European Journal of Therapeutics
DOI:10.54614/eurjther.2021.0029
Original Article

Manual Versus Automated Volume Reduction of Cord Blood

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ABSTRACT

Objectives: All cord blood banks all over the world follow a common procedure, concentrating progenitor cells by volume reduction, with the main purpose of optimizing the use of storage space. The main objective of this study was to compare CD34 and total nucleated cell recovery rates and red blood cell depletion efficiencies following cord blood processing using automated Sepax or manual CellEffic cord blood processing systems.

Methods: Nine cord blood units with high volumes were divided into 2 equal fractions and processed with CellEffic cord blood and Sepax. Total nucleated cell, mononuclear cells, $CD34^+$, red blood cell and total nucleated cell viability, and clonogenic assays were performed, and recovery rates were calculated on pre- and post-process cord blood units and after freeze/thaw process. In the comparison group, post-thaw differential cell counting was also performed.

Results: Our results showed that post-process total nucleated cell viability with CellEffic cord blood was slightly higher than Sepax, whereas Sepax post-process total nucleated cell/ mononuclear cell values were superior to CellEffic cord blood. Post-thaw red blood cell depletion was better for CellEffic cord blood. Post-thaw Sepax colony-forming unit counts were higher than CellEffic cord blood. In addition, $CD45^+CD71^+$ cells were lower, whereas $CD45^+CD34^+CD38^-$ cells were higher for the CellEffic cord blood system.

Conclusion: Despite the fact that there is a need for well-trained personnel for processing cord blood units with CellEffic cord blood, it may be an attractive alternative to Sepax system for cord blood processing, particularly for cord blood units with low volumes, at banks with low budget where the cord blood turnover rates are relatively low.

Keywords: Hematopoietic Stem cells, cord blood, blood banking

INTRODUCTION

Cord blood (CB) is a significant graft source for hematopoietic stem cell (HSC) transplantation for patients for whom a suitable human leukocyte antigen (HLA)-matched donor is missing.[1] Since 1988, more than 40 000 umbilical CB have been transplanted, both in children and adults.[2,3] Relapse as well as graft versus host disease risk after cord blood transplantation (CBT) is considerably low.[4,5] High quality of a CB unit (CBU) is strongly correlated with shorter engraftment period and rapid immune reconstitution, thus higher survival rates.[1,6] The quality of CBU is highly dependent on the laboratory procedures; mainly processing, cryopreservation, and storage conditions.[7-9] Currently, umbilical CBUs are processed via red blood cell (RBC) depletion and volume reduction. Basically, 2 approaches, automated and manual (centrifugation) processing, are being used worldwide.[10] Three major automated systems are in use for the depletion of excess plasma and RBC from CB, most commonly used are Sepax (Biosafe S.A. Eysins/Nyon, Switzerland), AutoXpress Platform (Cryo-Cell International Inc., Florida, USA, for mononuclear cells was also mentioned in the text as MNC.), and PrepaCyte-CB (Cryo-Cell International, Inc., USA) systems.[11,12] Both automated systems are proven to be efficient, yielding high total nucleated cell (TNC) and $CD34^+$ HSC recovery rates, retaining viabilities. The major advantage of these CB processing systems is the need for a fully closed operating environment, which minimizes the risk of contamination but increases cost. On the other hand, centrifugation may be harmful to quality and quantity of CB HSCs.[13-16] The need for potentially toxic chemical usage, such as hydroxyethyl starch (HES), is another drawback of closed systems.

A novel filtration system was described recently by KANEKA Corporation (2-3-18, Nakanoshima, Kita-ku, Osaka 530-8288, Japan). This filtration system uses a non-chemical-coated/non-woven polyester fabric filter, which traps $CD34^+$ cells through

Cite this article as: Yurdakul Mesutoğlu P, Gencer Öncül EB, Akın HY, Beksaç M. Manual versus automated volume reduction of cord blood. *Eur J Ther.* 2022;28(2):102–108.

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Received: March 14, 2021 **Accepted:** June 28, 2022

affinity without the need for centrifugation and allows manual processing of CB.[16] The main objective of this study was to compare CD34 and TNC recovery rates and RBC depletion efficiencies following CB processing using automated Sepax (Biosafe, S.A. Eysins/Nyon) or manual CellEffic CB technology (KANEKA Corporation, Japan).

