Qualitative and quantitative cell recovery in umbilical cord blood processed by two automated devices in routine cord blood banking: a comparative study

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Background. Volume reduction is a widely used procedure in umbilical cord blood banking. It concentrates progenitor cells by reducing plasma and red blood cells, thereby optimising the use of storage space. Sepax and AXP are automated systems specifically developed for umbilical cord blood processing. These systems basically consist of a bag processing set into which cord blood is transferred and a device that automatically separates the different components during centrifugation.

Methods. The aim of this study was to analyse and compare cell recovery of umbilical cord blood units processed with Sepax and AXP at Valencia Cord Blood Bank. Cell counts were performed before and after volume reduction with AXP and Sepax.

Results. When analysing all the data (n =1,000 for AXP and n= 670 for Sepax), the percentages of total nucleated cell recovery and red blood cell depletion were 76.76±7.51% and 88.28±5.62%, respectively, for AXP and 78.81±7.25% and 88.32±7.94%, respectively, for Sepax ($P < 0.005$ for both variables). $CD34^+$ cell recovery and viability in umbilical cord blood units were similar with both devices. Mononuclear cell recovery was significantly higher when the Sepax system was used.

Discussion. Both the Sepax and AXP automated systems achieve acceptable total nucleated cell recovery and good $CD34^+$ cell recovery after volume reduction of umbilical cord blood units and maintain cell viability. It should be noted that total nucleated cell recovery is significantly better with the Sepax system. Both systems deplete red blood cells efficiently, especially AXP which works without hydroxyethyl starch.

Keywords: cord blood, volume reduction, haematopoietic progenitors.

Introduction

Umbilical cord blood (UCB) banking has become a routine activity for providing hematopoietic progenitor cells for transplantation[1]. To date, more than 20,000 UCB transplants have been performed on children and adults[2]. UCB transplantation offers important advantages, including faster availability of banked units, lower incidence of acute Graft-versus-Host disease and lower risk of transmitting viral infections such as cytomegalovirus, compared to transplantation of hematopoietic progenitor cells from other sources. Many UCB banks have been established all over the world in order to facilitate transplantation activity[3]. More than 400,000 UCB units are currently available in over 50 UCB banks throughout the world[2]. One of the most important objectives of these banks is to cryopreserve and store high quality UCB units. For this reason, they must operate under strict quality conditions that guarantee optimal cell function[4]. Volume reduction is a widely used procedure to concentrate the progenitor cells by reducing the plasma and red blood cell (RBC) content in UCB units, thereby optimising the use of storage space. This procedure must ensure high cell recovery, cell viability and RBC depletion by reducing the UCB units to a standard volume. Although the first described procedure for this purpose was manual and used the sedimentation agent hydroxyethyl starch (HES), the current trend is to use automated systems[5]. Sepax (Biosafe S.A. Eysins/Nyon, Switzerland) and the AutoXpress Platform (AXP) (Thermogenesis Corp., Rancho Cordova, California, United States of America) are two automatic systems specifically developed for UCB processing[6,7]. Basically, they consist of a bag processing set into which cord blood is transferred and a device that automatically separates the different components during centrifugation. While the most commonly used protocol for the Sepax system involves the use of HES, AXP reduces the UCB to a precise volume (usually 20 mL) without HES[5].

Valencia Cord Blood Bank has used both devices consecutively in routine cord blood banking. The aim of this study was to analyse and compare cell recovery

of UCB units processed with Sepax and AXP. To our knowledge, this is the first study comparing cell recoveries of UCB units processed, for routine volume reduction and banking, with these two devices.

