Automated Cord Blood Processing:
A Follow-up to the Pilar Solves’ Study
Comparing the AXP® vs the Sepax®
Cord Blood Processing Systems

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Introduction

Safe, efficient, and reliable production through automation is the key to standardizing CBU processing.

Cord blood (CB) is a well-known and valuable source of progenitor cells, which can be used to treat disorders in the fields of oncology, hematology, and medical genetics that are currently most commonly treated with cells sourced from bone marrow (BM) and peripheral blood (PB). Though CB has many well-studied advantages over BM and PB, relative neutrophil and platelet engraftment times remain a challenge that does not easily offset the benefits provided by CB, which include the ease of procurement and reduced graft vs. host disease (GvHD) risk. In short, CB engraftment may take 2-3 times longer than BM, which could expose transplant recipients to an elongated and dangerous state of neutropenia.\(^1\) The delay in engraftment is due, in part, to suboptimal cell counts within a single unit of CB, the main limitation of CB use. Therefore, the target of CB therapeutic clinical trials has been focusing on CB cell expansion\(^2\)-\(^4\). Due to this limitation, the industry has focused CB primarily for use in pediatric indications where cell counts meet the required dose/kg prescription or, in cases where acceptable BM donors are not available, a double CB transplant is conducted at considerable cost to the patient or local healthcare system.\(^5\) The message, regardless of protocol type (direct transplantation vs. cell expansion), is the continued necessity to establish inventory of the highest possible number of potent progenitor cells from a given CB donor.

In order to keep pace with transplantation demands and future therapeutic innovation, cord blood banks (CBBs) are driven to continually improve the quality and standards of their CB collection, processing, and storage protocols. An early example of this was the adoption of volume reduction techniques developed in 1995 by Rubinstein et al. which allowed for improved storage efficiency;\(^8\) 3600 cord blood units (CBUs) can be stored in a single BioArchive\(^\text{®}\) Cryopreservation Dewar spanning only 4 feet in diameter due both to the advanced dewar design and to the smaller freezing bag designs which brought the final cryopreserved volume of a CBU to 25 mL as an industry standard. Volume reduction of the final CBU also allowed for a reduction in the volume of DMSO used for cryopreservation which allowed transplant centers the option to infuse cells without additional cell washing, a step associated with the loss of important transplantable cells.\(^8\) Moreover, volume reduction depletes red blood cells (RBCs) reducing the potential recipient’s reaction to ABO incompatibility and improving stem cell functionality.\(^5\),\(^7\)

Initial protocols for volume reduction of CBUs involved manual processing using agents such as hydroxyethyl starch (HES) to facilitate RBC sedimentation by rouleaux formation.\(^8\) However, both the use of HES and manual processing are increasingly viewed by some as suboptimal practices. The use of HES, for example, is not clinically...
approved or licensed in a number of countries including Canada, Portugal, and Japan: HES can be detected years after administration and safety concerns including severe pruritus, disseminated intravascular coagulopathy, and shock have led the field to search for alternative processing methods that do not incorporate the use of additives. In addition, the United States Department of Health and Human Services Federal Agency’s Food and Drug Administration (FDA) and the European Medicines Agency’s Pharmacovigilance Risk Assessment Committee (PRAC) both report risks associated with the use of HES products which include increased mortality and kidney injury in patients with sepsis, burn injuries, or critically ill patients. Manual processing of CBUs, typically performed using HES, can severely limit laboratory production efficiency, both temporally and economically, and further introduces user to user variability, making standardization and lean-manufacturing processes more challenging. The manual separation procedure relies on multiple technicians and technician-dependent technique, which requires comprehensive training to perfect and may walk out the door with the employee should they choose to change employment. Further, the manual process itself is relatively long and laborious due, in part, to a sedimentation step that can alone take up to 90 minutes.
Results and Discussion

Safe, efficient, and reliable production through automation is the key to standardizing CBU processing. Automation allows CBBs to implement lean manufacturing principles that drive consistency and quality across all CBUs and thus increases the likelihood of optimal inventories for clinical use. Presently, there are two leading automated systems for CBU processing with adequate published data available for analysis: the AXP® system, part of the AutoXpress® Platform (Thermogenesis®) of products offered by CESCA™ Therapeutics, and the Sepax® system (Biosafe® S.A.). Both systems volume reduce CBUs in a functionally closed approach to minimize contamination that can be introduced by other methods. Both automated systems reduce operator dependent variability and enable better standardization and reproducibility to ensure that the sample is banked under optimal conditions. Both systems are efficient in reducing processing times to around 30 minutes. The AXP and Sepax systems can be used with or without HES but the AXP system, typically used without HES, minimizes the risks and complications associated with potentially dangerous chemical additives while also reducing the cost of an added reagent.