METHODS

Collection of CB

Nine CBUs collected in utero from consented maternal donors with volumes >100 mL were included in this study. Cord blood plasma and RBC depletion were performed either with both Sepax/CellEffic CB systems (n = 9). Cord blood units with collection volumes higher than 100 mL were fractioned into 2 bags in equal volumes to be processed with both systems. All CBUs and maternal donors were negative for infectious disease markers, and CBUs were processed within 48 hours after collection. Apart from one CBU which was collected from vaginal delivery, CBUs were obtained from Caesarean section (C/S) and all 9 CBUs were split into 2 equivalent fractions. Thus, 18 units were processed by both systems.

CB Processing with Both Systems – Sepax and CellEffic CB

Nine CBUs were split into 2 bags in equal volumes. Cord blood units were processed as described by Sato et al.[17] Samples from each bag were taken for complete blood count and flow cytometric analyses, in order to evaluate TNC, RBC, mononuclear cell (MNC), neutrophil and CD34 cell counts, and cellular viabilities. Following cell count assessments for each split bag, half of the initial CB was processed with automated Sepax and the other half with CellEffic CB.

Cryopreservation, Thawing, and Recovery Assessment

Five microliters of 5% dimethyl sulfoxide (DMSO) was infused (at a 25 mL/h speed) into all buffy coat (v = 20 mL) products derived either from CellEffic CB or Sepax (n = 18). Cord blood unitswere then transferred into a controlled-rate freezer and relocated into cryogenic tank at liquid nitrogen vapor phase. Reference samples (either segment or vial) from all CB products were thawed after they had been cryopreserved for 45 days for post-thaw analysis.

A single segment attached to the CB product was used to determine HSC subpopulations in split CBUs processed with Sepax or CellEffic CB. Thawed segments were diluted in Roswell Park Memorial Institute (RPMI) (StemCell Technologies [Vancouver, Canada]) with 10% fetal bovine serum (Thermo Fisher Scientific [Waltham, Massachusetts, USA]) with a dilution factor of 1/9.

Main Points

- Sepax post-thaw total nucleated cell/mononuclear cell recovery rates as well as colony-forming unit counts were higher than CellEffic cord blood (CB). Nonetheless, CellEffic CB was by far superior in terms of red blood cell depletion.
- The main drawback of CellEffic CB seems to be the labor-intensive and longer hands-on time nature with the requirement of qualified technicians.
- CellEffic CB can be an alternative system for processing CB at a much lower cost in private as well as public CBB or for immediate use for CBBs with lower turnover rates.
- To recommend CellEffic CB for routine CB banking requires more experience from CBBs.

Before and after processing and thawing, TNC, $CD34^+$ cells, MNC, RBC, and neutrophil recoveries were calculated for each CBU, and the mean values were evaluated between the 2 groups at all stages. Viability assessment was also performed on all post-process and post-thaw CBUs. Differences among pre-process, post-process, and post-thaw mean values as well as the mean recovery rates calculated from each CBU were compared for the CBUs processed with both systems.

Cell Counting, Immunophenotyping, and Viability

Pre-process, post-process, and post-thaw TNC values were calculated using white blood cell (WBC) counts (Siemens Healthcare Diagnostic Inc [Wien, Austria]). Red blood cell, TNC, MNC, and neutrophil cell counts were assessed accordingly.

Likewise, $CD34^+$ cell counts, as well as viability assessment, were carried out using Beckman Coulter Navios Cell Sorting Device. 7-Aminoactinomycin D (7-AAD)-based viability detection and $CD34^+$ cell counting were performed via Stem Cell Enumeration Kit using FITC-labeled CD45 and PE-labeled CD34 monoclonal antibodies (Beckman Coulter Stem Kit, California, USA). The analyses were performed using International Society of Hematotherapy and Graft Engineering (ISHAGE) single test platform.

Hematopoietic stem cell subpopulations were assessed, with Sepax (n = 9) or with CellEffic CB (n = 9) on a single segment attached to the CBUs. Flow cytometry analyses were carried out using the Beckman Coulter FC500 device. Cell surface markers and staining dyes used were given as follows: CD45 (ECD/FITC), CD34 (PC7), CD38 (FITC), CD3 (PC5), CD19 (ECD), CD33 (PE), CD71 (PE), and 7-AAD (P5).

