Cytotherapy, 2011; Early Online, 1–8

Guidelines for the development and validation of new potency assays for the evaluation of umbilical cord blood

STEPHEN SPELLMAN[1], CAROLYN K. HURLEY[2], COLLEEN BRADY[1], LISA PHILLIPS-JOHNSON[10], ROBERT CHOW[3], MARY LAUGHLIN[4], JOHN MCMANNIS[5], JO-ANNA REEMS[6], DONNA REGAN[7], PABLO RUBINSTEIN[8], JOANNE KURTZBERG[9]

[1]*Center for International Blood and Marrow Transplantation Research, Minneapolis, Minnesota, USA,* [2]*Georgetown University Medical Center, Washington, District of Columbia, USA,* [3]*StemCyte, Covina, California, USA,* [4]*University of Virginia, Charlottesville, VA, USA,* [5]*The University of Texas MD Anderson Cancer Center, Houston, Texas, USA,* [6]*Puget Sound Blood Center, Seattle, Washington, USA,* [7]*SSM Cardinal Glennon Children's Medical Center, St Louis, Missouri, USA,* [8]*New York Blood Center, New York, New York, USA, and* [9]*Carolinas Cord Blood Bank, Durham, North Carolina, USA,* [10]*National Marrow Donor Program, Minneapolis, Minnesota, USA*

Abstract

The following commentary was developed by the National Marrow Donor Program Cord Blood Advisory Group and is intended to provide an overview of umbilical cord blood (UCB) processing, summarize the current state of potency assays used to characterize UCB, and define limitations of the assays and future needs of the cord blood banking and transplant community. The UCB banking industry is eager to participate in the development of standardized assays to uniformly characterize cellular therapy products that are manufactured in a variety of ways. This paper describes the desired qualities of these assays and how the industry proposes to co-operate with developers to bring relevant assays to market. To that end, the National Marrow Donor Program (NMDP) Cord Blood Bank Network is available to serve as a resource for UCB testing material, research and development consulting, and product/assay testing in an accredited UCB manufacturing environment.

Key Words: *umbilical cord blood banking, umbilical cord blood potency assessment, umbilical cord blood processing, umbilical cord blood transplantation*

Introduction

As the umbilical cord blood (UCB) banking community moves towards United States Food and Drug Administration (FDA) licensure, it is critical to define standardized testing methodologies that can be employed to characterize UCB, facilitate comparison of UCB between UCB banks, and assess potency prior to clinical use. The methodologies used currently have strong intralaboratory correlation, i.e. UCB evaluated within a UCB bank are tested in a consistent fashion and yield consistent results. However, the methodologies and assays can yield highly variable results in interlaboratory testing settings on identical material. The UCB banking community could benefit from the development of new potency assays that are highly reproducible and correlate with the outcome of interest, predominately hematopoietic reconstitution.

Potency is defined as the specific capacity of a cellular product to affect a given result. In the case of UCB, the community is interested in the capacity to engraft cells in a transplant recipient or to produce viable cells (e.g. viral or tumor-specific cytotoxic T lymphocytes or natural killer cells) for manipulation and administration for a desired effect. The UCB banking industry is eager to participate in the development of standardized assays to uniformly characterize cellular therapy products that are manufactured in a variety of ways. This paper describes the desired qualities of these assays and how the industry proposes to co-operate with developers to bring relevant assays to market.

History of clinical UCB transplantation

The first successful UCB transplantation (UCBTx) was performed in 1988 by Gluckman *et al.* (1) in a patient with Fanconi's anemia. The patient achieved stable engraftment of donor hematopoiesis

Correspondence: **Stephen Spellman,** Center for International Blood and Marrow Transplant Research, 3001 Broadway St. N.E., Minneapolis, Minnesota, USA, MN 55413. E-mail: sspellma@nmdp.org

(Received 19 October 2010; accepted 4 March 2011)

ISSN 1465-3249 print/ISSN 1477-2566 online
DOI: 10.3109/14653249.2011.571249

and survived without disease relapse (1). Because of the concern that UCB might not contain sufficient numbers of cells to engraft larger children or adults reliably, initial studies were limited to children. Three simultaneous studies that extended UCBTx to adults were reported in 1996 suggesting that cord blood transplantation (CBT) can be used successfully for this population (2–4). Following these early reports, there have been a number of studies using UCB for transplantation in adults. As of 2009, it is estimated that more than 20 000 UCBTx have been performed world-wide. In 2009, more UCBTx were performed than bone marrow transplants (5).