As automated systems, the AXP and Sepax systems can yield excellent recoveries that are comparable if not better than manual methods. The AXP system consistently yields MNC recoveries greater than 90% when processing CBUs with an average range of 70-85 mL without HES addition. Cord Blood Registry (CBR), one of the world’s largest and most experienced private cord blood banks, consistently obtains 95-99% recoveries for MNC and CD34+ populations using the AXP system in conjunction with lyophilized heparin. Side by side, the Sepax and AXP systems without the use of HES have never accurately been compared in a single peer reviewed publication. An attempt was made by Pilar Solves et al. in a retrospective study, but this evaluated the Sepax system using HES and the AXP system without the use of HES, which is not a study where both technologies were studied under equal conditions. While this study reported 2% more TNCs recovered with the Sepax system with the HES protocol, we can conclude that the AXP system without HES, was arguably equivalent and offers an additional advantage to the Sepax because it does not require HES to obtain these efficient recoveries.

“The AXP system without HES offers an additional advantage to the Sepax and Sepax II because it does not require HES to obtain efficient stem cell recoveries.”
Results and Discussion (continued)

Although most published data with the Sepax system includes the addition of HES,\textsuperscript{17,18} there is a study from Zingsem et al. in 2003 reporting the performance of the Sepax system without HES.\textsuperscript{19} To summarize, the Sepax system without the addition of HES volume reduced CBUs resulting in 77.4±27.8\% MNC and 83.6±32.5\% CD34+ recoveries. When compared to Takanshi et al.’s data from 2010, these values are lower than the recovery performance with the AXP system, likewise without HES, (97.3±11.2\% MNC and 93.4±12.0\% CD34+ recoveries) as seen in Figure 1.\textsuperscript{15,19} Additionally, the Sepax system without HES reduces RBCs by 47.5±9.1\% compared to 80±4.5\% as seen with the AXP system without HES (Figure 2).\textsuperscript{15,19} When HES is added the Sepax system RBC depletes at comparable values to the AXP system without HES (88.32±7.94\% and 88.28±5.62\% respectively) (Figure 3).\textsuperscript{18} Furthermore, in Solves et al.’s study, the Sepax system and not the AXP system showed an inverse correlation between TNC recovery and RBC depletion (rho= -0.225, P=0.000).\textsuperscript{18} This may be in part due to the inherent technological differences between both systems. The Sepax system starts with plasma extraction, followed by collection of theuffy coat to a defined volume before extracting RBCs to the RBC collection bag;\textsuperscript{19} whereas, the AXP system is designed to remove the RBC fraction first to consistently deliver a defined volume of packed RBCs into the freezing bag followed by the buffy coat and then utilizes the plasma to achieve the desired final volume, thereby achieving consistent hematocrit levels regardless of CBU processed.

\textbf{Figure 1.} Stem cell percentage recoveries (MNC and CD34+) for the AXP and Sepax systems both without HES.
In conclusion, the AXP system provides consistently high recoveries of TNC, MNC, and CD34+ cells without unnecessary exposure to HES to circumvent potentially serious adverse reactions in patients and does so while delivering consistently low RBC contamination, making it an attractive system for clinical applications. The trend in the restricted use of HES in subsets of patient populations and the ban of HES by some countries show the gravity of the risks outweighing the benefits worldwide. The AXP system’s proprietary processing technology also consistently reduces RBC contamination to ensure maximal post-thaw progenitor recoveries while minimizing RBC secreted factors which can impair invasion and colony forming unit capacities of stem cells.6,7 Maximizing cell counts, viabilities, and potencies in an automated manner are the key factors in building “bank-worthy” CBUs for therapies. With our ever-expanding technologies, CESCA™ Therapeutics (f.k.a. Thermogenesis® Corp.) is working to bridge the gap between the number of CBUs banked and the number of CBUs used for transplantation.
REFERENCES


