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History of the Brazilian Cellular Therapy Processing Group and initial survey on cellular therapy processing, quality control, and release criteria for cryopreserved peripheral blood stem cells in autologous transplantation

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ABSTRACT

There is considerable heterogeneity in peripheral blood stem cell (PBSC) cryopreservation protocols. In 2021, the Brazilian Cellular Therapy Processing Group was created to meet the need for technical specialists to exchange experiences, improve processes, and pursue international accreditation. This survey was developed to better understand national PBSC cryopreservation practices and to support the group's technical discussions. An online questionnaire was created using Google Forms and disseminated. A total of 61 cell processing centers (CPC) responded to the survey. Most CPC showed interest in voluntary technical accreditation (n = 45; 73.8%), served one or two bone marrow transplantation units (n = 46; 75.4%) and processed bone marrow, PBSC and lymphocytes (n = 21; 34.4%). PBSCs were mainly cryopreserved within 24 hours of collection (n = 45; 73.8%), using a cryopreservation solution containing 5% DMSO and hydroxyethyl starch (n = 35; 67.3%). Most institutions froze and stored the cells at -80°C until therapeutic use (n = 42; 80.8%). The maximum concentration of nucleated cells after cryopreservation varied widely across CPC. CD34+ cell quantification was most performed using the International Society for Hematotherapy and Graft Engineering dual-platform method (n = 25; 48.1%). The most frequently used post-cryopreservation quality control test was cell viability assessment using trypan blue exclusion (n = 34; 66.7%). This survey revealed considerable heterogeneity in PBSC cryopreservation practices among Brazilian CPC at the time the Cellular Therapy Processing Group was established. Since then, the group's meetings have promoted improvements in the quality, safety, and efficacy of cell therapy products, as well as the gradual standardization of practices among its members.

Keywords: Surveys and Questionnaires, Peripheral Blood Stem Cells, Cryopreservation, Transplantation, Autologous.

INTRODUCTION

Autologous hematopoietic stem cell transplantation has been successfully used to treat a variety of diseases[1]. These procedures are typically performed using peripheral blood stem cells (PBSCs) collected via apheresis. The collected cells are cryopreserved and stored for future use, with variable storage times depending on the treatment phase and the clinical progress of the patient.

In Brazil, the first bone marrow transplantation (BMT) unit was inaugurated in Curitiba, Paraná, where the first transplantation using hematopoietic stem cells was performed in 1979 by Ricardo Pasquini and his team. The second and third BMT units were established in Rio de Janeiro (1983) and São Paulo (1988), respectively[2]. To support the needs of these BMT units, cellular therapy processing laboratories, known in Brazil as cell processing centers (CPCs), were created. Initially, there was one CPC per BMT unit, and they both gradually increased in number and expanded nationwide.

Autologous hematopoietic stem cell transplantation is performed in public, private, or philanthropic hospitals. Philanthropic hospitals operate in partnership with the Brazilian Ministry of Health and receive tax incentives in return. In public hospitals, transplant procedures are typically reimbursed through the Brazilian Public Health System (Sistema Único de Saúde—SUS). In private or philanthropic hospitals, reimbursement may come from SUS, private health insurance, or directly from the patient.

The complexity of CPC operations varies according to the types of transplants performed at the associated BMT units. All CPCs must comply with Brazilian regulations, which are established by documents issued by the Ministry of Health[3,4] and the Brazilian Health Regulatory Agency (Agência Nacional de Vigilância Sanitária—ANVISA)[5]. These regulations define the minimum technical and sanitary requirements for good laboratory practices for therapeutic use, although they are generally less stringent than international accreditation standards. Currently, two international agencies provide technical guidelines for PBSC processing and regularly publish accreditation standards: the Foundation for the Accreditation of Cellular Therapy (FACT), the Joint Accreditation Committee of the International Society for Cellular Therapy and the European Society for Blood and Marrow Transplantation (JACIE); and the Association for the Advancement of Blood and Biotherapies (AABB), formerly known as the American Association of Blood Banks.

In 2012, during the annual congress of the Brazilian Society of Cellular Therapy and Bone Marrow Transplantation (Sociedade Brasileira de Terapia Celular e Transplante de Medula Óssea—SBTMO), the first workshop on laboratory practices, including cellular therapy processing, was held. This full-day event featured speakers from both FACT and AABB, who addressed technical aspects of their respective accreditation standards. In the previous year, an agreement[6,7] was established between AABB and the Brazilian Association of Hematology, Hemotherapy and Cellular Therapy (Associação Brasileira de Hematologia, Hemoterapia e Terapia Celular—ABHH) to improve the quality of Brazilian blood banks. In 2013, this partnership was extended to include cell therapy services[7].

