Red blood cell depletion with a semiautomated system or hydroxyethyl starch sedimentation for routine cord blood banking: a comparative study

Pilar Solves, Vicente Mirabet, Dolores Planelles, Ignacio Blasco, Alfredo Perales, Francisco Carbonell-Uberos, M. Angeles Soler, and Roberto Roig

BACKGROUND: The major problem with long-term cord blood (CB) banking is the required storage space. In this sense, many studies have been performed to establish techniques for volume reduction of CB units.

STUDY DESIGN AND METHODS: We compared two different methods for CB volume reduction in both development and routine phases: hydroxyethyl starch (HES) sedimentation and top-and-bottom fractionation with the Optipress II (Baxter Healthcare). Monitoring the total nucleated cell (TNC) count, lymphocytes, CD34+ cells, and colony-forming unit (CFU) content in both preprocess and postprocess CB units assessed the volume reduction process.

RESULTS: The CB units processed in both groups had comparable volume and cells counts before and after volume reduction, except for number of red blood cells (RBCs), which was significantly greater for the Optipress II group. Recoveries of CD34+ and RBC depletion were significantly better for the HES group. For routine processing, TNC and lymphocyte recoveries were significantly better for CB units processed by the Optipress II system. There was, however, significantly less depletion of RBCs for this group. The time required for CB processing with the Optipress II was significantly shorter than the time needed for volume reduction by addition of HES (25 ± 5 min vs. 55 ± 10 min).

CONCLUSION: The volume reduction method with the Optipress II is a closed time-saving system that allows good cell recoveries. In contrast, the main advantage of the HES method is the higher RBC depletion that influences CFU content. Reducing RBC content must be the object of further improvements for volume reduction using the Optipress II method.

Umbilical cord blood (CB) contains hematopoietic progenitor cells (HPCs) and has been successfully used as an alternative source of allogeneic HPCs for transplantation. Since 1989, when Gluckman and colleagues[1] reported the first successful umbilical CB transplant from an HLA-identical sibling in a child with severe Fanconi anemia, the number of CB transplants from both siblings and unrelated donors has dramatically increased. To date, an estimated 2000 patients have undergone umbilical CB transplantation. Although most CB transplants have been performed in children, unrelated donor-CB transplants in adults have been growing steadily in recent years.[2,3]

As a response to this increasing activity, many CB banks have been established worldwide. Since 1993, following the opening of the first unrelated CB bank in New York, the different banks have taken different operational approaches.[4] The major problem with long-term banking is the required storage space. In this sense, many studies have been performed to establish techniques for volume reduction of CB units. In many of these studies, processing was performed in the presence of additives like hydroxyethyl starch (HES) or dextran.[5-10] To improve cell recovery and standardize the process, other approaches with automated or semiautomated methods have also been developed.[11-13]

ABBREVIATIONS: CB = cord blood; CFU–GEMM = colony-forming unit–granulocyte-erythroid-macrophage-megakaryocyte; TNC(s) = total nucleated cell(s).

From the Valencia Cord Blood Bank, Valencia Transfusion Center, Valencia, Spain.

Address reprint requests: Pilar Solves, Valencia Transfusion Center, Avenida del Cid 65-A, 46014 Valencia, Spain; e-mail: solves_pil@gva.es.

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In our CB bank, we have recently substituted the HES method by top-and-bottom fractionation with the Optipress II (Baxter Healthcare, Deerfield, IL), to simplify the process and save time. With the aim of establishing the efficacy of the new technique, we have compared the two different methods in both development and routine phases.