CFU-GM Assay

Colony-forming unit-granulocytes and macrophages (GM) assay was performed using a commercially available methylcellulose medium [MethoCult H4445 Enriched without erythropoietin (EPO), StemCell Technologies, Canada]. Colony-forming unit-GM analyses were performed for all post-process and post-thaw CBUs. Colony-forming unit colonies were counted according to the manufacturer's recommendations with the same method used in the study by Gencer et al.[18]

Microbial Testing of the Processed CBUs

The RBC fraction was used for aerobic and anaerobic microbial testing (BACTEC Pediatrics Aerobic Plus/F Culture Vials (442194)/ BACTEC Plus Anaerobic Plus/F Culture Vials (442193). One to two microliters of RBC were used to inoculate aerobic bottles, whereas 8-10 mL of RBC fraction was seeded into anaerobic bottles, as recommended by the manufacturer. BACTEC bottles were loaded onto BD BACTEC 9240 Instrument and growth has continuously been tracked for 6 days.

Statistical Analysis

Statistical analyses for the differences between 2 processing methods were performed on Statistical Package for the Social

Table 1. Pre Process, Post Process, and Post–Thaw Data of Sepax and Celleffic Cb Systems

	Sepax			CellEffic CB		
	Pre Process (Mean ± SD)	Post Process (Mean ± SD)	Post Thaw (Mean ± SD)	Pre Process (Mean ± SD)	Post Process (Mean ± SD)	Post Thaw (Mean ± SD)
TNC ($\times10^7$/unit)	72.66 ± 27.29	58.16 ± 23.77	34.40 ± 19.76	72.87 ± 27.1	45.92 ± 14.15	29.91 ± 10.49
Neutrophil ($\times10^7$/unit)	35.95 ± 14.69	29.16 ± 12.15	18.2 ± 10.76	35.67 ±15.44	23.19 ± 7.63	15.31 ± 7.98
$CD34^+$ ($\times10^6$/unit)	2.68 ± 1.49	2.19 ± 1.49	1.15 ± 0.81	2.57 ± 1.48	2.05 ± 1.27	1.08 ± 0.63
MNC($\times10^7$/unit)	25.32 ± 15.21	23.78 ± 14.47	11.26 ± 12.14	25.48 ± 15.01	19.42 ± 9.17	13.81 ± 6.98
RBC ($\times10^{12}$/unit)	228.85 ± 52.93	94.92 ± 12.36	60.11 ± 24.93	227.79 ± 50.75	49.65 ± 7.44	39.42 ± 11.97
CFU Counts ($\times10^6$/unit)	NA	2.4 ± 1.73	1.91 ± 1.86	NA	2.73 ± 1.33	1.33 ± 0.77
Viability (%)	94.78 ± 4.94	92 ± 7.68	64.11 ± 8.27	95 ± 4.12	95.11 ± 4.01	68.01± 11.73

SD, standart deviation; CB, cord blood;TNC, total nucleated cells; MNC, mononuclear cells; RBC, red blood cells; CFU, colony forming unit.

Sciences for Windows version 20; (IBM Corporation, Armonk, NY, USA). Kolmogorov–Smirnov test and Shapiro–Wilk tests were taken into account for the assessment of the normality of the data. Paired samples *t*-test or Wilcoxon signed-ranks test was conducted to compare 2 processing methods for normally and non-normally distributed data, respectively. Results were interpreted as significant when a $P < .05$ was achieved.

RESULTS

Comparison of Processing Efficiencies and Recovery Rates of Sepax and CellEffic CB Systems

Nine splits (18 CBUs) were processed with both systems (median: 66.11; min-max: 54-94 mL). None of the pre-process parameters investigated yielded statistically significant differences between 2 groups [Sepax vs CellEffic CB]. Table 1 indicates pre-process, post-process, and post-thaw results in terms of TNC, neutrophil, $CD34^+$ cells, as well as MNC, RBC, and CFU mean counts with standard deviations (SD). Viabilities are also shown in Figure 1.

A total of 18 vials linked to associated units were thawed under similar conditions from the comparison group. Pre-process, post-process, and post-thaw data for this group are summarized in Table 1. The only major difference was that CellEffic CB system had better RBC depletion rates (Table 1).

