Establishing a stem cell culture laboratory for clinical trials

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Adult stem/progenitor cells are found in different human tissues. An in vitro cell culture is needed for their isolation or for their expansion when they are not available in a sufficient quantity to regenerate damaged organs and tissues. The level of complexity of these new technologies requires adequate facilities, qualified personnel with experience in cell culture techniques, assessment of quality and clear protocols for cell production. The rules for the implementation of cell therapy centers involve national and international standards of good manufacturing practices. However, such standards are not uniform, reflecting the diversity of technical and scientific development. Here standards from the United States, the European Union and Brazil are analyzed. Moreover, practical solutions encountered for the implementation of a cell therapy center appropriate for the preparation and supply of cultured cells for clinical studies are described. Development stages involved the planning and preparation of the project, the construction of the facility, standardization of laboratory procedures and development of systems to prevent cross contamination. Combining the theoretical knowledge of research centers involved in the study of cells with the practical experience of blood therapy services that manage structures for cell transplantation is presented as the best potential for synergy to meet the demands to implement cell therapy centers.

Keywords: *Stem cells; Tissue therapy; Cell culture techniques; Good manufacturing practices.*

Introduction

At the beginning of the twentieth century, *in vitro* tissue culture methods were developed to study the behavior of animal cells free of *in vivo* systemic variations. These cells could be tested under stable conditions imposed in the experiment. Cell culture methods were limited to applications that involved primary explants which at that time were the only source. In 1916, enzymatic dissociation of tissue was demonstrated by Rous & Jones[1] and a methodology of cell expansion became possible.

One hundred years after the discovery of these methods, researchers have changed the focus of cell culture procedures. In addition to cell studies into viral behavior and pharmacological agents, the current goal is *ex vivo* cell expansion. This approach will allow cells to be used in clinical treatments, the goal of cell therapy.

Another important achievement was the isolation of the first human cell line from a uterine cervical tumor (HeLa). This gave rise to experimental studies in cancer and other diseases, such as poliomyelitis[2]. In this area, stem cells played a prominent role, due to the transdifferentiation, self-renewal and paracrine effects that they display in some tissues and systems.

However, in many cases appropriate stem cells/progenitors are not available in sufficient quantities to restore organs and damaged tissues. To address this issue, numerous studies have developed ways to expand a limited number of cells in order to acquire a sufficient quantity for therapeutic use. Advancements from these studies might contribute to the development of regenerative therapies[3].

The complexity of these new technologies makes clinical applications difficult[4]. These technologies require adequate facilities, qualified staff, techniques to evaluate cell quality and well defined protocols for cell production. The rules to establish adequacy in these areas are at an advanced stage; they are based on national and international standards of good manufacturing practices (GMP). Generally, evaluation points have to be planned and require major investments from cell therapy centers[5].

Standards and legislation

Major breakthroughs in biotechnology, biology and medicine have promoted new therapeutic strategies in cell therapy, including stem cell transplantation. Biologically privileged stem cells have been used for research and for clinical applications. However, legislation and GMP standards are not uniform throughout the world.

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Several standards have been published for the structuring of cell processing centers. The Food and Drug Administration (FDA) is responsible to ensure the safety of biological products used in humans in the United States[6] and regulates the processing of *ex vivo* cell products[7]. These processes are divided into two categories: minimally manipulated and effectively manipulated. Minimally manipulated products are those that were only submitted to procedures that did not alter the basic features and function of cells. These procedures include cryopreservation and cell thawing, density gradient separation, washing and the dilution of cell products before reinfusion, cell selection and cell depletion. Effectively manipulated products are those submitted to procedures that might have altered the biological features of cells. This includes *ex vivo* expansion and genetic modification. The FDA uses a risk-based approach to regulate cell products in both categories. A facility that supplies minimally manipulated products should follow the current Good Tissue Practice (cGTP) guidelines as defined in the "Code of Federal Regulations" (CFR) title 21, part 1271[8]. A manufacturing laboratory that provides effectively manipulated cells should follow the cGMP guidelines defined by the CFR title 21, part 211[9], in addition to applicable items in the category of minimally manipulated products.