In 2014, the second laboratory practices workshop was held, in collaboration with AABB, FACT, and the Latin American Bone Marrow Transplantation Group. In 2018, a memorandum of understanding was signed between SBTMO and FACT to enhance the quality of hematopoietic progenitor cell transplantation in Brazil[8]. In 2020, SBTMO published its third consensus standards, which included a chapter offering guideline suggestions to help CPCs standardize practices across services[9]. Since then, annual workshops on laboratory practices have been held during the SBTMO congress.

The main barriers to international accreditation in Brazil are the high costs associated with obtaining and maintaining accreditation and certain technical requirements that are not mandatory under national regulations yet[10,11]. Although the standards have been translated into Portuguese, limited guidance exists on how to comply with these accreditation requirements in the Brazilian context.

The Brazilian Cellular Therapy Processing Group was formed in response to the need for technical experts to share experiences, improve processes, and pursue international accreditation. This survey was designed to describe PBSC cryopreservation practices across Brazil in 2021 and 2022, when the group officially began its activities. All CPCs that responded to the survey were registered participants of the Brazilian Cellular Therapy Processing Group.

METHODS

The survey titled "Cellular therapy processing, quality control, and release criteria of cryopreserved peripheral blood stem cells" was developed by the coordinators of the Brazilian Cellular Therapy Processing Group in April 2021. The online questionnaire was created using Google Forms, a web-based platform, and consisted of two sections[12]. The first section included 10 general questions about the CPCs, used for group registration purposes. The second section comprised 20 technical questions regarding PBSC cryopreservation procedures and product quality control. The survey was reviewed and, after minor adjustments, approved by the board of directors of the SBTMO in May 2021. Final formatting and dissemination were carried out by SBTMO's communication team.

Initially, participants were selected by convenience sampling. The survey link was shared via WhatsApp groups composed of transplant physicians and members of BrasilCord, the Brazilian public umbilical cord blood (UCB) bank network. Additionally, e-mails with the survey link were sent to a list of private UCB banks and CPCs. To increase the response rate, lab directors were also contacted individually via WhatsApp. The survey remained open for three months, from June to September 2021, and the Brazilian Cellular Therapy Processing Group officially began its activities in August 2021.

At the end of 2021, ANVISA published an interactive panel listing 67 active CPCs in Brazil. A new search was conducted to identify CPCs that had not participated in the initial survey, especially those involved in autologous PBSC processing. The survey was reopened from May to November 2022 to allow for the registration and data collection of these previously missing CPCs. Additionally, a new CPC not listed in the ANVISA panel requested to join the group and submitted its data. Each CPC was contacted at least twice (via e-mail and/or WhatsApp). Only the principal investigator had access to the CPC identification data, which were used solely to form the working group. Data analysis was limited to one response per institution per question. Descriptive statistical analysis was performed using Microsoft Excel for Microsoft 365. Results were reported as absolute numbers and frequencies (%) for each response item.