Recovery rates between pre-process and post-thaw steps were generally similar between the systems except for RBC depletion, which was superior for CellEffic CB as expected ($P = .005$). There was a trend for better CD34 recovery with CellEffic CB (73%) compared to Sepax (59.9%) ($P = .183$) (Figure 2). Total nucleated cell and MNC recoveries were similar for the 2 groups compared.

When post-process and post-thaw results were investigated within the same 2 groups, MNC recovery and RBC depletion rates differed significantly in favor of Sepax ($P = .018$ and $P = .066$, respectively). Recovery rates of post-process/post-thaw TNC viability for CellEffic CB group were slightly higher than after Sepax but did not reach any statistical significance ($P = .161$). CellEffic CB was surpassing Sepax in terms of all parameters in relation to post-process/post-thaw recovery rates (Figure 3).

CFU GM Analysis

A total of 35 CFU-GM analysis were performed for Sepax versus CellEffic CB group; however, 31 out of 35 were accomplished. Four individual units (2 CellEffic CB and 2 Sepax) did not show visible colony growth. When 2 systems were compared, although the number of post-process CFU-GM assays was not enough for statistical evaluation of the differences between the groups, in terms of post-thaw CFU-GM counts, Sepax was statistically

Figure 1. Process recovery rates of Sepax versus CellEffic CB.

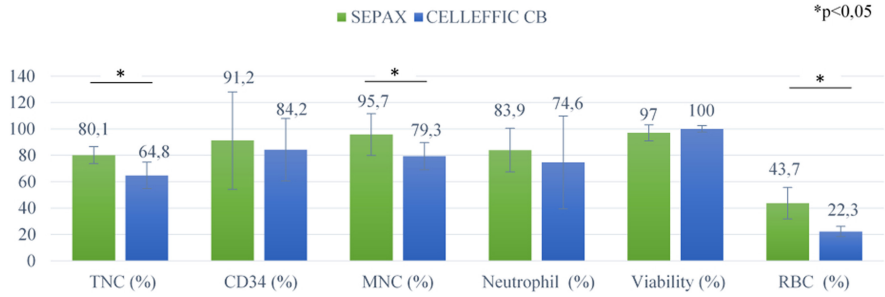


Figure 2. Pre process and post thaw recovery rates of Sepax versus CellEffic CB.

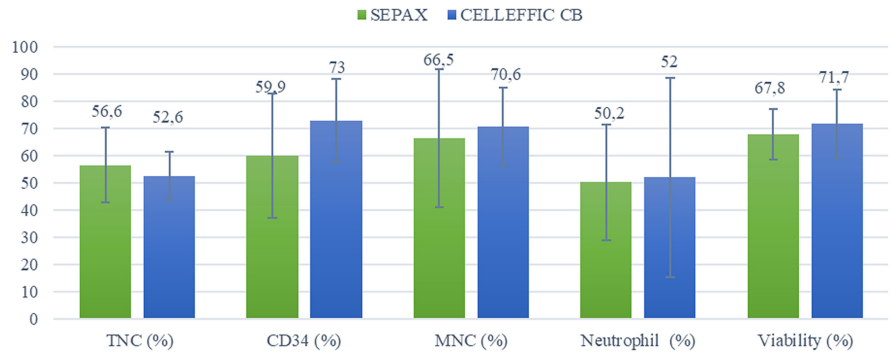


Figure 3. Post process and post thaw recovery rates of Sepax versus CellEffic CB.