Material and Methods

Umbilical cord blood collection

Maternal and neonatal pairs were evaluated during the antenatal period in the maternity wards at different hospitals collaborating with the UCB program in Valencia. Donors signed informed consent before delivery. UCB was collected from the umbilical vein by gravity into a simple bag (R MSC 1201 DU, MacoPharma, Tourcoing Cedex, France) containing 21 mL of citrate-phosphate-dextrose. This procedure was performed by trained midwives after delivery of the placenta. The UCB was stored at 4±2 ºC until processing for cryopreservation, within 48 hours of collection. UCB was transported to the bank at a temperature between 4 ºC and 22 ºC. The collection methodology was the same for all the units included in this study. Only UCB units with a total nucleated cell (TNC) count of at least 100×10^7 (from 2003), 120×10^7 (from 2009) or 140×10^7 (from 2010) were volume-reduced and cryopreserved. From January 2008 $CD34^+$ cell content was added as a selection criterion ($\geq30\times10^5$).

Volume reduction with the AXP automated system (2008-2009)

The AXP system consists of a microprocessor-controlled device and a disposable closed blood bag set. The device contains different compartments for housing the processing set and flow control optical sensors that are used to achieve the separation of a concentrated mononuclear cell (MNC) fraction of uniform volume. The UCB is transferred to the bag set by means of sterile dock tubing system (Terumo TSCD SC-201), through a clot filter and loaded into the AXP device. During the two-step centrifugation, whole blood is separated into three layers that are delivered into a RBC bag and a freezing bag. Plasma remains in the processing/plasma bag. The AXP device fits most standard blood bank centrifuge buckets. In Valencia Cord Blood Bank, two UCB units were centrifuged at the same time. The programmed final volume in the cryopreservation bag was 21 mL. Higher volume UCB units (≥170 mL) were split and processed in two different kits and cryopreserved in two bags. Samples were taken by sampling pillows integrated within the kit.

Volume reduction with the Sepax automated system (2009-2010)

The Sepax system consists of the main Sepax unit and the single-use kits. This system uses a rotating syringe technology that provides both separation of the syringe chamber (centrifugation) and component transfer through displacement of the syringe piston. An optical line sensor monitors the different components passing through the tubes. The processing kits are composed of a proprietary separation chamber, tubing and collection/by-product bags. These kits are compatible with standard input bags. This system can be used with several different protocols for UCB and other cell processing; the UCB-HES protocol is the one most frequently used in UCB banking. In the Valencia Cord Blood Bank, a solution of HES (Grifols, Barcelona, Spain) corresponding to 20% of the UCB input volume was injected into the input bag at a rate of approximately 0.5 mL/sec. The input bag was connected to the kit (CS-530.3) using the pre-installed spike connection in the kit input line. The processing kit was then placed in the main Sepax unit. Only one UCB unit could be volume reduced each time. The final product (usually 21 mL) was collected directly into the freezing bag.

Biological controls

TNC count, $CD34^+$ cell counts and CD34+ cell viability were determined on samples obtained from the UCB before and after volume reduction. Microbiological controls were performed after volume reduction and before cryopreservation. Nucleated cells were counted with an autoanalyser (Sysmex K800, Toa Medical Electronics, Japan) and the TNC count was calculated. The counts were not corrected for nucleated RBC. The $CD34^+$ cell content was quantified by flow cytometry. UCB (5×10^5 cells) was incubated with CD34 and CD45 monoclonal antibodies conjugated to fluorescein and phycoerythrin, respectively (Becton Dickinson, San Jose, CA, USA) and 7-aminoactinomycin D was used as a marker of DNA staining. Flow cytometric analysis was performed with computer software (Cell Quest, Becton Dickinson) and from January 2008 with DiVa software. Ethidium bromide and acridine orange were used to assess cell viability. Clonogenic assays were performed using a commercially prepared complete methylcellulose medium (Methocult GF H4434), supporting growth of CFU-GM, BFU-E and CFU-GEMM. Colony-forming units were calculated as the sum of these three kinds of colonies. Samples were drawn from a thawed segment attached to the bag, taking a previously calculated volume (between 0.010 and 0.020 µL/plate) according to the TNC and $CD34^+$ cell counts of the UCB units prior to cryopreservation. Cultures were plated in duplicate 35 mm diameter Petri dishes and incubated for 14 days in a humidified atmosphere at 37 ºC in 5% CO_2. Colonies, defined as aggregates of more than 40 cells, were counted under an inverted microscope. Only UCB units requested for quality control or HLA confirmation were used for clonogenic assays.