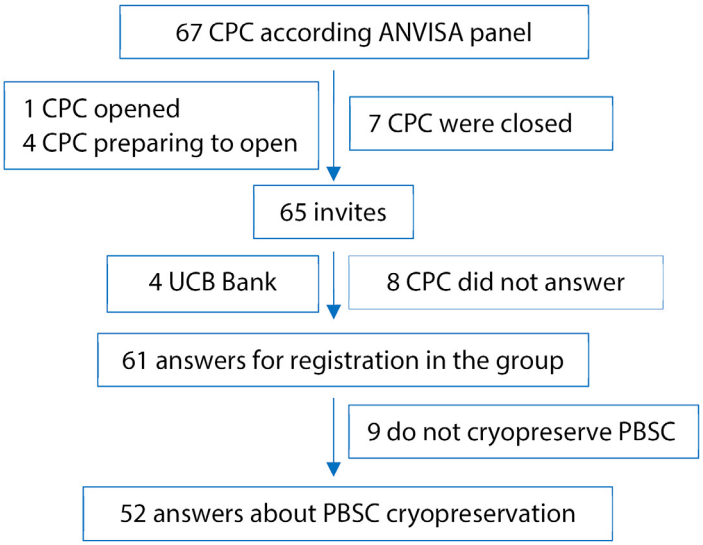
RESULTS

Demographics

All 67 CPCs listed in the ANVISA interactive panel (as of May 2022) were contacted. Their geographic distribution is presented in Fig. 1[13]. Between them, seven (10.4%) reported having ceased operations. Among the 60 remaining centers, 52 responded to the survey, yielding a response rate of 86.7%. One center was undergoing restructuring and was not processing PBSCs at the time of data collection. Additionally, four UCB banks and four CPCs in the process of implementation expressed interest in joining the Brazilian Cellular Therapy Processing Group and completed the first section of the survey for registration purposes (Fig. 2).

Table 1 summarizes the characteristics of participating CPCs. The majority were in the state of São Paulo (n = 21; 34.4%), followed by Minas Gerais (n = 7; 11.5%), Rio de Janeiro (n = 6; 9.8%), and Paraná (n = 6; 9.8%). The four CPCs in initial implementation phases were in the Midwest (n = 3; Campo Grande, Goiânia, and Brasília) and North (n = 1; Manaus) regions. Although only 19 CPCs (31%) were public institutions, 42 (68.8%) reported some form of reimbursement by SUS, while 16 (26.2%) operated exclusively with private or

Figure 1. Brazilian cell processing center distribution according to the Brazilian Health Regulatory Agency (Agência Nacional de Vigilância Sanitária—ANVISA) in 2022. The circle size stands for the cell processing centers number (17, 5, 3, 2 or 1) in each region[1].



CPC: cell processing center; ANVISA: Brazilian Health Regulatory Agency (Agência Nacional de Vigilância Sanitária); UCB: umbilical cord blood; PBSC: peripheral blood stem cells.

Figure 2. Brazilian Cellular Therapy Processing Group flow diagram of the search strategy and the centers inclusion.

Table 1. Characteristics of the 61 Brazilian cell processing centers that responded to the survey.

	Number	%
Localization		
Southeast Region	35	57.4
South Region	11	18.0
Midwest Region	8	13.1
Northeast Region	5	8.2
North Region	2	3.3
Type of institution		
Private	28	45.9
Public	19	31.1
Philanthropic	13	21.3
Military	1	1.6
Reimbursement type		
Public, insurance and/or private	26	42.6
Insurance and/or private	19	31.1
Public	16	26.2
Interest in accreditation in cell therapy?		
Yes	45	73.8
No	16	26.2
Which one? (N = 45)		
AABB	17	37.8
FACT/JACIE	14	31.1
Both	14	31.1
Number of BMT services directly served by the CPC		
1 or 2	46	75.4
3 or 4	7	11.5
More than 5	8	13.1
Type of cells		
BM, PBSC and lymphocytes	21	34.4
PBSC ± lymphocytes	14	23.0
UCB, BM, PBSC ± lymphocytes	13	21.3
BM and PBSC	4	6.6
UCB	4	6.6
Not working	5	8.2

AABB: Association for the Advancement of Blood & Biotherapies; FACT: Foundation for the Accreditation of Cellular Therapy; JACIE: Joint Accreditation Committee ISCT-Europe & EBMT; BMT: bone marrow transplantation; CPC: cell processing center; BM: bone marrow; PBSC: peripheral blood stem cell; UCB: umbilical cord blood.

insurance funding. Most centers (73.8%) expressed interest in voluntary technical accreditation, such as from AABB or FACT-JACIE. The majority served one or two BMT units (n = 46; 75.4%) and processed bone marrow, PBSCs, and lymphocytes (n = 21; 36.1%). Regarding the types of cells processed, most CPCs (n = 52; 85.2%) worked with PBSCs. Among them, 38 (73.1%) also processed bone marrow, 35 (67.3%) lymphocytes, and 12 (23.1%) UCB. CPCs that supported three or more BMT units (n = 15; 28.8%) were classified as regional centers. Of these, four were in the Northeast region, representing 80% of the CPCs in that region. The BrasilCord network comprises 13 public UCB banks, of which 11 (84.6%) responded to the survey. Among them, 10 (90.9%) also processed PBSCs and bone marrow, nine (81.8%) processed lymphocytes, and four (36.4%) were considered regional centers.