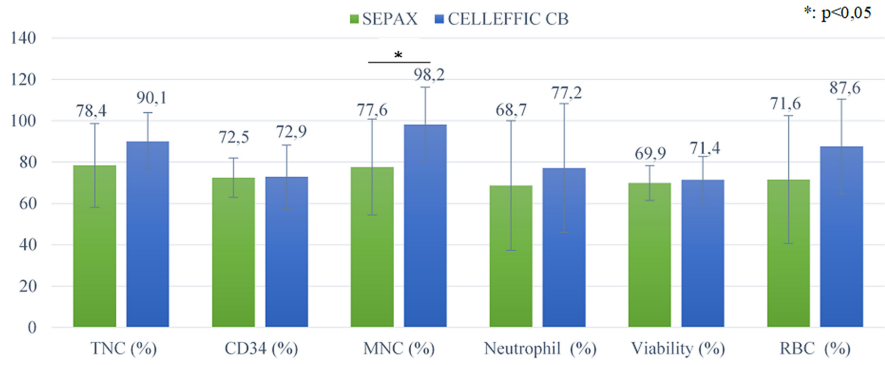
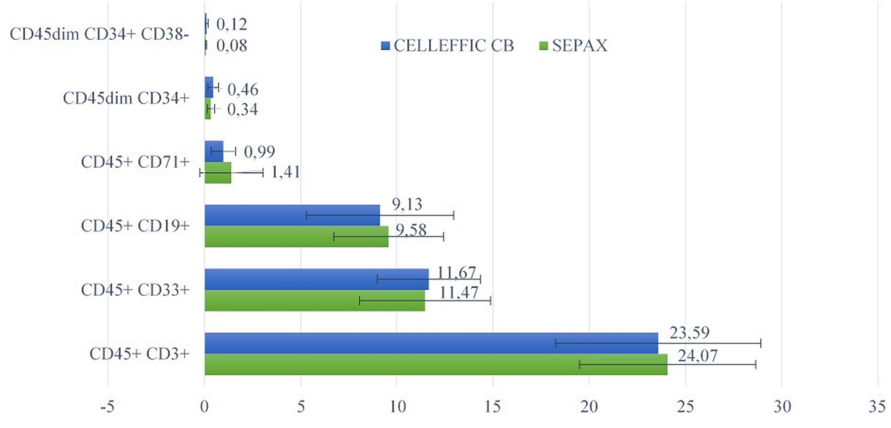


Figure 4. Post thaw cell populations (gated through viable CD45⁺ cells).



superior over the CellEffic CB group ($P = .048$), (Table 1). Colony-forming unit testing could not be performed on one of the units from CellEffic CB group.

CB HSC Subpopulation

Any likely impact of the observed processing systems on post-thaw CB HSC subpopulations was evaluated for all CBUs.

After the segments attached to the units had been thawed, mean percentages were calculated through the subpopulations gated from viable CD45⁺ population, and the results are summarized in Figure 4 which revealed no impact of the technology used.

CellEffic CB was advantageous in terms of RBC depletion ($P = .007$) and post-thaw TNC viability ($P = .017$), on the other

Table 2. Summary of CellEffic CB Versus Sepax Comparison [(+): Superior for Associated Item]

Item	Sepax	*P*	CellEffic CB
RBC depletion	(–)	.007	**(+)**
TNC recovery	**(+)**	<.001	(–)
MNC recovery	**(+)**	.016	(–)
Post process TNC viability	(–)	.017	**(+)**
Post thaw CFU counts	**(+)**	.048	(–)
Ease of use	**(+)**	–	(–)

CB, cord blood;RBC, red blood cells;TNC, total nucleated cells; MNC, mononuclear cells; CFU, colony forming unit.

hand, in terms of TNC and MNC recoveries, Sepax was superior to CellEffic CB ($P < .001$ and $P = .016$). Additionally, Sepax had a superiority over CellEffic CB ($P = .048$) for post-thaw CFU counts. While CellEffic CB was efficient for small volumes, Sepax was favorable with its user-friendly automated nature (Table 2).

Microbial Contamination

Totally 21 post-process microbial sterility testing was performed for CBUS using BACTEC system, and growth was observed on both aerobic and anaerobic bottles which were checked out daily for 6 days. None of the units showed any microbial growth regardless of the processing system used.

DISCUSSION

Processing and storage of high-quality CB are the primary goals of CB banking.[4,19,20] High post-thaw TNC/MNC, CD34 recovery rates, as well as viability, are the most important parameters to be maintained for a successful transplant.[21-23] A common procedure, volume reduction of CBU, is performed at all CB banks over the world. All FACT-NetCord accredited CB banks (including ours) have clearly defined acceptance criteria. A collection volume of ≥70 mL, a total TNC number being $\geq 100 \times 10^7$, and a previability of ≥90% are established. Provided that the CD34+ cell count is $\geq 1.5 \times 10^6$/unit, then CBU is accepted for processing. Additionally, all microbiological testing should come negative.