UCB samples consisting of mixed plasma and RBC (waste product) were screened for bacterial and

fungal contamination using an automated blood culture system (BacT/ALERT, Organon Teknika, bioMérieux, Hazelwood, MO, USA) at 35 ºC for 14 days.

Statistical analysis

Descriptive statistics are presented for UCB variables. Results are expressed as mean±SD. Computer software (SPSS, version 13, SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. The Kolmogorov-Smirnov test was employed to investigate the normality of the distribution of the variables. The Mann-Whitney U-test for continuous variables was used to compare the groups when applicable. Wilcoxon's test was used for the analysis of two related samples. The correlation between different variables was analysed by means of Spearman's correlation coefficient (rho). The chi-square test or Fisher's test was used to compare categorical variables between groups. A P value of less than 0.05 was considered to be statistically significant.

Results

Overall, 1,000 and 670 UCB units were processed with the AXP and Sepax systems, respectively. Table I shows the data for UCB units before and after volume reduction by the two systems. Analysis of these data showed statistically significant differences in initial volume and cell counts between UCB units processed with the two devices. In order to overcome this limitation of the overall data analysis, Table II shows the same parameters for those UCB units with a TNC count between 120 and 150×10^7. When the analysis was limited only to these units, the initial data were similar for units processed with both devices, except for the haematocrit. In all cases the final volume included 5 mL of cryopreservation solution. There were no statistically significant differences between the data in Tables I and II for TNC, lymphocyte, $CD34^+$ recovery and RBC depletion for units processed with the AXP system. For units processed with the Sepax system, the only statistically significant difference between the data in the two tables was for lymphocyte depletion (P =0.000).

Figure 1 shows the mean TNC recovery of routinely processed UCB units according to whether the AXP or Sepax system was used. TNC recovery was less than 60% in 5.5% and 2.2% of UCB units processed with the AXP and Sepax system, respectively (P =0.001). A haematocrit more than 40% was detected in 1.2% and 8.9% of UCB units processed with AXP and Sepax, respectively (P =0.00). The correlation between TNC count before and after volume reduction was statistically significant for AXP (rho=0.929, P =0.000) and Sepax (rho=0.932, P =0.000), as was the correlation between $CD34^+$ cell content before and after volume reduction (rho =0.865, P =0.000 for AXP and rho =0.867, P =0.000 for Sepax). When comparing the variables

Table I - Data from UCB units processed with AXP or Sepax. Results are expressed as mean±S.D.

	Before volume reduction		
	AXP	**Sepax**	**P**
N.	1,000	670	
Volume (mL)	108.28±23.29	114.95±24.44	0.000
TNC×10^7	144.28±43.62	165.00±47.02	0.000
Haematocrit	39.01±3.70	39.03±3.64	0.916
RBC×10^9/mL	3.50±0.37	3.50±0.36	0.875
Platelets×10^6	231.78±53.69	231.52±61.78	0.985
Lymphocytes (%)	35.53±7.73	35.57±7.50	0.760
$CD34^+$ cells (%)	0.43±0.23	0.43±0.21	0.536
$CD34^+$ cells×10^5	60.63±36.34	71.14±56.02	0.000
	After volume reduction		
	AXP	**Sepax**	**P**
Volume (mL)	24.61±3.64	24.06±1.30	0.000
TNCx10^7	105.23±31.81	124.45±35.07	0.000
TNC recovery (%)	76.76±7.51	78.81±7.25	0.000
TNC loss (%)	23.83 ± 7.83	21.19 ± 7.24	0.000
Haematocrit	27.01±8.34	27.56±9.04	0.275
RBC×10^9	2.20±0.36	2.39±0.80	0.000
RBC depletion (%)	88.28±5.62	88.32±7.94	0.002
Lymphocytes (%)	34.74±8.86	33.53±8.09	0.005
Lymphocyte recovery (%)	74.47±13.91	74.45±13.25	0.292
Lymphocytes loss (%)	25.53 ± 13.91	25.55 ± 13.25	0.292
$CD34^+$ cells (%)	0.49±0.30	0.50±0.30	0.557
$CD34^+$×10^5	51.79±37.17	63.17±44.30	0.000
$CD34^+$ cell recovery (%)	99.11±29.82	102.16±28.12	0.155
Viability (%)	89.72±6.70	90.19±5.41	0.829

