

Umbilical Cord Blood Processing Techniques and Their Comparative Advantages: A Review

Abstract

Background: Umbilical Cord Blood (UCB) has steadily gained prominence in haematopoietic stem cell transplantation (HSCT). Despite UCB advantages, the main disadvantage of UCB in haematopoietic stem cell transplantation (HSCT) is its limited cell dose. Initially, UCB used to be processed and then made to undergo cryopreservation as whole cord blood banking leading to the problem of storing sufficiently large number of cryoprotected UCB units which requires vast amounts of costly storage space in liquid nitrogen. The sole purpose of processing is to concentrate the stem cells and reduce the volume for storage. Different UCB processing methods have been developed.

Aim: This review is aimed at bringing together the literature on the different processing methods and highlighting the underlying principles of each method, the relative efficiency and advantages of the methods.

Methodology: The work involved mainly the critical review of all available academic, professional and industry documents on cord blood processing. The relevant information was obtained from textbooks, academic journals, conference proceedings, the internet among others. The major UCB processing methods include Plasma Depletion, Density Gradient Centrifugation (DGC), Hetastarch, PrepaCyte-CB and Sepax. A study of the potential impact of Hetastarch and PrepaCyte processing methods on transplantation outcomes revealed no difference that was significant was observed between patients receiving cells after the processing regimens were compared.

Results: A comparison of the engraftment time of PrepaCyte-CB with five other processing methods revealed a quicker engraftment time for PrepaCyte-CB processed cord blood units compared to other processing methods. PrepaCyte-CB also recovers significantly more viable stem cells than AutoXpress (AXP) and hydroxyethyl starch (HES) processing methods. Other workers demonstrated that Sepax depletion produces higher recovery of cells that are nucleated. The effect initial volume of cord blood had on the recovery of nucleated cells for the different method of processing were also compared. Recovery when using Sepax is reduced as the unit size processed increases. Hetastarch, which is a density gradient, and plasma depletion separation is also affected in like manner, however, processing done using PrepaCyte-CB was not affected by the initial volume of the collected unit. The advantage of Sepax is that it is fully automated and this allows for mass processing of samples, suitable for bigger cord blood banks. For erythrocyte removal, density gradient separation is a better method that is effective. PrepaCyte-CB is the second most efficient method for removing RBC. The result of Total Nucleated Cells (TNC) and Mono Nucleated Cells (MNC) recovery rate of Hespan and Sepax against AXP processing methods shows that both Hespan and Sepax reproducibly recover greater than 95% of the cord blood stem cells in a typical collection and result in a reduced final volume for final storage.

Conclusion: The five most popular processing techniques are Plasma Depletion, Density Gradient, Hetastarch, PrepaCyte-CB and Automated Centrifugal Machine (Sepax). Most methods involve centrifugation, sedimentation and/or filtration for reducing the red cell content, plasma volume, or both. The different UCB processing methods each has its advantages and disadvantages.

Key words: Umbilical cord blood (UCB), Haematopoietic stem-cell transplantation, UCB processing methods, Total nucleated cells, Mono nucleated cells, cord blood banks.

1. Introduction

“Despite its numerous advantages, the main setback for cord blood (CB) in haematopoietic stem cell transplantation (HSCT) is its limited cell dose”¹⁻². “The sole purpose of processing is to separate stem cells from the cord blood so that a sample is produced can concentrates the stem cells and that can be used safely. Before, CB used to be processed and cryopreserved as whole cord blood, and was referred to as zero generation process”.² “The first documented problem of cord blood banking was that banking a large number of sufficient cryoprotected CB units requires large amounts of expensive storage space that contains liquid nitrogen (LN)”³. “It was based on that that volume reduction processing of cord blood units was developed, with reduction of the bulk of the red cells and depletion of plasma. It is important to state that umbilical cord blood banks make use of two main methods to store frozen umbilical cord blood, red cell reduction, or plasma depletion. The red cell reduction method normally centrifuges cord blood in albumin or hetastarch in order to isolate 21 ml of cord blood containing majorly white blood cells, then adds 4 ml of 50 % dimethyl sulfoxide, before freezing the resulting 25 ml of cell suspension”⁴. “The platelet depletion method eliminates plasma, saves all viable cells, before freezing the cells in 10 % dimethyl sulfoxide (DMSO)”⁴.