Sepax is the mainstream closed automated system used worldwide which has been proven to be the most efficient method with highest TNC recovery yield.[10] Nonetheless, Sepax system has a main disadvantage of utilizing very expensive disposable kits that cannot be afforded by all banks. Having perks like considerable RBC depletion rates, low cost and *in house* optimization chances, manual systems operating in open settings are prone to contamination of the product. Last but not the least, they are generally labor-intensive and time-consuming. In general, automated systems are preferred over manual methods because of better standardization and reproducibility, as well as less operator dependency.[12,24]

CellEffic CB system, which was evaluated in this present study, is a manual semi-closed system claiming to cause less stress and thus less harm to cells. This enables the system to be a promising candidate for quality products to be transferred to clinical programs.

CellEffic CB was announced for the first time in this aforementioned paper.[17] Although similar in nature, they did use nonmatching separate CBUs for the comparison of 2 systems in contrast to ours. We involved exactly the same CBUs with high volumes equally divided into 2 fragments and evaluated data in the same unit. This is a striking difference which makes our interpretations more robust. To our knowledge, this is the first study that 2 processing systems were compared on equivalent split CBUs. The other main difference between theirs and ours is that we did not use HES.[17] Hydroxyethyl starch usage might have had a slight but negligible impact on the outcomes.

Recovery rate assessment was shown to be the best reflector of cell contents of a CB product thus allows comparison of CB processing systems. In this study, our post-process TNC recovery result was higher in favor of Sepax ($P < .001$). In contrast to ours, the paper from Sato et al.[17] in which CellEffic CB was announced for the first time and compared to Sepax, have reported no statistical significance in terms of TNC recovery rate (76.6% post process for Sepax and 73.14% for CellEffic CB Saline).[17] In the study from Basford et al.[10] in which 5 CB processing systems were compared, highest TNC recovery was found to be with Sepax similar to our findings .[10] Our results indicated significant difference in favor of post Sepax MNC recovery with 95.7% versus 79.3% ($P = .016$). In contrast to our findings, Sato et al[17] found no statistical significance for post-process MNC recovery. When CD34 post-process recovery rate was investigated, there was no statistical significance between 2 systems. Sato et al[17] denoted a considerable difference between CD34 recovery rates in favor of CellEffic CB; however, none of the post CD34 recovery results reached statistical significance; main reason for this difference might be HES usage along with Sepax system, which seems to be the one and only difference between their study and ours. When compared, post-thaw TNC viability was found to be similar with both systems (64.11% and 68.01% for Sepax and CellEffic CB). Sato et al.[17] opposite to us, have found statistical significance for post-thaw TNC viability rates (85.24% for CellEffic CB and 64.8% for Sepax).[17] Although CellEffic CB viability was slightly higher at their hands, post-thaw Sepax viability was in concordance with ours.

Plasma removal/RBC depletion is crucial to obtain pure MNC cells which may otherwise interfere with HSC population of the product. Additionally, depletion of RBC will lead to smaller volumes allowing more products to be banked.[25,26] Post-process RBC count was found to be higher in Sepax, similar to the study of Sato et al.[17] Consentient to our results, Basford et al[10] have found higher RBC count Sepax. When we analyzed RBC removal rates, CellEffic CB depleted more RBC than Sepax did ($P < .001$). Our results were similar to the findings from Sato et al.[17] although their results did not reach statistical significance.[17] Basford et al[10] reported better RBC depletion rates using manual CB processing methods over Sepax, ours and the results from Sato et al[17] showed better RBC depletion rates in favor of CellEffic CB.

As indicated in all studies, we mentioned above, although Sepax is better for TNC and MNC recovery, it is less efficient in terms of RBC depletion post process. When we analyze post-thaw RBC depletion rates, RBC count was revealed higher in Sepax, Both Sato et al[17] and Basford et al[10] have observed similar results, with Sepax being the most disadvantageous system in terms of excess RBCs in both processed and thawed CBUs.

Colony-forming unit-GM is essential for the assessment of functional the clonogenic and proliferative potential of HSC in vitro, a major FACT-NetCord quality standard at the same time.[18,27,28] The CFU-GM results in our study with both systems were generally in concordance. We found post-process CFU counts higher in CellEffic CB. Sato et al.[17] have found similar results like us for post process. Colony-forming unit-GM counts after CellEffic CB showed higher colonies compared to Sepax similar to ours in 2 different papers.[16,17] When we looked at CFU counts post-thaw, Sepax group was superior to CellEffic CB ($P = .048$). Consistent with our results, Sepax was superior to all other manual systems tested in the study of Basford et al.[10] In contrast to our result, Sato et al[17] and Shima et al[16] have observed higher post-thaw CFU counts for CellEffic CB. There was no significant difference between post-process CFU-GM counts for units processed with Sepax and CellEffic. Either way, the small number of observations in this analysis prevents any firm conclusion based on statistical results.