MATERIALS AND METHODS

CB collection

Maternal and neonatal pairs were evaluated during the prepartum period in the maternity ward at La Fe University Hospital (Valencia, Spain) by the obstetrical team. Donors signed informed consent before delivery, during the last months of pregnancy. For collection, umbilical cord was clamped immediately after baby delivery and cleaned with 70 percent alcohol and an iodine swab. Umbilical CB was collected from the umbilical vein by gravity in a closed sterile collection 350-mL double bag (R 1315, Baxter Healthcare) containing 23 mL of citrate-phosphate-dextrose-adenine anticoagulant or in a triple-bag system (R MQT 2205PU, Maco Pharma, Tourcoing Cedex, France) containing 21 mL of citrate-phosphate-dextrose. This process was performed by trained midwives before placental delivery. CB collections were stored at 4°C until processing[14] always performed within 48 hours from collection. Only CB units containing total nucleated cell (TNC) counts of at least 100×10^7 were volume-reduced.

Volume reduction with HES sedimentation

After removal of aliquots for routine testing, HES (Grifols, Barcelona, Spain) was added to the anticoagulated CB in a proportion of 1:4 HES solution to blood and then centrifuged at $45 \times g$ for 7 minutes at 10°C according to the method developed by Rubinstein and coworkers.[15] The removed supernatant white blood cell–rich plasma and 12 g of red blood cells (RBCs) were centrifuged again at $600 \times g$ for 15 minutes at 10°C. After discarding the supernatant, CB was adjusted to 25 mL for development study and 40 mL for routine CB banking.

Volume reduction with the semiautomated system Optipress II

The CB units collected in triple-bag system were centrifuged in oval buckets at $3000 \times g$ for 12 minutes at 22°C, ensuring that the bags were well supported to prevent disruption of the buffy-coat layer. A standard protocol programmed into the Optipress II (Baxter Healthcare), together with the standard backplate for buffy-coat preparation, was used to process the CB units. The program was set with the following settings: a buffy-coat volume of 40 mL, a buffy-coat level of 5.5, and a force of 25.

Cryopreservation

CB was cryopreserved separately in two 50-mL cryopreservation bags (Cryocyte R4R9951, Nexell Therapeutics Inc., Irvine, CA) containing 29 and 19 mL each. Cryopreservation of CB was performed by adding 50 percent dimethyl sulfoxide (DMSO) solution with dextran 40 (Rheomacrodex, Fresenius Kabi, Barcelona, Spain) for 15 minutes to reach a 10 percent DMSO final concentration. The cryocyte bags were placed in aluminum cassettes for immediate freezing. Units were frozen by controlled-rate freezing and stored in the vapor phase of liquid nitrogen.

A cryovial from each cryopreserved CB units was thawed after 1 week for clonogenic assays culture. After removal from the liquid nitrogen, the cryovial was immersed in a 37°C water bath and rapidly thawed.

Biologic controls

Samples for nucleated cell and CD34+ cell counts, sterility control, and progenitor cell assays (colony-forming unit [CFU]) were removed from the CB pre- and postvolume reduction.

Cell counts

Nucleated cells counts were performed with an autoanalyzer (Sysmex K800, Toa Medical Electronics, Kobe, Japan) and TNCs were calculated. The counts were not corrected for nucleated RBCs.

CD34 assay

CD34+ cells were quantified by flow cytometry. CB (5×10^5 cells) was incubated using monoclonal antibodies conjugated to CD34-fluorescein and CD45-phycoerythrin (Becton Dickinson, San Jose, CA) and 7-aminoactinomycin D as marker of DNA staining. Flow cytometric analysis was performed with computer software (Cell Quest, Becton Dickinson). A progenitor cell enumeration kit (ProCount, Becton Dickinson) was used in comparison with our standard protocol, giving similar results.[16]