Public banking of UCB was initiated in 1992 at the New York Blood Center (New York, NY, USA) (6–9). Because of the establishment of more public banks, the number of UCB units available for transplantation climbed from 44 000 in 1999 to 452 000 in 2008 (10). Early on, standards for defining the quality of a UCB unit began to evolve (11). This included not only defining standards for donor eligibility, but also for the collection, processing, storage, transport and transplantation of UCB. Over the past 18 years, the standards have undergone continuous modification as new technologies have been introduced and new information has been collected to improve the process. However, approximately 15–24% of patients receiving an unrelated UCBTx do not engraft (12,13). Although there are many clinical reasons why a patient may not engraft, the quality of the UCB unit, including potency, is likely to play a significant role. The potency of a UCB unit can be negatively impacted at several points in the manufacturing process, as discussed below.

Key steps in cord blood product manufacturing that could affect unit potency

While donor-specific factors probably impact on the potency of a UCB unit (14), the collection, processing, distribution and infusion stages may also affect the potency of this stem cell product, as described in Table I. Because of the central importance of cell dose in UCBTx, whatever processing technology a laboratory chooses, the goal is to achieve maximal recovery of nucleated cells, mononuclear cells, $CD34^+$ cells, colony-forming units (CFU), progenitor cells and stem cells. Typical UCB processing methods are described in Table II. In the future, other cell subsets, for example aldehyde dehydrogenase (ALDH)-bright or very small embryonic-like stem cells (VSEL) (15,16), may be determined as important too, and this development may require routine enumeration by processing laboratories. UCB units may be stored for an indefinite amount of time before use. Studies have demonstrated that cryopreserved UCB retrieved after > 15 years of storage can reconstitute hematopoiesis in NOD/SCID mice in a quantitative and qualitative manner consistent with freshly isolated UCB (17). While the overall cellular recovery declines as a result of the freezing process, the overall potency may be preserved for more than 15 years if the UCB is maintained below –150°C (17).

Testing of potency at the cord blood bank

The FDA Guidance for Industry (18) defines UCB potency by parameters measured on the UCB after processing but prior to cryopreservation. Currently the FDA recommends evaluation of potency through assessment of the following *in vitro* assays: total

Table I. Stages at which potency might be lost.

Stage	Process	Primary causes of loss of potency
Collection	*Ex utero* or *in utero* collection UCB (31)	Extremes in temperature during shipment
	Addition of anticoagulant[a]	
	Shipment to processing laboratory in validated container with temperature-monitoring equipment	
Processing	Volume reduction as described in Table II (32,33)	Reduction in cell number during volume reduction (36)
	Cryopreservation with 10% dimethyl sulfoxide (DMSO)	Temperature fluctuations during controlled-rate freezing, causing intracellular ice crystals (37)
	Liquid nitrogen storage (34,35)	Transient warming during storage
	Removal of samples for additional testing needed for unit selection	Transient warming during removal of samples
Distribution	Shipment to transplant center in validated dry shipper with temperature monitor	Complete or partial thawing during shipment; exposure to X-rays during shipment
	Temporary liquid nitrogen storage at transplant center	Transient warming during transfer to temporary storage
Infusion	Thawing of unit in 37°C water bath (27)	Exposure to high concentrations of DMSO over extended times during thawing (42)
	Dilution of product depending on protocol (32,33,38–41)	
	Infusion into patient	

[a]Anticoagulants commonly used include citrate phosphate dextrose, with or without adenosine (CPD or CPDA-1) or lyophilized heparin citrate phosphate dextrose, with or without adenosine (CPD or CPDA-1) or lyophilized heparin.

Table II. Summary of typical public UCB bank processing methods and product attributes.

