Weight:
Collection data	Procurement facility name: Donation identification number: Product description code and division code: Product name and attributes Blood cell separator model & software version: Date and time of procurement: Anticoagulant used: Product anticoagulant volume
Transportation data	Date: Date and time of departure and receipt: Duration: Departure and receipt temperature: Acceptable temperature during transportation? ☐ yes ☐ no Observations: Signature and stamp:
Receipt data	Receipt data and time: Product inspection upon receipt: Usual product appearance: ☐ yes ☐ no Visible evidence of contamination: ☐ yes ☐ no Container integrity? ☐ yes ☐ no Appropriate labeling? ☐ yes ☐ no Product classification ☐ acceptance ☐ rejection ☐ quarantine Disposition ☐ cryopreservation ☐ discard Responsible person (signature and stamp):
Temporary storage	Equipment: Date and time (start/endpoint): Duration: Observations

Cryopreservation registry

	Type	Identification code	Calibration[&#]	Preventive maintenance[#]
Equipment	Balance			
	Biological safety cabin			
	Centrifuge			
	Dry shipper			
	Plasma extractor			
	Sterile connection device			
	Programmable controlled-rate freezer			
	Refrigerator			
	Tubing sealer			
	Nitrogen tank			
	LN_2 source			

& if pertinent; # validity

	Type	Supplier[$]	Lot[$]	Validity date[$]	Numbered used[$]
Supplies and Reagents	Assay sample tubes				
	Blood culture medium				
	Cryogenic tubes				
	Disposable needles				
	Cryobags				
	Cryopreservation solution				
	Labels				
	Ice brick refrigerated				
	Sampling-site couplers				
	Sterile syringes				
	Storage cassettes				
	Transfer bags				

$ Mandatory registration if considered critical

Patient data	Name: Date of birth: Age: Weight:
Product data	Donation identification number: Product description code and division code: Product name and attributes: Procurement data and time Product anticoagulant volume
Temporary storage	Procedure start at: Duration between the completion of the procurement and cryopreservation process initial: _____ h Responsible person (signature and stamp):
Pre-procedure product inspection	Usual product appearance: ☐ yes ☐ no Visible evidence of contamination: ☐ yes ☐ no Clumps? ☐ yes ☐ no Container integrity? ☐ yes ☐ no Appropriate labeling? ☐ yes ☐ no Responsible person (signature and stamp):
Sample collection	Volume: _____ mL
Volume determination	Initial weight _____ g Calculated volume: _____ mL Responsible person (signature and stamp):
Cell concentration determination	TNC = _____ Cell concentrate final volume: _____ mL Number of bags to be cryopreserved: _____ Responsible person (signature and stamp):
Cryopreservation solution preparation	Volume to be prepared: _____ mL Reagents proportion: - DMSO 100%: _____ mL - Plasma or albumin: _____ mL - _____ mL Preparation time: _____: _____ Lot: _____ Bag insertion time into refrigerator: _____: _____ Bag removal time from refrigerator: _____: _____ Responsible person (signature and stamp): Responsible person (signature and stamp) for double check:
Plasma removal	Volume to be removed: _____ mL Calculated weight to be removed: _____ g Centrifugation time: Initial: _____: _____ end: _____: _____ Plasma extraction: Initial: _____: _____ end: _____: _____ Bag weight: _____ g Calculated plasma volume: _____ mL Cell concentrate bag weigh: _____ g Calculated cell concentrate volume: _____ mL Sample tests volume: _____ mL Cell recuperation: _____ % Responsible person (signature and stamp): Responsible person (signature and stamp) for double check:

Freezing	Cell concentrate and cryopreservation solution bags removal time from refrigerator: ____: ____ Cryopreservation solution addition to the cell concentrate times: Initial: _____: _____ end: _____: _____ Freezing times: Initial: _____: _____ end: _____: _____ Time between the completion of the cryopreservation solution addition and the initial freezing time: _____ min. Responsible person (signature and stamp): Responsible person (signature and stamp) for double check:
Storage	Time: _____: _____ Local: _____ Responsible person (signature and stamp):

Product transportation and shipping

Patient data	Name: Date of birth: Age: Signed request for sending? ☐ yes ☐ no Responsible person for checking (signature and stamp):
Cryopreservation data	Date: Procedure approved? ☐ yes ☐ no Cryopreservation curve approved? ☐ yes ☐ no Responsible person for checking (signature and stamp):
Product visual check	Cracks or bubbles? ☐ yes ☐ no Usual appearance? ☐ yes ☐ no Appropriate labeling? ☐ yes ☐ no Responsible person (signature and stamp):
Post-cryopreservation quality control	Cell viability: _____ %
Sending preparation	Select dry shipper identification: _____ Filling date and time: ___/___/___ ___: ___ Responsible person (signature and stamp): Labels preparation: ___/___/___ Responsible person (signature and stamp): Forms preparation: ___/___/___ Responsible person (signature and stamp):
Dry shipper preparation for sending	LN_2 excess removed (date and time): ___/___/___ ___: ___: Double identification check (bags and cryovials): Dry shipper weight: _____ g Temperature: _____ ºC Responsible person 1 (signature and stamp): Responsible person 2 (signature and stamp):
Dry shipper return	Visual check usual? ☐ yes ☐ no Temperature: _____ ºC Responsible person (signature and stamp): Transportation temperature graph: Responsible person (signature and stamp) for printing: Responsible person (signature and stamp) for its evaluation: _____