Clonogenic assays

Clonogenic assays were performed using a commercially prepared complete methylcellulose medium (Methocult GF H4434), containing recombinant cytokines and supporting growth of burst-forming unit–erythroid (BFU–E), colony-forming unit–granulocyte-macrophage (CFU–GM), and colony-forming unit–granulocyte-erythroid-

macrophage-megakaryocyte (CFU–GEMM). For the development phases, clonogenic assays were performed directly from the bags before and after volume reduction and plated at 1×10^4. Samples for routine CFU were drawn from a thawed cryovial, taking a volume previously calculated according to the TNC and CD34 cells content of CB units before cryopreservation. A volume between 0.006 and 0.015 mL of thawed CB was directly plated in culture medium. We employed this direct plating technique to save time and decrease variability. Cultures were plated in duplicate 35-mm-diameter petri dishes and incubated for 14 days at 37°C with 5 percent CO_2 in a humidified atmosphere. Colonies defined as aggregates of more than 40 cells were counted under an inverted microscope. CFUs were calculated by the sum of BFU–E, CFU–GM, and CFU–GEMM.

Viability

Ethidium bromide and acridine orange were used to assess cell viability. The nonviable cells stain deep orange. Viability was performed before cryopreservation.

Sterility control

CB samples consisting of plasma and RBCs were screened for bacterial and fungal contamination using an automated blood culture system (BacT/ALERT, Organon Teknica, bioMérieux, Hazlewood, MO) at 35°C for 14 days. Samples were drawn from waste products (4 mL of plasma plus 4 mL of RBCs).

Statistical analysis

Descriptive statistics are presented for CB variables. Computer software (SPSS, Version 10, SPSS Inc., Chicago, IL) was used to perform the statistical analysis. The Kolmogorov-Smirnov test was employed to investigate the normality distribution of the variables. The correlation between the cell counts of CB units before and after volume reduction were analyzed by means of Spearman correlation coefficient (rho [ρ]). The U test and Wilcoxon test for continuous variables were used to compare the groups when applicable. For categorical variables, the groups were compared by the chi-square test or the Fisher test. Multivariate analysis was performed by backward stepwise regression. A p value of less than 0.05 was considered to be significant.

RESULTS

Cell recovery

Only the variables RBC and lymphocyte percentage showed a normal distribution. Monitoring the TNCs, lymphocytes, CD34+ cells, and CFU content in both preprocess and postprocess CB units assessed the volume reduction process. Table 1 shows the results of CB units that were volume-reduced during the development stage. The CB units processed in both groups had comparable volume and cell counts before and after volume reduction, except for RBCs, which were significantly greater for the Optipress II group. Recoveries of CD34+ and RBC depletion were significantly better for the HES group.

Cell recovery was analyzed for all but the CD34+ and CFU when this method was introduced into the routine processing laboratory. Cells counts for routine processing are shown in Table 2. The correlation between TNC before and after volume reduction for both groups is shown in Fig. 1. TNC and lymphocyte recoveries were significantly better for CB units processed by the Optipress II system. There was, however, significantly less depletion of RBCs for this group. There was difference for all variables before and after volume reduction, except for lymphocyte percentage in HES group, which showed similar results. Figure 2 shows the mean recoveries of TNCs, lymphocytes, and RBCs for both groups ($p < 0.001$ for all variables). Total CD34+ cells and viability after volume reduction were $4.2 \times 10^6 \pm 3.1 \times 10^6$ and 92 ± 4 percent for the HES group and $4.5 \times 10^6 \pm 1.4 \times 10^6$ and 93 ± 5 percent for the Optipress II group (not significant [NS]). When analyzing the TNC, lymphocyte, and RBC recoveries according to the