In Europe, countries that are members of the European Union (EU) define cell-based medicinal products (CBMP) as any viable cells (including stem cells) used in clinical treatment[10]. The European Medicine Agency (EMEA), established in London by Regulatory Council nº 2309/93 of 22 July 1993, is responsible for coordinating existing scientific resources for country members that seek assessment and supervision in the production of medicinal products for human and veterinary use[11-13].

CBMPs are heterogeneous in origin, cell type and complexity. A living organism has stem cells, progenitor cells (with a higher degree of differentiation) and terminally differentiated cells (with a defined physiological function). In addition, cells can be genetically modified, used alone or in association with biomolecules or chemical substances, or combined with structural materials (combined products)[14-17].

The European and American legislation on GMP defines qualities that are required for cells used in the treatment of patients. These qualities include donor suitability, cell product traceability, follow-up of the donor and the patient over time and quality control systems with documentation that must be maintained by the handler[18-20].

Many institutions have recently built or are interested in building manufacturing facilities to support clinical research in Brazil and around the world. However, for facilities to be classified "GMP compliant", it is not sufficient just to build the facility according to the prevailing building standards (CFR part 211). These standards include eleven sub-parts and the building structure is only one item[21].

For laboratories designed to prepare effectively manipulated cells, the standards take into account the following critical points: general planning, organization and human resources, facilities and edifications, equipment, component controls, containers, manufacturing and process control, packing and labeling, storage and distribution, laboratory controls, records and reports, returned products and remaining products.

In Brazil, the Regulatory agency, ANVISA, recently published Resolution nº 9 of March 14th, 2011[22]. This resolution provides guidelines for the operation of cell technology centers (CTCs) that prepare human cells and derivatives for the purpose of clinical research and therapy. The guidelines set the minimum requirements for these CTCs.

The Brazilian legislation has two classifications for CTC facilities:

- CTC Type 1: An establishment that only performs activities with human adult cells that are autologous, fresh or cryopreserved without cultivation and have been only minimally manipulated for use in clinical research or therapy or both.
- CTC Type 2: An establishment that performs activities with human stem cells, adult or embryonic, autologous or allogeneic, fresh or cryopreserved, with or without cultivation, and with or without extensive manipulation for use in clinical research or therapy or both.

The project must adhere to the mandates in Resolution RDC/ANVISA nº 50 of February 21st 2002 [23], which regulates the planning, programming, elaboration and assessment of physical projects in healthcare facilities. The structure should be used and accessed exclusively for the approved purpose. To avoid cross-contamination, the flow of raw materials, biological materials, professionals and waste should be independent; in addition, allowances should be made for thorough cleaning and maintenance of equipment and tools.

The deployment process: experiences of a Brazilian cell technology center

Planning and project elaboration

Researchers in basic research with proficiency in cell cultivation were consulted to create a project that would be developed in a CTC. Despite great technical contributions in laboratory processes, to fulfill the aim of manipulating cells for clinical applications, it was necessary to consult a professional with extensive knowledge of practical and management aspects of project design.

Thus, a team was created that had extensive experience in managing the scientific, technical, and administrative aspects of medical services in the area of hemotherapy and bone marrow transplantation. The objective of the transfusion treatment was to provide blood cell components that would restore cellular elements in a patient with a deficiency. The transfusion would be derived from total blood either from the patient or from other donors[24].

In bone marrow transplantation, the hemotherapy would be performed with bone marrow, peripheral blood or umbilical cord blood[25-26]. The hemotherapy would involve hematopoietic stem cell harvesting, processing, cryopreservation and infusion after chemotherapeutic conditioning[27].

Because Brazil lacked specific national standards, the final CTC project design was based on a review of all legislation available at that time that regulated activities in this area. Thus, the project was compatible with requirements in the Brazilian scenario.

Physical structure

The CTC was designed to conduct all procedures related to stem cell preparation. These included harvesting, cell processing, storage, quality control of cells, disposal, release for use and transport[28-30].