PBSC cryopreservation practices

The PBSC storage, processing, and cryopreservation practices are summarized in Table 2. Most CPCs (n = 39; 75%) cryopreserved PBSCs within 24 hours of collection and did not perform overnight storage manipulation (n = 35; 67.3%). The most common cryopreservation solution used was a combination of 5% dimethyl sulfoxide (DMSO), hydroxyethyl starch (HES), and a human protein source (albumin or plasma), reported by 67.3% of centers. Among centers using HES, two distinct approaches were identified: 19 CPCs (43.2%) used manipulated HES with molecular weights ranging from 130,000 to 200,000 Da and final concentrations between 5–6%, while others used commercial solutions such as Voluven (130,000 Da) or Plasmin (450,000 Da) at concentrations of 1–2%.

Table 2. Evaluation of the peripheral blood stem cell cryopreservation process.

	Number	%
When is it performed?		
Usually in the same day of the collection	7	13.5
Until 24 hours of the collection	39	75.0
Between 24 and 48 hours of the collection	6	11.5
Do you manipulate the cells for overnight storage?		
No	35	67.3
Yes, to achieve a nucleated cell concentration less than 2 x10^8/mL	4	7.7
Yes, to achieve a nucleated cell concentration between 2–4 x10^8/mL	5	9.6
Yes, to achieve a nucleated cell concentration less than 5 x10^8/mL	6	11.5
Other	2	3.8
Type of cryopreservation solution		
10% DMSO + human albumin or plasma	5	9.6
10% DMSO + human albumin or plasma + HES	9	17.3
5% DMSO + human albumin or plasma + HES	35	67.3
Other	3	5.8
Which HES? (N = 40)		
Voluven® (MW 130,000 Daltons)	12	27.3
Plasmin® (MW 450,000 Daltons)	13	29.5
Manipulated (MW 130–200,000 Daltons)	19	43.2
Which is the HES final concentration?		
Between 1 and 2%	20	45.5
Between 2 and 5%	5	11.4
More than 5%	19	43.2
Which is the maximum nucleated cell final concentration?		
Not measured	7	13.5
Until 2,5 x10^8/mL	7	13.5
Until 3 x10^8/mL	16	30.8
Until 4 x10^8/mL	7	13.5
Until 5 x10^8/mL	12	23.1
Other	3	5.8
Is the final bag volume fixed?		
Yes	13	25.0
No	39	75.0
Which method is used for freezing?		
Controlled rated	7	13.5
Mechanical freezer	42	80.8
Both	3	5.8
Where are the cells stored?		
Mechanical freezer (-86°C)	42	80.8
Mechanical freezer (-150°C)	2	3.8
Liquid nitrogen or vapor phase tank	6	11.5
Mechanical freezer (-86°C) or liquid nitrogen tank	2	3.8

HES: hydroxyethyl starch; MW: molecular weight; PBSC: peripheral blood stem cell.

The maximum nucleated cell concentration after cryopreservation was highly variable, ranging from ≤ 1.5×10^8/mL to ≤ 5×10^8/mL. Some CPCs did not measure final nucleated cell concentration. Most centers (75%) did not define a fixed cryobag volume and cryopreserved products using a -80°C mechanical freezer (n = 42; 80.8%). Only 10 CPCs (19.2%) used controlled rate freezing equipment, but four of them still stored grafts in mechanical freezers. Nitrogen storage was used by only eight centers (15.3%), with five storing in the liquid phase and three in the vapor phase. Among these, four were part of the BrasilCord network, four were regional CPCs, and six used controlled rate freezing.

PBSC quality control practices

Quality control procedures are detailed in Table 3. CD34+ cell quantification was performed using the International Society for Hematotherapy and Graft Engineering (ISHAGE) protocol in 46 CPCs (88.5%). However, six centers (11.5%) outsourced this analysis and were unaware of the method used. Among those

Table 3. Evaluation of the peripheral blood stem cell cryopreservation quality control.