TABLE 1. Results of volume reduction processing for initial development groups*

Variable†	Before processing	After processing	Recovery (%)
Volume (mL)			
A	101 ± 15	25	NA‡
B	96.5 ± 17.3	41.9 ± 3.9	
P value	NS		
TNCs ($\times 10^7$)			
A	91.4 ± 46	72.5 ± 39.4	78.5 ± 10.2
B	95.2 ± 30	72.3 ± 25.4	78 ± 10.8
P value	NS	NS	NS
RBCs ($\times 10^{11}$)			
A	4.9 ± 2.3	0.03 ± 0.07	24.3 ± 15.3
B	3.2 ± 1	1.5 ± 3	50.5 ± 12
P value	<0.05	<0.005	<0.001
CD34+ cells ($\times 10^5$)			
A	26.3 ± 18.4	31.4 ± 25.2	105 ± 25
B	32.6 ± 21.3	27.9 ± 19.9	89.5 ± 23
P value	NS	NS	<0.05
CFUs ($\times 10^4$)			
A	120.8 ± 92.4	104.6 ± 80.22	102.5 ± 81.1
B	137.11 ± 83.1	125.93 ± 83.15	92.6 ± 27.8
P value	NS	NS	NS

* Data are reported as mean ± SD. NS = not significant.
† A = HES method (n = 50); B = Optipress II method (n = 32).
‡ NA = not applicable.

initial volume of the units of less than 80 mL and at least 80 mL, we found no differences of TNC recovery for any group. For the HES method, however, units containing more than 80 mL had lower lymphocyte recovery (81 ± 5 vs. 74 ± 9, $p < 0.005$). For the Optipress II units lymphocyte recovery was similar for both groups (82 ± 10 vs. 81 ± 16; NS).

Multivariate analysis performed on HES group showed that the main significant factors negatively affecting lymphocyte recovery were TNC ($p < 0.05$) and lymphocyte percentages ($p < 0.005$). For the Optipress II group, TNC and lymphocyte percentages positively influenced TNC recovery ($p < 0.05$).

The time required for CB processing with the Optipress II was reduced to 25 ± 5 minutes. This was significantly shorter than the time needed for volume reduction by addition of HES (55 ± 10 min).

RBC depletion

For the development group, the mean RBC depletion was 75 ± 15 percent for HES sedimentation and 55.4 ± 9 percent for Optipress II fractionation ($p < 0.001$). The mean RBC depletion for the routine group was 80.1 ± 8.3 for HES and 56.4 ± 14.4 for the Optipress II ($p < 0.001$). For both groups, the RBC depletion was higher for larger units (>80 mL). A significant correlation between initial volume and RBC depletion for each group was observed, as shown in Fig. 3.

Multivariate analysis showed that for the HES group the total number of RBCs ($p < 0.05$) determined RBC depletion. For CB units volume-reduced with the Optipress II, RBC depletion was influenced by volume ($p < 0.001$) and RBC content ($p < 0.05$).

Quality control of cryopreservation

Quality control of the cryopreservation procedure was evaluated by clonogenic assays that were cultured directly from a thawed cryovial. Results are shown in Table 3. When analyzing all cases, a negative significant correlation was observed between CFU–GM and RBCs before cryopreservation, as shown in Fig. 4. This correlation was significant also for the Optipress II group, but not for the HES group.

TABLE 2. Cell counts for routine processing*

Variable†	Before processing	After processing	Recovery (%)
Volume (mL)			
A	111 ± 25.9	48 ± 5	NA‡
B	110.2 ± 25.2	48 ± 6	
TNC ($\times 10^8$)			
A	14.1 ± 4.2	10.6 ± 3.2	74.7 ± 8.2
B	14.3 ± 5	11.3 ± 4	78.7 ± 7.8
P value	NS	0.043	<0.001
RBC ($\times 10^{11}$)			
A	3.87 ± 1.05	0.72 ± 0.22	19.8 ± 8.3
B	4.02 ± 1.1	1.64 ± 0.32	43.5 ± 14
P value	NS	<0.001	<0.001
Hct			
A	38.5 ± 16.1	16.7 ± 8.9	45 ± 24.5
B	38.1 ± 1.8	35.6 ± 6.4	97 ± 14
P value	NS	<0.001	<0.001
Lymphocytes (%)			
A	32.2 ± 13.2	35.6 ± 7.4	99.5 ± 10.3
B	35.9 ± 9.7	37.1 ± 9.7	103.2 ± 17.2
P value	0.048	NS	<0.001
Lymphocytes ($\times 10^8$)			
A	4.5 ± 2.4	3.7 ± 1.2	74.5 ± 9.4
B	5.1 ± 2.1	4.1 ± 1.4	80.9 ± 16.3
P value	<0.05	<0.001	<0.001

* Data are reported as mean ± SD.
† A = HES method (n = 350); B = Optipress II method (n = 325).
‡ NA = not applicable.