Processing method	Additives (what are the typical concentrations?)	Final volume (mL)	Hematocrit (%)	RBC volume (mL)
Plasma depletion/reduction	*c.* 14% CPD, *c.* 1% dextran, *c.* 10% DMSO	≥60	<40–90%	*c.* 45
RBC and plasma depletion/ reduction	1% Hydroxyethyl starch	≤25	<10–45%	0.6–12.5
	OR *c.* 14% PrepaCyte, *c.* 14% CPD, 10% DMSO			

RBC, red blood cell.

nucleated cell count (TNC), cell viability and CD34 analysis. Additional measurements not specifically recommended by the FDA include nucleated red blood cell (nRBC) count, white blood cell (WBC) differential and CFU. Typically, TNC are measured using automated hematology analyzers, the CD34 assay is performed using flow cytometry, and the CFU assay is accomplished using a methylcellulose-based media impregnated with cytokines. The CFU assay may be performed using various cytokines and may enumerate several cell types of different lineages, yielding granulocytes, macrophages, erythrocytes or megakaryocytes. Usually, a volume of 5–6 mL of whole UCB is available to carry out these assays. Information from the results of these assays for the UCB unit is available to transplant centers considering the unit as a stem cell source.

There is increasing evidence that UCB unit potency should be assessed post-cryopreservation because events associated with freezing and storage can affect subsequent cellular viability and overall UCB potency. Ideally, assays are performed on approximately 100 μL found in an attached segment of the UCB prior to release to a transplant center. Sample availability for potency assessment has varied over time. Current NetCord-Foundation for the Accreditation of Cellular Therapy (FACT) standards (19) require that a minimum volume of at least 200 μL must be stored in at least two segments, with each sealed and integrally attached to the freezing bag. A significant problem in using attached segments for potency assays is that a segment cell viability and CFU does not uniformly correlate with the UCB unit (20). The contents of each reference sample must be representative of the UCB unit as a whole. When a UCB is requested by a transplant center, one segment is used for additional histocompatibility testing and, in some banks, for cell viability and/or potency analysis. In addition, standards require that additional samples of a minimum of 2×10^6 nucleated cells should be stored in at least two vials or additional contiguous segments, and a minimum volume of 3.6 mL serum or plasma from a non-heparinized sample should be stored in at least two vials. Further, storage of suitable material for preparation of at least 50 ng genomic DNA is required. Thus each UCB unit has multiple reference samples available for potency testing (21).

Limitations in assays currently used to assess UCB potency

A lack of consensus regarding which potency assays to perform remains an issue within the UCB banking and transplantation community. The ultimate goal is to identify a quality-control assay that would always be able to predict the engraftment capability of the graft before the cells are infused. Thus potency assays are performed at the UCB bank either pre- or post-cryopreservation and/or at the transplant center, either prior to infusion of the unit or retrospectively. A number of *in vitro* assays are performed, including TNC count, cell viability, nRBC count (22), WBC differential, CD34 analysis and CFU. The association of these *in vitro* values with clinical transplant center outcome was first evaluated by Rubinstein *et al.* (23) at the New York Blood Center. Their studies demonstrated that TNC/kg and total CD34 cells/kg infused were predictive of engraftment (24).

Various groups have looked into the correlation between potency measurement using different assays and their predictability of engraftment. Among these studies, the measurement of CFU and determination of percentage CD34 viability using a novel flow cytometry gating strategy in single- and double-UCBTx, respectively, have been listed as the most significant predictors of engraftment (25,26). In general, the association of these quality-control parameters and engraftment appears to be a continuum: as the number increases, the chances of engraftment increases. An absolute minimum number at which no patients would engraft has not been established. In summary, there is currently a lack of consensus regarding which assays should be performed to evaluate potency.

At present, there is a lack of standardization of potency assays among UCB banks and transplant centers and issues persist regarding the optimization of these assays for the varying types of UCB products. Assays may suffer from laboratory artifacts, especially when red cell-containing products are being assessed.