Legend TNC: total nucleated cells; RBC: red blood cells.

Table II - Data from UCB units containing between 120 and 150 TNC × 10^7 processed with AXP or Sepax. Results are expressed as mean±S.D.

	Before volume reduction		
	AXP	**Sepax**	**P**
N	328	330	
Volume (mL)	105.58±16.83	104.53±18.53	0.284
TNC×10^7	134.19±8.79	134.28±8.62	0.873
Haematocrit	39.02±3.31	38.44±3.34	0.039
RBC×10^9/mL	3.50±0.33	3.45±0.35	0.052
Lymphocytes (%)	35.56±8.08	35.34±7.58	0.732
$CD34^+$ cells (%)	0.45±0.23	0.41±0.18	0.174
$CD34^+$×10^5	60.71±31.90	55.48±24.19	0.233
	After volume reduction		
	AXP	**Sepax**	**P**
Volume (mL)	24.07±0.36	24.06±1.41	0.001
TNC×10^7	102.54±12.57	106.10±10.89	0.001
TNC recovery (%)	76.41±7.85	79.05±6.76	0.000
TNC loss (%)	23.58±7.85	20.94±6.76	0.000
Haematocrit	26.91±3.31	27.75±9.41	0.699
RBC×10^9	2.19±0.24	2.28±0.79	0.764
RBC depletion (%)	88.66±4.03	87.29±8.94	0.392
Lymphocytes (%)	34.89±8.91	34.22±7.91	0.410
Lymphocyte recovery (%)	75.00±16.17	76.76±12.23	0.001
Lymphocyte loss (%)	25.01±16.11	23.30±12.06	0.001
$CD34^+$ cells (%)	0.48±0.28	0.48±0.27	0.902
$CD34^{+x}10^5$	51.68±30.68	53.60±30.25	0.469
$CD34^+$ cell recovery (%)	99.45±23.39	104.62±25.30	0.238
Viability (%)	87.48±9.55	88.31±8.98	0.840

Legend TNC: total nucleated cells; RBC: red blood cells.

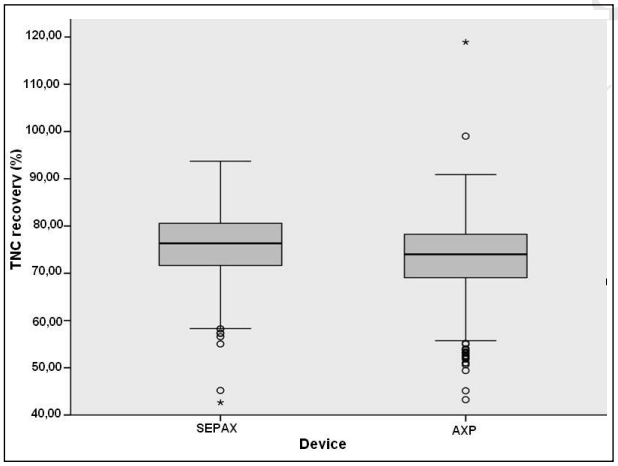

Figure 1 - Box plot showing mean TNC recovery according to the volume reduction device used: AXP (n =1,000) or Sepax (n =670) (P <0.001).