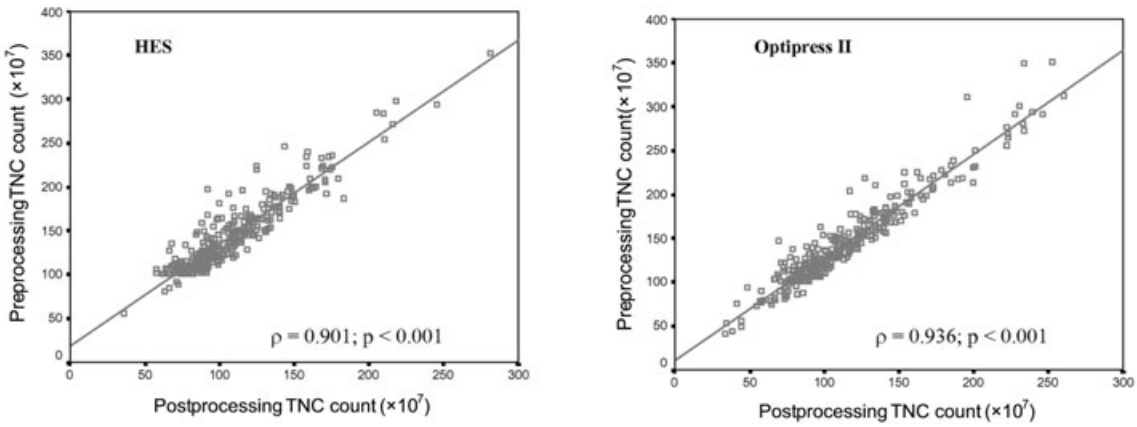

Fig. 1. Relationship between TNC before and after volume reduction for both methods.

Microbiologic results

Sterility control was positive for 13 of 350 (3.7%) for the HES method and 4 of 325 (1.2%) for the Optipress II method ($p = 0.042$).

DISCUSSION

The main goal of CB banks is to provide quality-processed units that can be transplanted into the largest possible number of patients. A major concern of CB banking is the limited storage space available in nitrogen tanks. The reduction of volume not only technically maximizes storage space, but also clinically alleviates the toxicity resulting from the infusion of larger volumes of DMSO and hemolyzed products in the unfractionated infusates.[17] Thus, a cost-effective processing procedure is the ultimate objective of clinical CB banking.

Many techniques have been tested for volume reduction purposes, but to date most transplants have involved HES-processed CB from the New York Blood Center.[18,19] In fact, better recovery of HPCs has been demonstrated with

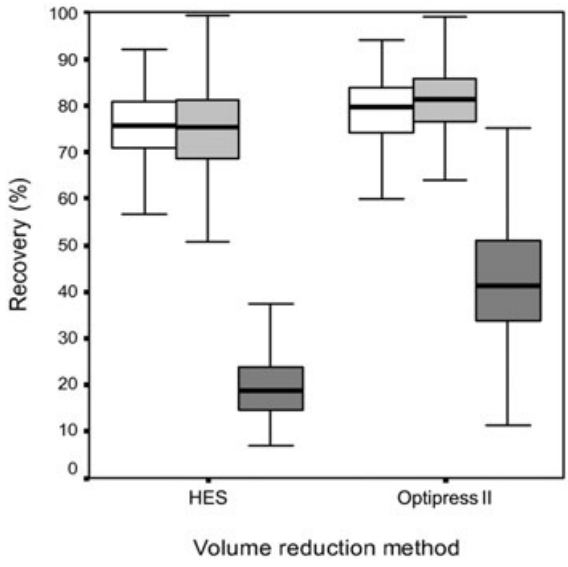

Fig. 2. Boxplot showing the differences for TNC (□), lymphocyte (▨), and RBC (■) recoveries according to the volume reduction method ($p < 0.001$ for all three variables).