“It was reported that the red cell reduction method of processing increases the number of units that can be stored in the same freezer space by as high as ten-fold, and thus provided huge economic advantage”³. “Volume-reduced process involves reducing the size of the sample by removing the harmful waste products (roughly 90% of the sample), then isolating and protecting the valuable stem cells in storage”⁵. “Waste products refer to red blood cells within a cord blood sample”⁵. “They do not cope well in storage and can rupture and release a toxin into the sample. This can kill off valuable stem cells, meaning higher cell loss as well as being toxic for the recipient in transplant. In umbilical cord blood banking, volume and RBC reduction of the collected UC blood allows more efficient long-term storage and decreases infusion-related hemolysis and DMSO toxicity”⁵. To establish an adequate panels of CB units, therefore, the haematopoietic cells of placental cord blood units must be concentrated into units of smaller volumes.

2. Umbilical Cord Blood Processing Techniques

“Presently, umbilical cord blood processing laboratories make use of a variety of techniques for reduction in volume: removal of red cells, plasma depletion, or both. Most methods involve

sedimentation, centrifugation, and/or filtration for reducing red blood cell content, plasma volume, or both”⁶. Five main separation methods used are Density Gradient, Plasma Depletion, Hetastarch, Automated Centrifugal Machine (Sepax) and PrepaCyte⁷⁻¹⁰. MacoPress and AutoXpress (AXP), are the machines that performs the volume reduction processes.

2.1 Plasma Depletion

“Limited cell dose hampers wider use of Cord Blood Transplantation (CBT). Plasma Depletion (PD) was developed by Chow and others in the late 1990s”⁴. “In the PD method, the UCB is centrifuged to separate the cells and plasma. The plasma is squeezed into a separate plasma bag, and 50% DMSO is added until the final DMSO concentration is 10% in the cord blood. The cord blood is then transferred to a freeze bag”⁴. “By reducing plasma but not red cells during processing, cells that are nucleated is reduced to <0.1 % which increases significantly the proportion of high cell dose products by three-fold”¹¹. “A retrospective audited analysis performed on 118 Plasma Depleted (PD) cord blood transplantation demonstrated that plasma depleted cord blood transplantation is effective and save, and that eliminating red blood cell reduction or depletion improves cell recovery during cord blood processing, resulting in a larger proportion of the inventory with high number of nucleated cells”¹¹. “Many other workers have compared the performance of PD with other processing methods”^{4,12-14}. “Plasma depleted UCB units are more troublesome to thaw and wash, due to their larger and variable volume. However, when they are properly thawed and washed, PD units not only have more total nucleated cells, colony-forming units, CD34+ cells, than RCR units but also have high engraftment rates which may likely be more effective for treating β -thalassemia”^{2,4}.

2.2 Density Gradient Centrifugation (DGC)

“Density gradient centrifugation (DGC) and the density gradient ultracentrifugation (DG-UC) method are widely used for UCB processing”¹⁵. “They work by applying a density gradient to the sample, by which the components, move to their equilibrium density (relative to the medium), separating them based on size and mass density in the case of top-down gradients, or solely based on mass density in the case of bottom-up gradients, with denser soluble constituents collecting at the bottom. To enable the desired separation of different samples different gradient

media, such as Ficoll, Percoll, Ficoll-Paque were developed. The Ficoll method was first used in 1968”¹⁶.

“Some Authors made comparison between the separation procedures based on differences in density gradients in order to obtain the highest reduction of red cells, while maintaining the highest recovery of progenitor cells”¹⁷. Three different densities of Percoll (1.069 g/ml, 1.077 g/ml, 1.084 g/ml) was compared, sedimentation over polygeline, sedimentation over polygeline, followed by separation over Ficoll-Paque. Sedimentation over polygeline followed by Ficoll-Paque allowed the highest reduction of red cells (hematocrit of final cellular suspension 0.4 +/- 0.1%) while maintaining high recovery of CD34+ cells (85.3 +/- 5.6%) and total recovery for burst forming unit-erythroid (BFU-E), colony forming unit-granulocyte-erythrocyte-monocyte-megakaryocyte (CFU-GEMM), and colony forming unit-granulocyte-macrophage/monocyte (CFU-GM).

Another group of authors compared four protocols for processing CB, using different combinations of density-gradient centrifugation, hydroxyethyl starch (HES) and ammonium chloride (NH₄Cl) treatment, regarding the yields of CD45+, CD34+/CD133+ and colony-forming cells and stated that the highest yields of nucleated and progenitor stem cells were obtained with a two-step processing of cord blood¹. The CD133+ cells obtained by this method are expected to yield enough hematopoietic progenitors for potential allogeneic transplantation.

“Sedimentation methods reduce the number of RBC to be infused. Red blood cell reduction reduces the risk of incompatible reaction. Sedimentation reduces side-effects of the DMSO cytotoxicity”¹⁸.