TNC, RBC, lymphocytes and $CD34^+$ cell absolute contents before and after volume reduction, the differences were statistically significant for both devices (P =0.000 for all variables). When analysing all cases, significant correlations were detected between initial volume and RBC depletion (rho =0.623, P =0.000) and between TNC and RBC depletion (rho=0.443, P =0.000). The higher the TNC content and volume, the greater the RBC depletion. These correlations were maintained when analysing each device separately. There were also significant correlations between TNC counts, lymphocytes and $CD34^+$ cell recovery for both devices (P =0.000). There was a significant inverse correlation between TNC recovery and RBC depletion, only for UCB units processed with Sepax (rho= –0.225, P =0.000).

Table III shows the different recovery of leucocyte populations in the UCB units processed with AXP and Sepax. The mean mononucleated cell (monocytes + lymphocytes) loss for the UCB units processed with AXP (n=413) was 26.10±7.86 % while that for the UCB processed with Sepax (n=276) was 24.50±6.90% (P =0.002).

Table IV shows the UCB parameters after volume reduction with the AXP and Sepax systems according to the initial volume and TNC, focusing on cell recoveries. The higher the initial TNC content and greater the volume of the UCB units, the greater the RBC depletion and the lower the lymphocyte recovery for both devices.

Clonogenic assays after cryopreservation and thawing showed that there were 105.15±71.96×10^4 CFU in UCB units processed with AXP (n =15) and 113.75±23.94×10^4 in units processed with Sepax (n =10) (P =0.80).

The percentage of UCB units refused because of problems during processing was 6.5% for AXP (8.8% during the first year and 3.6% during the second) and 1.0% for Sepax (P =0.00). The main problems leading to refusal were a TNC recovery below 60% and a higher than planned final volume. Microbiological cultures

Table III - Different recoveries of leucocyte populations with AXP and Sepax. Results are expressed as mean±S.D.

	Before volume reduction		
	AXP	**Sepax**	**P**
N.	413	276	
TNC×10^7	144.46±37.98	162.03±41.52	0.000
Neutrophils (%)	53.51±7.99	53.34±7.66	0.574
Lymphocytes (%)	35.28±7.41	35.33±7.09	0.747
Monocytes (%)	11.07±3.10	11.32±3.16	0.301
Platelets×10^6	228.76±49.31	222.42±45.84	0.044
	After volume reduction		
	AXP	**Sepax**	**P**
TNC×10^7	105.36±27.50	124.91±32.74	0.000
Neutrophils (%)	54.54±8.92	55.50±8.25	0.157
Neutrophil recovery (%)	77.82±12.64	82.85±10.24	0.000
Neutrophil loss (%)	22.17 ± 12.64	17.14 ± 10.24	0.000
Lymphocytes (%)	34.43±7.89	33.33±7.24	0.053
Lymphocyte recovery (%)	73.60±7.38	74.63±5.91	0.045
Lymphocyte loss (%)	26.39 ± 7.38	25.36 ± 5.91	0.045
Monocytes (%)	10.98±3.79	11.15±4.03	0.995
Monocyte recovery (%)	76.33±23.57	78.98±21.17	0.074
Monocyte loss (%)	23.66 ± 23.57	21.01 ± 21.17	0.074
Mononucleated cell recovery (%)	73.89±7.86	75.49±6.90	0.002
Mononucleated cell loss (%)	26.10 ± 7.86	24.50 ± 6.90	0.002
Platelets×10^6	942.49±297.76	646±184.63	0.000
Platelet recovery (%)	100.43±14.16	65.74±11.54	0.000

Legend TNC: total nucleated cells.

Table IV - Volume and cell recovery after volume reduction with AXP or Sepax according to initial volume and total nucleated cell content of the USB units. Results are expressed as mean±S.D.