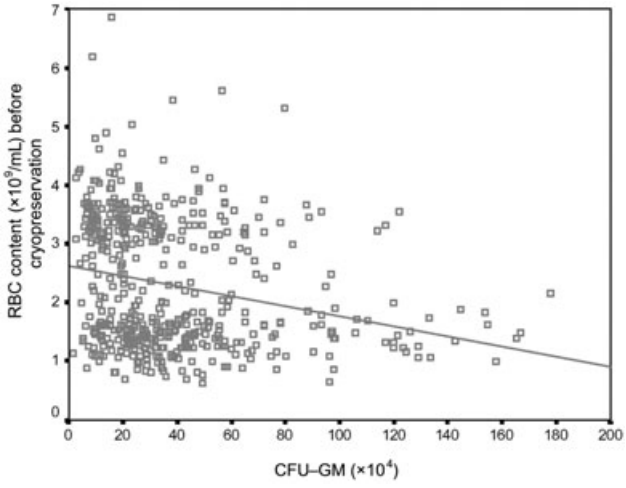

Fig. 4. Relationship between the CFU–GM and RBC content of CB units ($n = 675$; $\rho = -0.277$; $p < 0.001$).

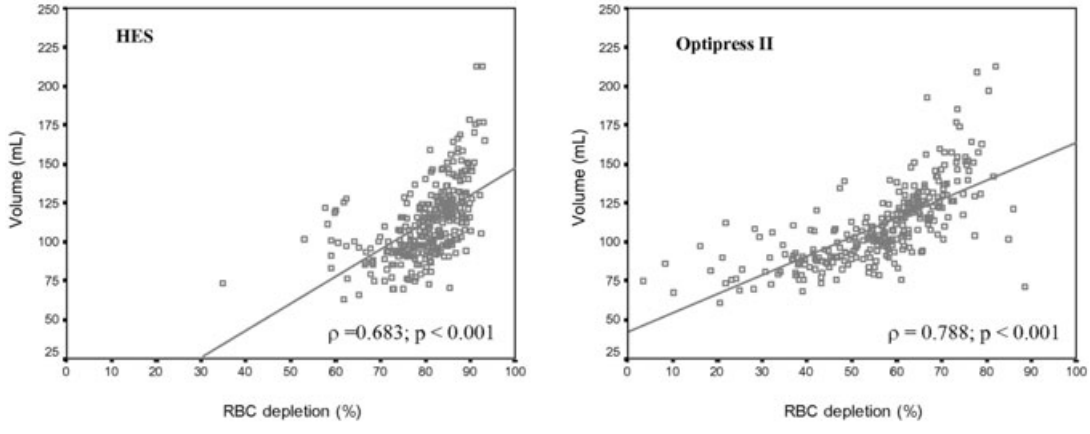

Fig. 3. Relationship between the volume and RBC depletion of CB units according to the volume reduction method.

TABLE 3. Clonogenic assays results according to the volume reduction method

Volume reduction method	BFU–E (×10⁴)	CFU–GM (×10⁴)	CFU–GEMM (×10⁴)	CFU (×10⁴)
HES (n = 350)	54 ± 36.3	43.3 ± 34.2	6.1 ± 8.5	102.8 ± 72.5
Optipress II (n = 325)	53.3 ± 34.9	29.7 ± 23.4	4.6 ± 5.4	87.2 ± 60.2
P value	NS	<0.001	NS	<0.001

HES compared to Ficoll and Percoll.[8] In this work, we have compared two closed systems for volume reduction of CB units: a method of HES sedimentation and a semi-automated method with the Optipress II. To our knowledge, there is no study comparing the benefits and disadvantages of these two methods for routine CB banking.