According to the classification of clean room types established in the national standards RDC 9 of 2011 (cited above), centers with existing stem cell processing labs were considered CTC type 1. Thus, these were considered establishments that only performed activities with human autologous, fresh or cryopreserved adult stem cells, without cultivation for use in clinical research and/or therapy.

The purpose of the current project was to achieve a laboratory with type 2 classification.

According to international standards, the classification of air quality in each area is defined by the nature of cell products that are manipulated in the laboratory. Currently, there are two accepted classifications in the world: the air classification of the US Federal Government Standard and the International Organization for Standardization (ISO)[31]. Table 1 illustrates the differences between these classifications.

Table 1 - Air classification standards: comparison between US and ISO guidelines on viable count action levels[32]

Clean room classification - US guidelines (Particles 0.5 µm/ft³)	ISO designation	Number of particles > 0.5 µm/m³	Active air action levels* (CFU viable/m³)
100	5	3,520	1
1000	6	35,200	7
10,000	7	352,000	10
100,000	8	3,520,000	100

*Plates of microbiological culture with diameter of 90 mm; CFU/4 h

ISO = International Organization for Standardization; CFU = Colony Forming Unit

When cell processing is conducted in an open system, it should be performed in a biological cabinet that conforms to ISO-5 specifications (US Class 100) placed in an environment classified as ISO-7 (US Class 10,000). When product manipulation is conducted in a closed system, with minimal manipulation and aseptic validation, it may be performed in a non-classified environment, but with a controlled air system. Cell culture laboratories must have positive pressure relative to the surrounding areas to avoid the inflow of contaminated air.

To comply with international standards related to the physical structure of a clean room, it was necessary to select engineering companies with specific experience in building and installing GMP facilities. Because no national legislation existed to regulate this area, company selection was a long, difficult process, due to discrepancies in the information obtained. Thus, the company selected proposed a project that fully complied with US regulations utilizing the existing structure.

Considering that this was the only structure of its kind in Brazil, the next task was to set up an information and experience exchange network with international sites of excellence in cell processing for human use. For example, correspondence was established with the MD Anderson Cancer Center (Houston, Texas, USA), the New York Blood Center (New York, USA) and the Duke University Medical Center (Durham, USA).

After selecting the engineering company, the next step was to acquire the proper equipment to purify the room air. The equipment had to generate an environment with the recommended number and quality of air particles. The accepted guidelines mandated inert particles in this environment, with no detectable microbiological activity. Therefore, we acquired equipment that was available and approved for the project. This was the most time consuming part of the project, because most equipment was imported, and the deadlines could not always be met. The location of equipment in controlled environments also had to follow specific standards that were included in the project.

To suit international standards, the laboratory was built with one class 100,000 (ISO 8) clean room and two class 10,000 (ISO 7) clean rooms. Access from the external to the internal environments required passage through two separate rooms. One of these rooms was for washing the hands and the other was for putting on clean laboratory attire. To access the class 10,000 environments, we also built a specific area to prevent cross-contamination. In each access room, stickers were fixed to the floor to retain particles that could be dragged between rooms.

The air treatment system and air purification equipment was designed and installed in a protected environment. The environment was turned into a clean room with HEPA filters and air compressors to substitute environmental air. Particle count sensors were also installed at strategic points to provide continuous air quality monitoring and automatic activation of an insufflation and aspiration air system. Humidity sensors and air pressure monitors were also installed.

Dedicated equipment was installed inside the class 100,000 laboratory. The water treatment system (Ultrapurification Water System, Milli-Q) produced ultrapure water with pass-through ultraviolet radiation. Precision scales were also included as were a freezer to store reagents, refrigerators, biological safety cabinets (Class 100; ISO-5), a refrigerated centrifuge and an anaerobic CO_2 cabinet.