“It has been demonstrated that mononuclear cells are quickly isolated by density gradient centrifugation”¹⁹. “In Ficoll-Paque density gradient centrifugation, anticoagulant-treated and diluted cord blood is layered on Ficoll-Paque solution before being centrifuged. When centrifuging, RBC and WBC sediment at the bottom. Lower density lymphocytes, together with other slowly sedimenting cells such as monocytes and platelets, are retained at the interface

between plasma and the Ficoll-Paque, where they are collected and subjected to further isolation of hematopoietic stem cells or culture of mesenchymal stem cells”¹⁹.

2.3 Hetastarch

Hetastarch is a synthetic colloid made from natural sources of starch. The chemical name for Hetastarch is Hydroxyethyl Starch (HES)²⁰. “Hespan is a brand name of Hetastarch. Hydroxyethyl starch processing of umbilical cord blood has been the standard method right from 1988, and so many transplants using HES-processed cord blood has been done successfully. The use of sedimenting agents such as gelatin, HES, poligeline, and dextran has been the most common means of reducing red cell content has been”²¹.

“Erythrocyte reduction is important to reduce the cord blood unit volume for commercial banking. Red cell sedimentation using hetastarch is a standard procedure and the most common protocol in cord blood banks”²². “The quality of umbilical cord blood volume reduction is guaranteed by minimum manipulation of cord blood samples in the closed system”²³. “These authors, carried out a study aimed at analyzing and comparing cell recovery and viability of UCB processed using the Sepax automated system in the presence and absence of HES and showed that processing of UCB using the Sepax system with the without-HES protocol due to the lower manipulation of samples could be used as an eligible protocol to reduce the volume of UCB”²³. “It was demonstrated that incubation time of HES sedimentation increases cell recovery in umbilical cord blood processing by automated system”²⁴.

2.4 PrepaCyte-CB

In year 2009, a new advanced technology for the processing of cord blood known as PrepaCyte-CB was developed. A completely closed and sterile system with capacity to greatly reduce the contamination when processing²⁵. PrepaCyte-CB a two-bag device, interconnects with any freezing/storage bag of choice. The first bag is mainly pre-filled with PrepaCyte-CB separation solution, while the second bag is used for separating the different blood components while processing. The system is interconnected such a way that the closed-bag set limits cell manipulation and helps reduce contamination and identification errors^{9,26}.

“CryoCell International became the first cord blood bank to use this technology, which is known to produce maximum recovery of healthy stem cells and also provide superior red cell reduction over the other methods²⁵. PrepaCyte-CB has shown to lead to earlier engraftment. For conditions where chemotherapy affect the immune system are utilized, an earlier engraftment time implies the patient will spend less time in the critical stage, where their immune system capable of fighting pathogens is incapable of doing so. It can also translate to less time in hospital and less worry and stress waiting for the patient to recover²⁵. In one study²⁶ showed that “PrepaCyte-CB offers high recovery of TNC, particularly after removal of granulocytes”. “This is because, as yet, current technology is not advanced enough to allow granulocytes to survive the freeze–thaw process^{3,27}”.

2.5 Sepax

“Historical data on the use of Sepax revealed an average TNC recovery of approximately 80% for CBUs with a processed volume of <220 ml. However, as volume increased to 270 ml, TNC recovery fell to <50%²⁸. “Sepax runs with the new program showed an increased TNC recovery for large volume units in comparison with similar historical runs, although not to the recovery seen in lower volume UCB. The addition of RBC removal allowed for the desired high TNC recovery²⁸”.

Sepax-2 automated cell concentration was developed for concentrating thawed cell whiling removing the DMSO. Initial performance qualification runs showed promising results in achieving this purpose while removing > 95% of DMSO². They reported a reproducibility of the process when used to serially wash and concentrate two thawed UCB bags. Automation of the cell concentration of post-thaw cells with the use of Sepax-2 device was introduced in routine practice about 2013.

It has been reported that 352 UCB transplants comprising 65 adults and 287 pediatrics, performed between 2000 and 2017, with a cumulative incidence (CI) of primary graft failure (PGF) of approximately 17 % in pediatrics and approximately 28 % in adults. They showed that there was a trend that favoured lower PGF in pediatric Sepax-processed UCB and improved time outcome to neutrophil engraftment for UCB processed with Sepax²⁸.

Table 1 outlines the clinical outcomes for Sepax and Non-Sepax processed UCB transplants.

Table 1: Clinical Outcomes for Non-Sepax and Sepax Processed Umbilical Cord Blood Transplant (2000-2017).