Some nRBC have physical properties (including low hemoglobin concentration) (27) that make them similar to lymphocytes, resulting in poor separation of mononuclear, progenitor and stem cells on traditionally used gradients (e.g. Ficoll). The proper optimization and validation of potency assays for red cell-containing products may include assessment of the potency assay at various dilutions of the product, to demonstrate the red cell effect and determine the optimal test conditions. These studies usually reveal an improved ability to enumerate cell or colony numbers properly at reduced red cell concentrations.

In addition, interlaboratory variability has been identified as a serious flaw in many recent studies. The reliability of using these parameters to select a unit for clinical transplantation is dependent upon the reproducibility of the results between testing sites. This is particularly troublesome when discrepancies exist between the results of the UCB bank and the transplant center receiving the unit. It has been reported that reasonable reproducibility can be obtained for TNC results when determined using automated hematology analyzers (28).

The intralaboratory reliability of CD34 and CFU analysis is very good. However, proficiency testing results obtained through the National Marrow Donor Program (NMDP; Minneapolis, MN, USA) proficiency testing program over the past several years suggest that there is considerable interlaboratory variability for CD34 and CFU assays. Therefore it is difficult to compare the quality of UCB units among different banks and between the UCB bank and transplant center.

In an attempt to address the issue of interlaboratory variability, the NMDP has established a proficiency testing (PT) program for all member banks. The aim of the program is to improve the reliability and the interbank comparability of tests used to characterize and rank UCB units during the search process (e.g. TNC count, UCB growth assays and $CD34^{+}$ cell counts). To date, there have been five separate PT analyses (2006–2010), where samples of fresh UCB, obtained from one UCB donation, were shipped to a number of centers throughout North America. The participants were asked to process the sample on arrival and measure the following: (a) TNC count; (b) CFU count; and (c) percentage of CD34 cells. A brief comparison of all five analyses is shown in Table III. The coefficient of variation (CV) for the concentration of TNC decreased substantially in the January 2010 analysis to 9.8%, a level consistent with the previous analyses. Two of the three CFU subset analyses continued to decrease in variability in 2010 compared with the previous analyses. For example, the CV for CFU- granulocyte–erythrocyte–macrophage and megakaryocyte progenitor (GEMM) were 129%, 100%, 121%, 70%, and 64% in 2006, 2007, 2008, 2009, and 2010, respectively. The CV for total CFU was consistent with previous PT analyses. Taken together, these results suggest that more robust and consistent assays are required to allow UCB banks and transplant centers to obtain comparable results for potency.

Expectations for the next generation of assays

UCB products come in many shapes, sizes and compositions. In some cases, these characteristics are linked to the UCB bank providing the UCB unit. In others, the same UCB bank may distribute multiple types of products. The transplant center, processing laboratory, assay developer and any other parties handling the product must be familiar with the product types, the configuration of the cryobags they are stored in, the types of access ports on the cryobag, the number of segments on the bag (in order to best accommodate the storage of and access to the unit shipped for their patient) and, most importantly, the proper method to thaw and infuse the particular product. When developing an assay that could be useful in predicting UCB potency and/or transplant center outcomes, the developer should ideally create protocols for testing all types of UCB products. If this is not possible, then the developer must specify the types of products for which the assay can be performed.

Expectations for new assay systems

The previous section described the current complement of assays utilized to qualify a UCB unit for clinical use, and the observed limitations. The evaluation of TNC (CV 6.1–16.9%) is fairly consistent in inter- and intralaboratory proficiency testing (Table III). CFU assessments considered indicative of the engraftment potential of the UCB produce less consistent results. Next-generation assays must address the limitations of the current testing systems in the following areas:

- standardized methodologies
- reproducibility with limited variability between testing sites
- automated testing outputs (objectivity)
- high throughput for UCB banks
- rapid turnaround time
- single sample test for transplant centers.

Assay developers should focus efforts on the development of new assay systems that meet the requirements noted above, but also consider novel approaches to defining the potency potential of UCB. The existing

Table III. Summary of NMDP proficiency testing results.