Once the structure was ready and the equipment was properly installed and tested, the qualifying phase of the qualitative and quantitative control of laboratory air was started. To ensure the reliability of results, organizations that were not connected to the engineering company that installed the air treatment system were hired. One company was engaged to check and certify the sterile conditions of the laboratory environment, including the control of air particle density and the air outflow system and other companies to assess the microbiological control.

Standardization of laboratory procedures

After concluding the structural evaluation, another fundamental phase of cGMP structure maintenance was begun[33-35]. This included the standardization of methods, procedures and materials to perform the activities and maintain hygiene in the controlled environments[36].

Cross-contamination

Asepsis is the most important requirement that distinguishes cell culture protocols for materials to be used in humans from laboratory techniques[37-40]. However, establishing sterile areas requires high investment. The areas must maintain strict hygienic controls (dirt removal with special products), restricted access, an established flow of authorized individuals, and proper attire dedicated for use in this area. These structures should also incorporate a unidirectional flow of raw materials, components, products, employees, and discharges to ensure maximum segregation and minimum intersection.

Cleaning

The sanitization of clean rooms requires the use of suitable pharmaceutically pure water or water purification systems that meet USP standards. Solutions for cleaning and sanitizing equipment within these facilities must be produced with suitably pure water.

Movement of individuals

In designing the physical area of the clean rooms, it was important to scale the room sizes to the number of employees assigned in order to control room cleanliness, the time required for each shift, and what types of culture would be developed. To ensure site cleanliness, norms were established to control the number of people allowed inside and the flow of people. This design was also useful to determine the number of biological safety cabinets and the types of equipment. Another important issue was to avoid a large number of professionals and equipment operating at any one time. This decreases air circulation, and consequently, minimizes the risk of contamination by microbial particles.

Quality control of the system and standardization of protocols

To implement the laboratory routine, it was important to map processes, identify and delineate critical procedures and establish procedures to monitor the products and expected results. In monitoring products for clinical use (in humans), it is essential to have laboratory tests for the quality control of cultured cells. Flow cytometry was performed to characterize the cells and cytogenetic analysis was used to monitor chromosomal alterations. Tests for *in vitro* cell differentiation and sterility, among others, were also standardized.

Parameters considered critical for proper procedures were analyzed and validated. For example, the transportation time of biological materials, cultivation protocols, laboratory garments, aseptic procedures, materials, reagents etc. Results were presented and discussed in a scientific committee to gain institutional approval.

Traceability was established for the entire procedure, materials used, and professionals employed through standardized registration forms and a system for packaging, storage, and distribution. A program for waste management was established according to current national standards.

Discussion

An important future direction of medicine is the development of cell-based regenerative medicine. As, in many cases, humans cannot regenerate damaged tissue, there is a clear need for stem cell isolation and *ex vivo* expansion[41,42]. These activities require an appropriate laboratory environment to ensure adequate safety during cell processing to eliminate risk of mortality[43].

The new Brazilian legislation describes the minimum environmental conditions required for centers that offer cell technology. However, this is only the first step in regulating the complex processes involved in cultivated stem cell delivery.

The main difficulties in deploying a Brazilian CTC were related to discrepant information that arose due to the lack of regulations in force in Brazil. Some difficulties were addressed by the selection of specialized engineering companies. Building proposals were presented to the team responsible for the project. Technical issues ranged from maintaining air quality to problems related to coatings on equipment. International exchange allowed us to assess similar laboratories; this provided crucial information for decision making on key issues.

The GMP guidelines for cell harvesting, processing, cryopreservation, testing, distribution, and the use of blood products enabled the implementation of a safe production system for cultured cells. Another important consideration was quality control, which must be applied not only to the final product (the cells), but also to each procedure involved.

Beyond the technology, the most important goal is patient safety. The team management experience allowed us to achieve the project and implement safe procedures for processing stem cells from various sources for use in studies involving humans.