	Number	%
What assays do you perform to quantify the CD34+ cells?		
ISHAGE double platform	25	48.1
ISHAGE single platform	21	40.4
External lab	6	11.5
What assays do you perform to quantify the cell viability before the cryopreservation?		
Flow cytometry-based permeability marker	45	86.5
Trypan blue dye exclusion	6	11.5
External lab	1	1.9
How many sample aliquots do you freeze together with the bags?		
> 2	26	50.0
2	19	36.5
1	6	11.5
0	1	1.9
Where are these samples stored? (N = 51)		
Cryovial	31	60.8
Bag segment	18	35.3
Both or other	2	3.9
Are these samples routine stored for what purpose? (N = 51)		
A) Quality control close to the therapeutic use	34	66.7
B) Quality control after short-term storage	6	11.8
Not routine used	6	11.8
Both (A + B)	2	3.9
Other	3	5.9
What assays do your routine perform to evaluate the product quality after the cryopreservation?		
A) Trypan blue dye exclusion	25	49.0
B) Flow cytometry-based permeability marker	20	39.2
A and B	2	3.9
A or B + Stem cell culture assays	4	7.8
None	1	2.0
If necessary, can you perform a stem cell culture assay?		
No	37	71.2
Yes	15	28.8
Do you use post-cryopreservation quality control tests as a product release criterion?		
Yes	35	67.3
No	17	32.7
Which bottles for microbiological culture are used in the routine of the service?		
Standard aerobic and/or pediatric	41	78.8
Standard aerobic and/or pediatric + anaerobic	8	15.4
Pediatric or standard aerobic + standard anaerobic + filamentous fungi	2	3.8
Pediatric or standard aerobic + filamentous fungi	1	1.9

ISHAGE: International Society for Hematotherapy and Graft Engineering.

using ISHAGE single-platform methodology, 17/21 (81%) applied a commercial kit. Pre-cryopreservation cell viability was predominantly assessed using flow cytometry with 7-aminoactinomycin D (7-AAD) staining (n = 44; 84.6%). Most CPCs (n = 45; 86.5%) cryopreserved at least two aliquots under the same conditions as the main graft, primarily in cryovials (n = 31; 60.8%) for subsequent quality control testing. The most frequently used post-cryopreservation test was cell viability (n = 51; 98.0%), while only four CPCs (7.7%) routinely performed colony-forming unit (CFU) assays. Nonetheless, 18 CPCs (34.6%) reported the infrastructure and expertise to perform CFU testing if needed. Among these, eight were part of the BrasilCord, nine were regional centers, 13 were located in the Southeast region, and 10 were public or philanthropic institutions. A total of 35 CPCs (67.3%) reported using post-cryopreservation quality control data as a criterion for product release. Concerning microbiological testing, only three CPCs (5.8%) routinely used specific culture bottles for filamentous fungi, and eight (15.4%) used bottles for anaerobic organisms. The vast majority (n = 41; 78.8%) used only pediatric and/or standard aerobic culture bottles.

Transportation and cell washing practices

Questions regarding internal and external transportation of cellular therapy products and washing procedures were also included. Internal transportation methods were reported by 38 CPCs (73%). Most used dry ice (n = 25; 65.8%), followed by vapor-phase nitrogen shippers (n = 7; 18.4%), ice packs stored at -80°C (n = 5; 13.2%), and conventional coolers (n = 2; 5.2%). For external transportation (n = 24), dry ice remained the most common method (n = 20; 83.3%), followed by vapor-phase shippers (n = 7; 29.2%) and, in one case, liquid nitrogen (4.2%). Some CPCs reported using multiple transportation methods internally (3/38) or externally (4/24).

PBSC washing before infusion was not a routine practice for most centers (n = 40; 76.9%). Among the CPCs that performed washing (n = 12), the main indications were DMSO dose > 1 g/kg recipient weight (n = 7; 58.3%), renal impairment (n = 6; 50%), frozen red blood cell volume > 0.5 mL/kg (n = 4; 33.3%), pediatric recipients (n = 3; 25%), and other clinical conditions such as hepatic or cardiac dysfunction, aged UCB units, or medical request (n = 5; 41.7%).

DISCUSSION

This national survey was developed to assess the practices of Brazilian CPCs regarding PBSC cryopreservation in 2021 and 2022. The results provided a detailed overview of Brazilian practices and highlighted significant heterogeneity in cryopreservation protocols. To our knowledge, this is the first report of a laboratory-focused survey on PBSC cryopreservation practices in Brazil.

Historically, there has been a concentration of BMT units and CPCs in the Southeast and South regions, particularly in Curitiba, Rio de Janeiro, and São Paulo, the cities where the first BMT units were established. Each early BMT center operated with its own processing laboratory, shaping the original organizational structure of cellular therapy services in Brazil. In recent years, several efforts have been made to expand access to autologous BMT across other regions, especially in the North and Midwest, where four CPCs were in the process of implementation during the survey period. In the Northeast region, 80% of active CPCs functioned as regional centers, processing cells for three or more BMT units. This reflects a shift toward resource optimization, in which a single, more complex laboratory supports multiple clinical services.