	2006		2007		2008		2009		2010	
Assay parameter	*n*	% CV	*n*	% CV	*n*	% CV	*n*	% CV	*n*	% CV
TNC/mL	28	6.1	38	16.8	33	7.5	42	16.9	40	9.8
% $CD34^{+}$ cells	ND		ND		ND		24	22.9	31	27.8
BFU-E	27	40.6	35	45.5	28	49.2	42	35.2	34	45.4
CFU-GM	29	65	40	54.5	28	50.4	44	42.9	34	29.8
CFU-GEMM	26	129	34	100	27	121.3	41	69.9	34	63.9
Total CFU	28	34.4	37	36.6	28	36.3	42	31.6	41	32.8

ND, not done; *n*, number of participating entities; BFU-E, blast-forming units–erythrocyte progenitor; CFU-GM, CFU-granulocyte–macrophage progenitor.

testing systems either identify a cell-surface marker that has been tied to hematopoietic stem cells (CD34) or measure the growth of cells in response to specific growth factors (CFU). Neither of these approaches directly measures the quantity or quality of the hematopoietic stem cells within the stored UCB product. However, pending further discovery, the existing surrogates provide an acceptable evaluation of the engraftment/growth potential of UCB.

Validation and implementation of new assay systems

The gold standard for assay validation is to determine the new system's correlation with an existing test and, if no current test exists, correlate with the transplant center's outcome of interest (18,29). All assessments should include a statistical evaluation of reliability, which is the measurement variability attributed to different UCB units relative to the total measurement of variability (including the variability from UCB unit to UCB unit, and inter- and intralaboratory variability). A test should have a target reliability of 80–90% to support comparisons across UCB units, test runs and laboratories. Below are issues that should be considered in the validation process.

- Define expectations/intended uses of the test as it relates to the type of samples to be evaluated. Consider the: impact of various anticoagulants and additives in the product; age of testing material; type of testing material, i.e. fresh versus post-thaw, plasma- and red cell-depleted versus plasma-depleted; matrix of the product, i.e. the biologic features in the product that may impact the assay, either through enhancement or inhibition; sample concentrations and limits of detection.
- Evaluate/verify that the test performance is not affected by the different types of samples as defined above.
- Determine/verify the stability of the test end-point and impact of temperature and time on testing.
- Determine the test reliability within intra- and interlaboratory evaluations. Define the test reliability requirements and determine the appropriate sample size to confirm. Perform parallel testing for validation against existing assays to measure equivalence and quantify benefits of the new system.

The evaluation methods and documentation requirements for new assays introduced to the manufacturing process for licensed products must meet the specifications dictated by the accreditation and regulatory agencies. The US FDA requirements for licensing addresses the acceptance criteria for potency assay validation and other assays for inclusion in a biologics licensure application (BLA) (30). In addition, it is important that any new assay system produce results that correlate with assessments performed previously on cryopreserved UCB. UCB suitability for a particular patient is currently assessed based on the degree of HLA match and the cell dose determined primarily by TNC.

NMDP Cord Blood Bank Network: a resource for assay and instrument evaluation

The NMDP serves as the Cord Blood Coordinating Center under the C. W. Bill Young Cell Transplantation Program and has developed a network of leaders in public UCB banking and transplantation in North America. The network has a broad geographical distribution and employs diverse methods of UCB collection and processing. This network can provide support for assay development in a number of ways (Table IV).

First, the network can provide access to UCB units for test development and validation. The research units are predominately composed of UCB that does not qualify for storage because of low volume or cell counts, but is suitable for laboratory evaluation. The material is available as fresh or cryopreserved units and can be tailored to the needs of the request. For example, UCB can be prepared in multiple segment

Table IV. Support provided by the NMDP Cord Blood Bank Network.

Contractual aspects	Access to centralized contract and payment system
Technical aspects	Access to research UCB units tailored for assay development and validation from FACT/AABB-accredited manufacturers Access to UCBTx experts to act as consultants in assay and validation design Access to UCB bank processing laboratories to perform assay validation Assistance with statistical modeling and data interpretation

AABB, American Association of Blood Banks.

aliquots to simulate the testing of segments at the time of UCB release for transplant.

Second, the network can provide a source of information on UCB banks, transplant centers and UCB experts who may be available to serve as consultants at all stages of the assay design, development and validation process.