Conclusion

Cell therapy is a medical area on the rise. This has increased the demand for CTCs that can supply stem cells suitable for clinical protocols. In Brazil, the legal framework for regulating these structures was recently established. Previously, CTCs were rarely prepared to meet all the requirements for the production of cells for use in humans. The solution presented here is a practical response to the needs of basic research specialties that deal with cells. We described the practical management concerns involved in establishing an institution that must also meet demands for constant improvement and exchanged ideas with other institutions involved in this process. We demonstrated that a theoretical knowledge of site requirements combined with practical experience in hemotherapy services provides the best synergy for meeting the demands of establishing a viable CTC.

References

1. Rous P, Jones FS. A method for obtaining suspensions of living cells from the fixed tissues, and for the plating out of individual cells. J Exp Med. 1916;23(4):549-55.
2. Scherer WF, Syverton JT, Gey GO. Studies on the propagation in vitro of poliomyelitis viruses. IV. Viral multiplication in a stable strain of human malignant epithelial cells (strain HeLa) derived from an epidermoid carcinoma of the cervix. J Exp Med. 1953;97(5):695-710.

3. Coutinho LH, Testa NG, Chang J, Morgenstern G, Harrison C, Dexter TM. The use of cultured bone marrow cells in autologous transplantation. Prog Clin Biol Res. 1990;333:415-32; discussion 433.
4. Schallmoser K, Bartmann C, Rohde E, Reinisch A, Kashofer K, Stadelmeyer E, et al. Human platelet lysate can replace fetal bovine serum for clinical-scale expansion of functional mesenchymal stromal cells. Transfusion. 2007;47(8):1436-46.
5. Sensebé L, Bourin P. Producing MSC according GMP: process and controls. Biomed Mater Eng. 2008;18(4-5):173-7.
6. Weber DJ. Navigating FDA regulations for human cells and tissues. Bio Process Internat. 2004;2(8):22-6.
7. Gastineau DA. Will regulation be the death of cell therapy in the United States? Bone Marrow Transplant. 2004;33(8):777-80.
8. U.S. Food and Drug Administration. CFR- Code of Federal Regulations Title 21. Human cells, tissues, and cellular and tissue-based products. Part 1271 [Internet]. Washington, DC: FDA; 2008. [cited 2011 Dec 12]. Available from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=1271&showFR=1
9. U.S. Food and Drug Administration. CFR- Code of Federal Regulations Title 21. Current good manufacturing practice for finished pharmaceuticals Part 211 [Internet]. Washington, DC: FDA; 2008. [cited 2011 Dec 12] Available from: http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?CFRPart=211
10. The Commission of the European Communities. Commission Directive 2003/63/CE. Community code relating to medicinal products for human use. Brussels: Commission of the European Communities; 2003. [cited 2009 Jun 27]. Available from: http://eur-lex.europa.eu/Notice.do?mode=dbl&lang=en&ihmlang=en&lng1=en,pt&lng2=bg,cs,da,de,el,en,es,et,fi,fr,hu,it,lt,lv,mt,nl,pl,pt,ro,sk,sl,sv,&val=285425:cs&page=
11. The European Agency for the Evaluation of Medicinal Products. Committee for human medicinal product (CHMP). Guideline on human cell-based medicinal products [Internet]. London: EMEA; 2007. [cited 2012 Mar 10]. Available from:http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003898.pdf
12. The Commission of the European Communities. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use [Internet]. Brussels: Commission of the European Communities; 2001. [cited 2010 Jun 12]. Available from: http://www.emea.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/10/WC500004481.pdf
13. The European Agency for the Evaluation of Medicinal Products. Committee for Proprietary Medicinal Products (CPMP). Committee for Veterinary Medicinal Products (CVMP). Note for guidance on minimizing the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products (EMEA/410/01 rev.2- October 2003. Official Journal of the European Union [Internet]; 2003. [cited 2010 Jun 23]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:C:2004:024:0006:0019:EN:PDF