Sustaining the essential cellular content of the CB product ensures successful transplantation.[29] In light of this information, we wanted to evaluate any likely impact of the investigated processing systems on post-thaw CB HSC subpopulations after cryopreservation. Early HSCs, namely $CD45^{dim}$ $CD34^{+}$ $CD38^{-}$ cells, were 0.08% and 0.12% of viable $CD45^{+}$ cells in Sepax and CellEffic CB, respectively. Although minimal, this difference may highlight the lack of centrifugation for a higher yield of viable HSC was slightly higher. Whereas, erythroid progenitors were found to be lower in CellEffic CB as expected, since RBC depletion rates were also higher in this group.

A CB processing system which avoids centrifuge stress on cells with better RBC depletion and TNC/MNC recovery rates will highly likely to be effectively used in the field. Moreover, RBC depletion pre-cryopreservation is crucial since removing RBCs was shown to improve post-thaw CD34+ cell viability.[30] Post-thaw CD34+ cell viability is one of the most important parameters for a successful transplant outcome. As a result, the occurrence of viscosity and clumping in the product may be another disadvantage for CB transplants.[17]

To our knowledge, this is the first study that 2 processing systems were compared on equivalent split CBUs. CellEffic CB was advantageous in terms of RBC depletion ($P = .007$) and post-thaw TNC viability ($P = .017$), on the other hand, in terms of TNC and MNC recoveries Sepax was superior to CellEffic CB ($P < .001$ and $P = .016$). Additionally, Sepax had a superiority over CellEffic CB ($P = .048$) for post-thaw CFU counts. While CellEffic CB was efficient for small volumes, Sepax was favorable with its user-friendly automated nature (Table 2).

The major drawback of our study is the sample size. Due to the valuable nature of CB, only discarded ineligible units were included in this study. Moreover, of the discarded units, only the ones with sufficient volumes were selected; those suitable for a split. Low volume is among the most common non-conformities leading to disposal, thus only restricted number of CB was available for the comparison of split units.

CONCLUSION

A CB processing system avoiding centrifuge stress on cells with better RBC depletion and TNC/MNC recovery rates on top will highly likely to be effectively used in the field. CellEffic CB was surpassing Sepax in terms of all parameters in relation to post-process recovery rates. CellEffic CB seems to be particularly useful for CBUs with lower volumes and high $CD34^{+}$ cell counts, which are generally subject to be not applicable to automated systems. The main 2 differences in favor of Sepax were post-thaw TNC/MNC recovery rates as well as CFU counts. Nonetheless, CellEffic CB was by far superior in terms of RBC depletion. The main drawback of CellEffic CB seems to be the labor-intensive and longer hands-on time nature with the requirement of qualified technician. CellEffic CB can be an alternative system for processing CB at a much lower cost in private as well as public CBB or for immediate use for CBBs with lower turnover rates. Thus, to recommend CellEffic CB for routine cord blood banking requires more experience from CBBs.

Ethics Committee Approval: Ethical committee approval was received from the Ankara University School of Medicine Ethics Committee. (Date: December 22, 2014, Decision no: 21-882-14).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept – P.Y.M.; Design – M.B.; Supervision – E.B.G.O., H.Y.A.; Funding – P.Y.M., E.B.G.O., H.Y.A., M.B.; Materials – P.Y.M., E.B.G.O., H.Y.A., M.B.; Collection and Processing – H.Y.A.; Analysis and/or Interpretation – E.B.G.O.; Literature Review – P.Y.M., E.B.G.O.; Writing – P.Y.M.; Critical Review – P.Y.M., E.B.G.O., H.Y.A., M.B.

Acknowledgments: This work was supported by KANEKA Corporation, Japan. The authors would like to thank Dr. Nobuhiko Sato from Kaneka Corporation-Japan, Osaka, Japan and Dr. Cornelia Fricke from Kaneka Pharma Europe German Branch, Eschborn, Germany for their assistance and contribution during and after the research process.

Declaration of Interests: The authors declare that they have no competing interest.

Funding: This work was supported by KANEKA Corporation, Japan.

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