In Brazil, SUS plays a fundamental role in ensuring access to transplant services, including the reimbursement of procedures. Most CPCs reported SUS-related reimbursement, which underscores the system's capacity and its contribution to expanding patient access to cellular therapy.

Although seven of the 13 UCB banks in the BrasilCord network (one of which did not participate in this survey) had achieved AABB accreditation, only three Brazilian CPCs had international accreditation at the time of the study, two for AABB/ABHH (which did not respond to the survey)[14,15] and one for FACT[16]. The successful accreditation of the BrasilCord UCB banks was supported by a government-led project that provided

dedicated funding for the accreditation process, including consultancy and the translation of requirements. These banks, often associated with blood centers, had a more robust structure, and 28.5% also functioned as regional labs.

Several factors help explain the strong interest in AABB accreditation in Brazil: the possibility of conducting audits in Portuguese with local auditors trained under AABB supervision, the acceptance of documentation in Portuguese, and lower costs compared to FACT accreditation. On the contrary, FACT was more often pursued by private or philanthropic CPCs linked to one or two BMT units. These dynamics suggest that accreditation decisions in Brazil are influenced by institutional structure, resource availability, and strategic priorities.

While CPCs have traditionally been directly affiliated with BMT units, the BrasilCord network encouraged the establishment of laboratories integrated with the national blood system. These centers were designed to process PBSCs, bone marrow, and UCB, often acting as regional hubs. Private institutions have also contributed to this decentralization by establishing CPCs that serve multiple BMT units. Consequently, there has been a noticeable increase in the number of laboratories supporting three or more centers. The variety of cell types processed by each CPC reflects both regionalization and the technical complexity of their operations. Laboratories handling multiple cell sources typically require more comprehensive infrastructure and equipment, which also enables more extensive quality control procedures.

Although best practices recommend immediate cryopreservation after PBSC collection[17], overnight refrigerated storage before freezing remains common in Brazil and Europe[1]. This logistical strategy facilitates workload management, allowing staff to operate during regular hours and in better conditions. However, it requires careful monitoring of nucleated cell concentrations, as elevated counts, particularly in products stored beyond 24 hours, have been associated with graft failure. For example, one product processed 16 hours post-collection showed a nucleated cell count of 903×10^3/μL and a CD34+ viability of only 68% (KLP, personal communication). Recommendations suggest limiting nucleated cell concentration to $\leq 200 \times 10^3$/μL when storage exceeds 24 hours[1,17,18]. Validation of in-house storage protocols and the implementation of additional viability assessments beyond basic testing are essential, as some cells may appear viable but are functionally compromised[17].

Cryopreservation protocols in Brazil vary widely[18], as in other regions. The European Bone Marrow Transplantation (EBMT) recommends a maximum nucleated cell concentration of $\leq 400 \times 10^3$/μL, although some Brazilian CPCs have validated protocols with thresholds of $\leq 500 \times 10^3$/μL[19] or $\leq 600 \times 10^3$/μL[20]. Higher concentrations reduce the number of cryopreservation bags and associated costs, including those linked to DMSO and HES toxicity.

Most Brazilian CPCs used a cryopreservation solution composed of 5% DMSO plus HES, with freezing and storage in -80°C mechanical freezers (non-programmable or passive freezing). In contrast, European centers predominantly use 10% DMSO[1,17,18], controlled rate freezing, and nitrogen storage[1]. Despite the European preference, HES is restricted in Europe due to regulatory concerns. In Brazil, only commercial HES solutions with 6% initial concentration are available, necessitating dilution or manipulation when different final concentrations are required.

Notably, most CPCs did not define a fixed cryobag volume, although this parameter, along with cell concentration, directly affects the freezing rate[21] and must be standardized during cryopreservation protocol validation. Controlled rate freezing (1–2°C per minute) and nitrogen vapor storage at ≤ -140°C are considered gold-standard practices[17,18], but their high costs limit feasibility in Brazil. Uncontrolled-rate freezing offers a lower-cost alternative, with proven safety and efficacy, especially when long-term storage does not exceed five years[17]. However, care must be taken to avoid transient warming events, which can jeopardize cell viability.