Finally, once an assay is ready for testing, the NMDP Cord Blood Advisory Group has developed a process to assist assay and instrument developers with the validation of their products through the NMDP Cord Blood Bank Network. The goal of the process is to provide access to a dedicated network of UCB unit-processing laboratories and UCB bank. The network utilizes a centralized contracting mechanism for rapid deployment and implementation of assay validation in a controlled environment to manufacturer protocol specifications. The network is available to assist with all phases of product development that support the expectations for new assay systems and instrumentation as outlined above. The NMDP UCB bank network provides:

- access to established high-quality accredited UCB unit manufacturers
- centralized contract and payment management
- assistance with study design, statistical modeling, data analysis and interpretation.

The network may be accessed by contacting the NMDP at www.marrow-donor.org/CONTACT/Submit_a_Question/index.html.

Summary

Fifteen to 24% of patients receiving UCBTx do not engraft. One potential cause of engraftment failure is a loss of reconstituting hematopoietic stem cell potency that may have occurred at some stage during UCB collection, processing, storage, shipment and infusion. The best measure of potency is still unclear and current assays are limited by a lack of consistency among laboratories performing the assays. Potency assays available to UCB banks as they evaluate their products both pre- and post-cryopreservation, and to transplant centers as they monitor the UCB units they have received for infusion, are needed to address these critical questions. The NMDP Cord Blood Advisory Group can assist in the development of assays by providing resources in terms of UCB, expertise and access to a network for testing assays, once developed, in the field.

Acknowledgement

This project has been supported by funding from the National Marrow Donor Program and the Department of the Navy, Office of Naval Research Grant #N00014-10-1-0204 and the Health Resources and Services Administration Contract No. HHSH234200637021C to the National Marrow Donor Program. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the Office of Naval Research or the Health Resources and Services Administration.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

1. Gluckman E, Broxmeyer HA, Auerbach AD, Friedman HS, Douglas GW, Devergie A, et al. Hematopoietic reconstitution in a patient with Fanconi's anemia by means of umbilical-cord blood from an HLA-identical sibling. N Engl J Med. 1989;321:1174–8.
2. Wagner JE, Rosenthal J, Sweetman R, Shu XO, Davies SM, Ramsay NK, et al. Successful transplantation of HLA-matched and HLA-mismatched umbilical cord blood from unrelated donors: analysis of engraftment and acute graft-versus-host disease. Blood. 1996;88:795–802.
3. Kurtzberg J, Laughlin M, Graham ML, Smith C, Olson JF, Halperin EC, et al. Placental blood as a source of hematopoietic stem cells for transplantation into unrelated recipients. N Engl J Med. 1996;335:157–66.
4. Laporte JP, Gorin NC, Rubinstein P, Lesage S, Portnoi MF, Barbu V, et al. Cord-blood transplantation from an unrelated donor in an adult with chronic myelogenous leukemia. N Engl J Med. 1996;335:167–70.
5. Broxmeyer HE. Umbilical cord transplantation: epilogue. Semin Hematol. 2010;47:97–103.
6. Rocha V, Broxmeyer HE. New approaches for improving engraftment after cord blood transplantation. Biol Blood Marrow Transplant. 2010;16:S126–32.
7. Rubinstein P. Placental blood-derived hematopoietic stem cells for unrelated bone marrow reconstitution. J Hematother. 1993;2:207–10.
8. Rubinstein P, Rosenfield RE, Adamson JW, Stevens CE. Stored placental blood for unrelated bone marrow reconstitution. Blood. 1993;81:1679–90.