14. The European Agency for the Evaluation of Medicinal Products. Committee for Proprietary Medicinal Products (CPMP). Note for guidance on the quality, preclinical and clinical aspects of gene transfer medicinal products [Internet]. London: EMA; 2001. (CPMP/BWP/3088/99); [cited 2010 Jun 21]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500003987.pdf
15. The European Agency for the Evaluation of Medicinal Products. Committee for Proprietary Medicinal Products (CPMP). Note for guidance on use of bovine serum in the manufacture of human biological medicinal product [Internet]. London: EMA; 2002 (CPMP/BWP/1793/02) [cited 2010 Oct 15]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003675.pdf
16. The European Agency for the Evaluation of Medicinal Products. Committee for Proprietary Medicinal Products (CPMP). Points to consider on xenogeneic cell therapy medicinal products [Internet]. London: EMA; 2002 (CPMP/1199/02) [cited 2010 Nov 21]. Available from: http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003893.pdf
17. The European Agency for the Evaluation of Medicinal Products. Committee for Proprietary Medicinal Products (CPMP). Note for guidance on plasma-derived medicinal products [Internet]. London: EMA; 2001. (CPWP/BWP/269/95, rev.3) [cited 2010 Jun 21]. Available from: http://www.emea.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003613.pdf
18. U.S. Food and Drug Administration HHS. Eligibility determination for donors of human cells, tissues, and cellular and tissue-based products; final rule. Fed Regist. 2004;69(101):29785-834.
19. The Commission of the European Communities. Commissions Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells. Official Journal of the European Union [Internet]; 2006. [cited 2010 Jun 12]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2006:038:0040:0052:EN:PDF
20. The Commission of the European Communities. Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells. Official Journal of the European Union [Internet]; 2004. [cited 2010 Jun 12]. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2004:102:0048:0058:EN:PDF
21. Burger SR. Design and operation of a current good manufacturing practices cell-engineering laboratory. Cytotherapy. 2000;2(2):111-22.
22. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução – RDC n.9, de 14 de março de. Dispõe sobre o funcionamento dos Centros de Tecnologia Celular para fins de pesquisa clínica e terapia e dá outras providências [Internet]. Diário Oficial da República Federativa do Brasil, Brasilia (DF): 2011 Mar 16 [cited Jun 2010]. Available from: ftp://ftp.saude.sp.gov.br/ftpsessp/bibliote/informe_eletronico/2011/iels.mar.11/Iels49/U_RS-MS-ANVISA-RDC-9_140311.pdf
23. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução – RDC nº 50, de 21 de fevereiro de 2002. Dispõe sobre o regulamento técnico para planejamento, programação, elaboração e avaliação de projetos físicos de estabelecimentos assistenciais de saúde [Internet]. Brasilia (DF): ANVISA; 2002. [cited 2010 Jun 23]. Available from: http://bvsms.saude.gov.br/bvs/saudelegis/anvisa/2002/res0050_21_02_2002.html
24. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução - RDC nº 57, de 16 de dezembro de 2010. Determina o Regulamento Sanitário para serviços que desenvolvem atividades relacionadas ao ciclo produtivo do sangue humano e seus componentes e procedimentos transfusionais [Internet]. Diário Oficial da República Federativa do Brasil, Brasilia (DF): 2010 Dez 17 [cited Jun 2010]. Available from: http://pegasus.fmrp.usp.br/projeto/legislacao/rdc_57_161210.pdf
25. U.S. Food and Drug Administration. Human tissue intended for transplantation: final rule. Fed Regist. 1997;62:40429-47.
26. Harvath L. Food and Drug Administration's proposed approach to regulation of hematopoietic stem/progenitor cell products for therapeutic use. Transfus Med Rev. 2000;14(2):104-11.