	Volume (mL)			TNC×107		
	≤140	**>140**	**p**	**≤140**	**>140**	**p**
N						
AXP	907	91		537	453	
Sepax	563	103		221	441	
Volume (mL)						
AXP	24.14±1.49	29.36±10	0.000	24.13±0.73	25.20±5.30	0.943
Sepax	24.04±0.93	24.17±2.52	0.336	24.01±0.52	24.09±1.56	0.461
P	0.00	0.00		0.04	0.00	
TNC recovery (%)						
AXP	76.10±7.84	76.71±7.83	0.280	76.40±8.03	75.80±7.58	0.245
Sepax	78.68±7.04	79.39±8.32	0.302	79.26±6.48	78.54±7.60	0.324
P	0.00	0.02		0.00	0.00	
RBC depletion (%)						
AXP	84.29±5.28	89.82±4.06	0.000	82.66±5.56	87.29±3.99	0.000
Sepax	84.14±7.91	88.43±4.25	0.000	82.11±9.66	86.14±5.96	0.000
P	0.90	0.03		0.34	0.00	
Lymphocyte recovery (%)						
AXP	74.53±13.29	73.76±20.04	0.026	75.71±10.11	72.90±17.45	0.000
Sepax	75.14±13.34	70.55±12.20	0.000	77.81±12.72	72.67±13.25	0.000
P	0.98	0.22		0.09	0.83	
CD34+ cell recovery (%)						
AXP	99.27±30.74	97.61±18.92	0.747	101.17±24.56	96.68±35.32	0.013
Sepax	101.73±26.35	104.56±38.59	0.846	103.10±31.24	101.47±26.34	0.517
P	0.17	0.32		0.98	0.14	
Viability (%)						
AXP	87.46±8.94	88.94±7.67	0.308	88.96±6.95	90.64±6.27	0.000
Sepax	90.17±5.47	90.33±5.11	0.888	87.99±9.04	89.44±8.17	0.017
P	0.01	0.05		0.32	0.05	

Legend TNC: total nucleated cells; RBC: red blood cells.

were positive in 3.4% (n =34) of the UCB units volume reduced with the AXP system and in 4% (n =27) of those processed with Sepax (P =ns).

The time for processing one UCB unit was 45±10 minutes and was similar for both volume reduction devices.

Discussion

From the time when UCB banks were established up to now, various different procedures have been described for volume reduction. These procedures are based on plasma and RBC depletion and the cell recovery is highly variable[8-11]. In recent years, automatic systems (Sepax and AXP) have been specifically developed for the purpose of reducing the volume of UCB units[12,13]. In general, these are preferred to manual methods because of better standardisation and reproducibility and less influence from the operator. Although there are substantial differences in working and management, this study is focused mainly on the analysis of cellular recovery.

The TNC dose, $CD34^+$ cell count and clonogenic assays are currently considered the most important parameters associated with UCB transplantation outcome[14]. While there is large inter-laboratory variability in the latter two variables, TNC is a well-standardised variable that is a surrogate marker of the hematopoietic progenitor cell content[15]. In fact, the probability and speed of engraftment decrease significantly if the infused UCB TNC content is below 2.5×10^7/Kg[5]. UCB banks must, therefore, optimise the procedures (mainly reduction volume) to recover as many cells as possible from UCB collections, usually to achieve a recovery of more than 75-80% TNC. In our experience, TNC recovery was significantly higher for UCB units processed with Sepax rather than with AXP. The average TNC recovery with Sepax was in the range of 78-87%, while that for AXP was lower, in the range of 76-84%[16,17] in different UCB banks. Our data for both devices are at the low limit of these results. In our hands, there was a TNC loss of about 21% with Sepax and around 23% with AXP, in routine processing. This statistically significant difference was present in both the overall data analysis (Table I) and in the homogeneous sample analysis (Table II). It has been showed that the higher the initial cell count, the lower the cell recovery after volume reduction for HES and top and bottom systems[18]. This is not, however, the case for AXP or Sepax. Differences in TNC recovery are consistent with the data in Table IV which show that UCB volume and cellularity do not affect this parameter, but do affect lymphocyte recovery. Haematopoietic progenitor cell recovery ($CD34^+$ cells) was similar for both devices and not influenced by initial cell content. The CD34+ cell recovery was lower for UCB units with a higher TNC content only for those units processed with AXP. Taking into account the low level of standardisation of the test, this result should be considered with caution.