9. Harris DT, Schumacher MJ, Rychlik S, Booth A, Acevedo A, Rubinstein P, et al. Collection, separation and cryopreservation of umbilical cord blood for use in transplantation. Bone Marrow Transplant. 1994;13:135–43.
10. Welte K, Foeken L, Gluckman E, Navarrete C. International exchange of cord blood units: the registry aspects. Bone Marrow Transplant. 2010;45:825–31.
11. Fraser JK, Cairo MS, Wagner EL, McCurdy PR, Baxter-Lowe LA, Carter SL, et al. Cord Blood Transplantation Study (COBLT): cord blood bank standard operating procedures. J Hematother. 1998;7:521–61.
12. Eapen M, Rocha V, Sanz G, Scaradavou A, Zhang MJ, Arcese W, et al. Effect of graft source on unrelated donor haemopoietic stem-cell transplantation in adults with acute leukaemia: a retrospective analysis. Lancet Oncol. 2010;11:653–60.
13. Eapen M, Rubinstei P, Zhang MJ, Stevens C, Kurtzberg J, Scaradavou A, et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. Lancet 2007;369:1947–54.
14. Cairo MS, Wagner EL, Fraser J, Cohen G, van de Ven C, Carter SL, et al. Characterization of banked umbilical cord blood hematopoietic progenitor cells and lymphocyte subsets and correlation with ethnicity, birth weight, sex, and type of delivery: a Cord Blood Transplantation (COBLT) study report. Transfusion. 2005;45:856–66.
15. Kucia M, Halasa M, Wysoczynski M, Baskiewicz-Masiuk M, Moldenhawer S, Zuba-Surma E, et al. Morphological and molecular characterization of novel population of CXCR4$^+$ SSEA-4$^+$ Oct-4$_+$ very small embryonic-like cells purified from human cord blood: preliminary report. Leukemia. 2007; 21:297–303.
16. Zuba-Surma EK, Klich I, Greco N, Laughlin MJ, Ratajczak J, Ratajczak MZ. Optimization of isolation and further characterization of umbilical-cord-blood-derived very small embryonic/epiblast-like stem cells (VSELs). Eur J Haematol. 2010;84: 34–46.
17. 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;100:645–50.
18. Center for Biologics Evaluation and Research, Rockville, MD. Minimally Manipulated, Unrelated Allogeneic Placental/Umbilical Cord Blood Intended for Hematopoietic Reconstitution for Specified Indications. FDA Guidance for Industry. US Department of Health and Human Services, Food and Drug Administration; 2009. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Blood/UCM187146.pdf. Accessed date: 14 April 2010.
19. NetCord-FACT, Omaha, NE. International Standards for Cord Blood Collection, Banking, and Release for Administration. 4th ed. NetCord-FACT; 2010. http://www.factwebsite.org/uploadedFiles/News/4th_Edition_Cord_Blood_Standards_4.0.pdf. Accessed date: 14 April 2010.
20. Goodwin HS, Grunzinger LM, Regan DM, McCormick KA, Johnson CE, Oliver DA, et al. Long term cryostorage of UC blood units: ability of the integral segment to confirm both identity and hematopoietic potential. Cytotherapy. 2003;5:80–6.
21. Rodriguez L, Garcia J, Querol S. Predictive utility of the attached segment in the quality control of a cord blood graft. Biol Blood Marrow Transplant. 2005;11:247–51.
22. Stevens CE, Gladstone J, Taylor PE, Scaradavou A, Migliaccio AR, Visser J, et al. Placental/umbilical cord blood for unrelated-donor bone marrow reconstitution: relevance of nucleated red blood cells. Blood. 2002;100:2662–4.
23. Rubinstein DB, Leblanc P, Wright DG, Guillaume T, Strotchevoi A, Boosalis M. Anti-CD34$^+$ Fabs generated against hematopoietic stem cells in HIV-derived combinatorial immunoglobulin library suggest antigen-selected autoantibodies. Mol Immunol. 1998;35:955–64.
24. Migliaccio AR, Adamson JW, Stevens CE, Dobrila NL, Carrier CM, Rubinstein P. Cell dose and speed of engraftment in placental/umbilical cord blood transplantation: graft progenitor cell content is a better predictor than nucleated cell quantity. Blood. 2000;96:2717–22.
25. Prasad VK, Mendizabal A, Parikh SH, Szabolcs P, Driscoll TA, Page K, et al. Unrelated donor umbilical cord blood transplantation for inherited metabolic disorders in 159 pediatric patients from a single center: influence of cellular composition of the graft on transplantation outcomes. Blood. 2008;112:2979–89.
26. Scaradavou A, Smith KM, Hawke R, Schaible A, Abboud M, Kernan NA, et al. Cord blood units with low CD34$^+$ cell viability have a low probability of engraftment after double unit transplantation. Biol Blood Marrow Transplant. 2010; 16:500–8.
27. Brown B. Hematology: Principles and Procedures. 6th ed. Philadelphia: Lea & Febiger; 2006.
28. Moroff G, Eichler H, Brand A, Kekomaki R, Kurtz J, Letowska M, et al. Multiple-laboratory comparison of in vitro assays utilized to characterize hematopoietic cells in cord blood. Transfusion. 2006;46:507–15.
29. International Conference on Harmonisation. Harmonised Tripartite Guideline: Validation of Analytical Procedures: Text And Methodology Q2(R1) Current Step 4 version, Parent Guideline dated 27 October 1994 (Complementary Guideline on Methodology dated 6 November 1996 incorporated in November 2005), Ness Ziona, Israel. http://www.bioforum.org.il/Uploads/Editor/karen/q2_r1_step4.pdf. Accessed date: 14 April 2010.
30. Center for Biologics Evaluation and Research, Rockville, MD. Potency Tests for Cellular and Gene Therapy Products. FDA Draft Guidance for Industry. US Department of Health and Human Services; 2008. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/CellularandGeneTherapy/ucm078687.pdf. Accessed date: 14 April 2010.
31. Lasky LC, Lane TA, Miller JP, Lindgren B, Patterson HA, Haley NR, et al. In utero or ex utero cord blood collection: which is better? Transfusion. 2002;42:1261–7.
32. Jaing TH, Chen SH, Tsai MH, Yang CP, Hung IJ, Tsay PK. Transplantation of unrelated donor umbilical cord blood for nonmalignant diseases: a single institution's experience with 45 patients. Biol Blood Marrow Transplant. 2010;16:102–7.
33. Chow R, Nademanee A, Rosenthal J, Karanes C, Jaing TH, Graham ML, et al. Analysis of hematopoietic cell transplants using plasma-depleted cord blood products that are not red blood cell reduced. Biol Blood Marrow Transplant. 2007;13: 1346–57.
34. American Association of Blood Banks. Standards for Blood Banks and Transfusion Services, Bethesda, MD. 26th ed. AABB Standard: 5.18.1.2.4. American Association of Blood Banks; 2009.
35. NetCord-FACT, Omaha, NE. International Standards for Cord Blood Collection, Banking, and Release for Administration. 4th ed. FACT standard D7.5.1. NetCord-FACT; 2010.
36. Takahashi TA, Rebulla P, Armitage S, van Beckhoven J, Eichler H, Kekomaki R, et al. Multi-laboratory evaluation of procedures for reducing the volume of cord blood: influence on cell recoveries. Cytotherapy. 2006;8:254–64.
37. Yang H, Acker JP, Hannon J, Miszta-Lane H, Akabutu JJ, McGann LE. Damage and protection of UC blood cells during cryopreservation. Cytotherapy. 2001;3:377–86.