27. Brasil. Ministério da Saúde. Agência Nacional de Vigilância Sanitária. Resolução - RDC n° 56, de 16 de dezembro de 2010. Dispõe sobre o regulamento técnico para o funcionamento dos laboratórios de processamento de células progenitoras hematopoéticas (CPH) provenientes de medula óssea e sangue periférico e bancos de sangue de cordão umbilical e placentário, para finalidade de transplante convencional e dá outras providências [Internet]. Diário Oficial da República Federativa do Brasil, Brasilia (DF): 2010 Dez 16 [cited Jun 2010]. Available from: http://portal.anvisa.gov.br/wps/wcm/connect/c8272000474597529fcadf3fbc4c6735/RDC_n%C2%BA_56.pdf?MOD=AJPERES

28. U.S. Food and Drug Administration. Guidance for industry: regulation of human cells, tissues, and cellular and tissue-based products (HCT/Ps); small entity compliance guide [Internet] Rockville (MD): FDA; 2007. [cited 2011 Oct 21]. Available from: http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm062592.pdf

29. U.S. Food and Drug Administration. Guidance for industry: Sterile drug products produced by aseptic processing - current good manufacturing practice [Internet]. Rockville (MD): FDA; 2004. [cited 2011 Oct 21]. Available from: http://www.bcg-usa.com/regulatory/docs/2003/FDA200309A.pdf

30. U.S. Food and Drug Administration. Guidance on applications for products comprised of living autologous cells manipulated ex vivo and intended for structural repair or reconstruction. Rockville (MD): FDA; 1996. (Docket No. 95N-0200). [cited 2011 Jun 23]. Available from: http://www.gpo.gov/fdsys/pkg/FR-1996-05-28/pdf/96-13386.pdf

31. International Organization for Standardization. ISO 14644-1, 1999. Cleanrooms and associated controlled environments—Part 1: Classification of air cleanliness. Geneva: Switzerland: ISO; 1999.

32. Areman EM, Loper K, editors. Cellular therapy: principles, methods, and regulations. Bethesda (MD): American Association of Blood Banks; 2009

33. International Organization for Standardization. ISO 10993-1:2009. Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process. Geneva; Switzerland; ISO; 2009.

34. International Organization for Standardization. ISO 10993-18:2005. Biological evaluation of medical devices- Part 18: Chemical characterization of materials. Geneva; Switzerland; ISO; 2005.

35. International Organization for Standardization. ISO 10993-19:2006. Biological evaluation of medical devices- Part 19: Physico-chemical, morphological and topographical characterization of materials. Geneva; Switzerland; ISO; 2006.

36. Food and Drug Administration. Guidance for industry: Class II special controls guidance document: Cord blood processing system and storage container. Fed Regist. 2007;72(21):4715.

37. European Medicines Agency. ICH topic Q5A (R1). Quality of biotechnological products: viral safety evaluation of biotechnology product derived from cell lines in of human or animal origin (CPMP/ICH/295/95) [Internet]. London: 1995. [cited 2011 Sep 15]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002801.pdf

38. European Medicines Agency. ICH topic Q5D. Quality of biotechnological products: Derivation and characterisation of cell substrates used for production of biotechnological/biological products (CPMP/ICH/294/95)[Internet]. London: 1998. [cited 2011 Sep 15]. Available from: http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500003280.pdf

39. European Medicines Agency. ICH topic Q6B. Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products. CPMP/ICH/365/96) [Internet]. London: 1998. [cited 2011 Sep 15]. Available from http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002824.pdf

40. Mannello F, Tonti GA. Concise review: no breakthroughs for human mesenchymal and embryonic stem cell culture: conditioned medium, feeder layer, or feeder-free; medium with fetal calf serum, human serum, or enriched plasma; serum-free, serum replacement nonconditioned medium, or ad hoc formula? All that glitters is not gold! Stem Cells. 2007;25(7):1603-9

41. Wagner JE, Verfaillie CM. Ex vivo expansion of umbilical cord blood hemopoietic stem and progenitor cells. Exp Hematol. 2004;32(5):412-3.

42. Broxmeyer HE, Srour EF, Hangoc G, Cooper S, Anderson SA, Bodine DM. High-efficiency recovery of functional hematopoietic progenitor and stem cells from human cord blood cryopreserved for 15 years. Proc Natl Acad Sci USA. 2003;100(2):645-50.

43. Whiteside TL, Griffin DL, Stanson J, Gooding W, McKenna D, Sumstad D, et al. Shipping of therapeutic somatic cell products. Cytotherapy. 2011;13(2):201-13.

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