Brazilian regulations[5] mandate that PBSC quality control be performed both before cryopreservation and, ideally, prior to clinical use[18]. Most CPCs followed the ISHAGE protocol[22,23] for CD34+ quantification and viability testing by flow cytometry. The single-platform ISHAGE method[17] is considered more standardized[23] but it is more expensive than the double-platform version, explaining its lower adoption.

While Brazilian regulations[5] require at least two reference samples per product, the EBMT recommends three[18]. Most CPCs reported using these samples for quality control testing close to clinical application and using the results as release criteria. Trypan blue exclusion and flow cytometry were the most used viability tests. We support the EBMT[18] recommendation to conduct post-thaw quality control on all products and to repeat testing prior to clinical use depending on storage duration. In some cases, new collection and cryopreservation may be necessary to ensure transplant efficacy.

The low use of CFU assays among CPCs is not surprising, as only a few centers worldwide routinely perform this functional test[1,17]. CFU assays evaluate the *in-vitro* proliferative capacity of hematopoietic stem cells[17] and are primarily used for UCB products. Their limited adoption is due to cost, technical demands, and time constraints. In Brazil, CFU assays are more commonly performed by CPCs linked to the BrasilCord network, in which training and infrastructure are available.

Microbiological testing of cryopreserved PBSCs, including cultures for aerobic, anaerobic, and fungal organisms, is required by national[5] and international regulations[24]. In 2021 and 2022, only two commercial brands of culture bottles were available in Brazil, with only one offering specific bottles for fungal detection. As a result, only three of 52 CPCs routinely screened for filamentous fungi, while most used standard aerobic or pediatric bottles, which also detect *Candida* species. Although PBSC contamination has been infrequently associated with patient infections[24-26], current testing protocols warrant review. There is an urgent need for improved methodologies and clearer guidelines for microbial safety in cellular therapies.

Regarding transportation, Brazilian regulations[5] allow PBSCs stored at -80°C to be transported on dry ice, whereas products stored in liquid or vapor nitrogen require specialized shippers. Given Brazil's geographic diversity, transportation practices vary considerably. While some CPCs are located adjacent to BMT units, others are hundreds of kilometers away. This variability justifies the diversity of transportation methods reported in the survey.

None of the CPCs routinely removed DMSO from PBSC products prior to infusion. A minority (12/52; 23%) reported washing cells under specific clinical conditions, a frequency comparable to European centers[1].

This study represents the most comprehensive national overview of CPC practices for PBSC cryopreservation in Brazil to date. Its dual purpose was to establish a national registry of participating CPCs and to document current practices, providing the foundation for technical dialogue and quality improvement.

The Brazilian Cellular Therapy Processing Group began hosting monthly one-hour virtual meetings in August 2021 (except January) and in-person sessions during the annual SBTMO congress. Discussions during the first year focused on the technical and regulatory challenges faced by CPCs. In the following years, meetings explored the results of this survey and their scientific context. Attendance averaged 60–80 participants online and over 100 at in-person events. In accordance with SBTMO governance, group leadership changed after three years to ensure transparency and continued development.

Feedback has been overwhelmingly positive. Participants consistently report that the group's discussions have improved their technical knowledge and laboratory practices. When the initiative began in 2021, the objective was to enhance and standardize cell processing in Brazil and to support centers seeking international accreditation. After four years, the group has not only met this objective but also became a reference for quality in cellular therapy in Brazil.

This national survey revealed significant heterogeneity in PBSC cryopreservation practices across Brazilian CPCs. Despite structural and resource limitations, many centers demonstrated commitment to improving quality and pursuing international accreditation. The creation of the Brazilian Cellular Therapy Processing Group has fostered collaboration, knowledge exchange, and standardization efforts. Continued investment in training, infrastructure, and regulatory alignment is essential to ensure the safety and effectiveness of cellular therapy in Brazil.

CONFLICT OF INTEREST

Nothing to declare.

DATA AVAILABILITY STATEMENT

Supplementary Material are available at Zenodo. https://doi.org/10.5281/zenodo.17258684

AUTHORS' CONTRIBUTIONS

Substantive scientific and intellectual contributions to the study: Prata KL, Kondo AT, Custer B, Belisário AR. **Conception and design:** Prata KL, Kondo AT, Belisário AR. **Analysis and interpretation of data:** Prata KL, Custer B, Belisário AR. **Statistics analysis:** Prata KL, Custer B, Belisário AR. **Manuscript writing:** Prata KL, Belisário AR. **Final approval:** Prata KL.

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