38. Chow R, Law P, Wang B, Rosenthal J, Nademanee A, Karanes C, et al. Negative impact of post-thaw washing on the overall survival (OS) and disease free survival (DFS) of patients receiving plasma depleted (PD) cord blood (CB) transplantation. Biol Blood Marrow Transplant. 2009;15(Suppl 2) Abstract no: O-39:17.
39. Regan DM, Wofford JD, Wall DA. Comparison of cord blood thawing methods on cell recovery, potency, and infusion. Transfusion. 2010;50:2670–5.
40. Barker JN, Abboud M, Rice RD, Hawke R, Schaible A, Heller G, et al. A 'no-wash' albumin-dextran dilution strategy for cord blood unit thaw: high rate of engraftment and a low incidence of serious infusion reactions. Biol Blood Marrow Transplant. 2009;15:1596–602.
41. Cord Blood Transplantation Study (COBLT), Rockville, MD. Cord Blood Bank Standard Operating Procedures. Carolinas Cord Blood Bank-Duke University & University of California-Los Angeles Cord Blood Bank. https://web.emmes.com/study/cord/sop.htm.
42. Rubinstein P, Dobrila L, Rosenfield RE, Adamson JW, Migliaccio G, Migliaccio AR, et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad Sci USA. 1995;92:10119–22.