The HES method is a manual technique that requires two centrifugation steps. In contrast, the Optipress II is usually used for top-and-bottom separation of blood components in transfusion centers.[20] Using the Optipress II, the London Cord Blood Bank achieved partial removal of both plasma and RBCs from the CB collections after one centrifugation step with the standardization of all CB units to 25 mL.[21] The clinical usefulness of these units has also been proved.[22] The CB collections from London Cord Blood Bank, however, were transferred to an Optipac triple system from which the anticoagulant had been removed under sterile conditions. To avoid this step, we used a triple-bag system with adjusted anticoagulant for CB collections. This method requires only one centrifugation step and significantly reduces the handling and time of processing.

Similar recoveries of TNCs and CFUs were demonstrated in development group for both methods. Only the CD34+ cell recovery was significantly better for the HES group. Similar CD34+ contents after volume reduction but significantly higher recovery of TNCs and lymphocytes, however, were observed for the Optipress II in routine groups when the process was systematically repeated. Besides, recovery of lymphocytes percentages was 103.2 ± 17.2 percent, which means that the percentage of lymphocytes was systematically higher after volume reduction with the Optipress II than before processing. The negative influence of TNC and lymphocyte counts on lymphocyte recovery in CB volume-reduced with HES was noteworthy. The RBCs may entrap mononuclear cells during the process of HES sedimentation and could explain the lower cell recoveries. In contrast, higher TNC and lymphocyte counts allow better cell recoveries for CB processed by the Optipress II. For those units processed with the Optipress II, RBC reduction in the final product is similar to that reported by Armitage and associates[11] and other authors who used automatic devices[13] but significantly less effective than the RBC reduction obtained by HES centrifugation.[23] For the Optipress II method, volume influences RBC depletion, which means the greater volume of CB the higher the RBC depletion. Taking into account the fact that larger units are selected for banking, volume reduction with the Optipress II not only increases the quality of stored units but also optimizes the method of volume reduction.

In spite of more TNC and lymphocyte contents and similar content of CD34+ cells in CB units processed with the Optipress II, CFU contents after cryopreservation were lower than for the HES method. We investigated the causes of this difference, and we found a negative influence of RBC content in CB sample before cryopreservation on CFU counts. Other authors, also with a direct plating method, have found the negative influence of RBC content on CFU–GM growth if a concentration of greater than 0.02×10^9 per mL was present in the CFU medium.[24] We have not determined the concentration of RBCs in medium, and we have no explanation for these data. A possible influence of HES in protecting HPCs during the cryopreservation process could explain these results. Whether these in vitro results have in vivo repercussion remains unknown.

The main factor influencing results of microbiologic control of CB units is the volume of the sample cultured.[25] Taking the same volume for both groups, we observed a lower contamination rate in CB units processed with the Optipress II, as previously reported.[21,25]

In recent years, automatic devices have been developed to process low-volume cell suspensions as CB collections.[13] The main advantages of these systems are the standardized cell processing, the significantly reduced processing time, and the closed system for performing the complete preparation. The main disadvantage is the cost that makes it difficult to acquire for smaller CB banks. We think that the Optipress II is an inexpensive alternative to these methods and especially suitable for a CB bank integrated in a transfusion center.

In conclusion, the volume reduction method with the Optipress II is a closed, time-saving system that allows good cell recoveries. In addition, this procedure allows standardization of the process and also reduces the microbiologic contamination rate. In contrast, the main advantage of the HES method is the higher RBC depletion that influences CFU content. CB banks must choose the volume reduction method according to their characteristics and objectives. Reducing RBC content must be the subject of further improvements for volume reduction using the Optipress II method. Addition of HES and posterior semi-automated fractionation could be the subject of further studies.

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