The results of clonogenic cultures must be considered approximate because of the low number of cases.

It has been reported that the loss of up to 30% of neutrophils is the main cause of the decreased TNC recovery with the AXP system, while more than 95% of mononuclear cells are recovered[5]. We also detected a greater loss of neutrophils for UCB processed with AXP as compared to Sepax, but also a lower mononuclear cell recovery. Neutrophil recovery, but also mononuclear cell recovery (75.49%) was greater with Sepax than with AXP (mononuclear cell recovery of about 74%). These data show a poorer TNC recovery for the AXP device and also significantly lower recovery than previously published[5]. However, $CD34^+$ cell recovery and viability were similar for both devices. $CD34^+$ is a hematopoietic progenitor cell marker, but again the lack of a standardised method for $CD34^+$ cell quantification makes it difficult to compare results among banks. It should be noted that platelet counts were significantly higher in those UCB units processed with AXP. Higher platelet counts can cause aggregation problems during the thawing procedure.

An important difference between the evaluated devices is the use of HES. This is a sedimentation agent that increases the volume of the UCB and improves the separation of cells and RBC depletion. The most widely used protocol with the Sepax device requires the addition of HES to the UCB unit[6,12,18], while AXP currently operates without this agent[19]. Zingsem *et al.*[20] reported a TNC recovery of 78.6±24.9% for Sepax working without HES, which is not very different from our results with UCB units processed with AXP. This TNC recovery has been clearly improved by adding HES[6,12]. The addition of HES to UCB units processed with AXP also enhances average mononuclear cell recovery to more than 90%[17,21]. In general, the use of HES improves TNC recovery and RBC depletion with the two devices. Nevertheless, the use of HES is controversial. In addition to improving RBC depletion, it has a cryoprotective effect, mainly in non-controlled rate freezing[22], but it has the important disadvantage of being an exogenous product that must be added and the resulting open system.

The AXP system requires that UCB units of 170 ml or more are split and processed separately. In our hands, the high quality units (TNC $\geq140\times10^7$ or volume ≥140 mL) have better RBC depletion and lower lymphocyte recovery. These results are found with both devices, so they seem to work similarly in some aspects. An important difference occurring only with the Sepax system is that increased cell recovery is associated with decreased RBC depletion efficiency. It is not usually recommended to split large volume UCB units when using the Sepax device, although some authors have

showed better results when single, large-volume UCB units are split into two half subunits before processing[23].

RBC depletion is another goal of volume reduction that has some clear advantages. RBC depletion decreases ABO incompatibility reactions at the time of transplantation and some authors have suggested that the RBC contained in the cord blood product negatively influence the function of the progenitor cells recovered after thawing[24]. With regards to this issue, both devices are highly efficient, achieving RBC depletion of greater than 80% with haematocrits consistently below 30% in the volume-reduced UCB units. In view of these results, we consider that AXP is especially effective for the RBC depletion of UCB units in the absence of HES. A RBC depletion of 44.5±14.6% for Sepax operating without HES has been reported[20], which is significantly lower than the RBC depletion achieved with AXP, also without HES. In fact, the proportion of UCB units with a haematocrit above 40% is higher among the Sepax-processed units than among the AXP-processed ones.

Stem cell transplant recipients are at a high risk of infection. The sterility of the volume reduction process is, therefore, very important. The rate of microbiological positivity was similar for both devices and similar to previously published and acceptable results. In spite of the addition of HES and subsequent manipulation, the rate of microbiological positivity was not significantly higher with the Sepax system.

In conclusion, the Sepax and AXP automated systems both provided acceptable TNC recovery and good $CD34^+$ cell recovery after UCB unit volume reduction, while maintaining cell viability. It should be noted that TNC recovery is significantly better for Sepax. Both devices are efficient in RBC depletion, especially AXP operating without HES.

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