Clinical Characteristics	Non-Sepax	Sepax
Number of UCB Transplant	329	23
Cumulative incidence (CI) of primary graft failure (PGF)	20.4%	4.5%
CI of PGF: Adult UCB Transplant	27.4%	33.3%
CI of PGF: Pediatric UCB Transplant	18.7%	0%
Time to neutrophil engraftment: Median (range) days	20 (6-141)	18 (12-35)
Time to platelet engraftment : Median (range) days	45 (18-184)	38 (9-76)
CI of Acute GVHD	50.7%	60.8%
CI of Chronic GVHD	28.5%	21.7%
Overall survival	48%	64%

Log rank analysis. All other *p*-values by Fisher's Exact Test. GVHD- Graft versus host disease

Source²⁸

From this study²⁸ also reported great success in engraftment with no cases of primary graft failure recorded in 20 consecutive pediatric UCB transplants when Sepax processing was used. Also, Sepax umbilical cord blood processing has the advantage of producing prolonged product viability (> 94 % at 24 hours post processing) and removal of > 95 % of DMSO prior to infusion²⁸.

3. Comparison of Umbilical Cord Blood Processing Techniques

“The processing technique may influence the final concentration of the haematologic measurements and can be adjusted according to the operator’s necessity. Therefore, it will be necessary to validate the process to obtain a good cellular recovery and the establishment of a quality standard for these red blood cells units”³⁰. Many workers have compared the effectiveness of different UCB processing techniques in achieving different purposes^{9,2,31,32,25}.

3.1 PrepaCyte-CB Versus Hespan Grafting Success

Saint Louis Cord Blood Bank – SLCBB, one of the largest public cord blood banks in the globe utilize both Hetastarch and PrepaCyte-CB UCB processed units for transplantation. A group of scholars³² studied the likely impact of these methods of processing on transplantation results. One-year overall survival revealed no difference significant enough between patients receiving cells from each regimen-(processing). Neutrophil engraftments done using Hespan and PrepaCyte-CB cord blood units were compared, and engraftment was defined based on the achievement of ≥ 500 absolute neutrophil count by post-transplant day 42.

Figure 1 is a comparison of patient engraftment data for processed units using Hespan to that of PrepaCyte-CB. It was observed that median time to engraftment was similar, as shown in the interquartile ranges. PrepaCyte-CB and Hespan had engraftment success of 98.1 % and 94.5 % respectively. Among the engrafted cells 9.3 % died by day 42 in the Hespan processed samples while a slightly less percentage (7.5 %) died in the PrepaCytes-CB samples.

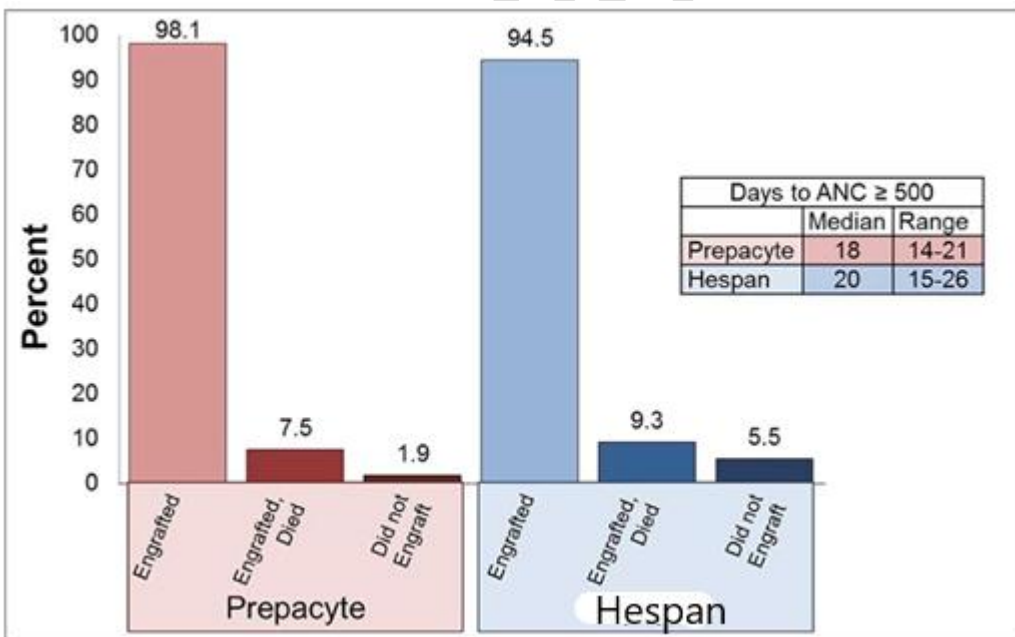


Figure 1: Day 42 Neutrophil Engraftment State: PrepaCyte-CB Versus Hespan.

Source³²

3.2 Engraftment Time: PrepaCyte-CB Versus Five Other Processing Methods

CryoCell International²⁵ used data from five cord blood banks in the United State to compare the engraftment time of PrepaCyte-CB with five other UCB processing methods. As shown in Figure 2, the data revealed a faster time of engraftment for PrepaCyte-CB processed UCB units compared to other processing methods.

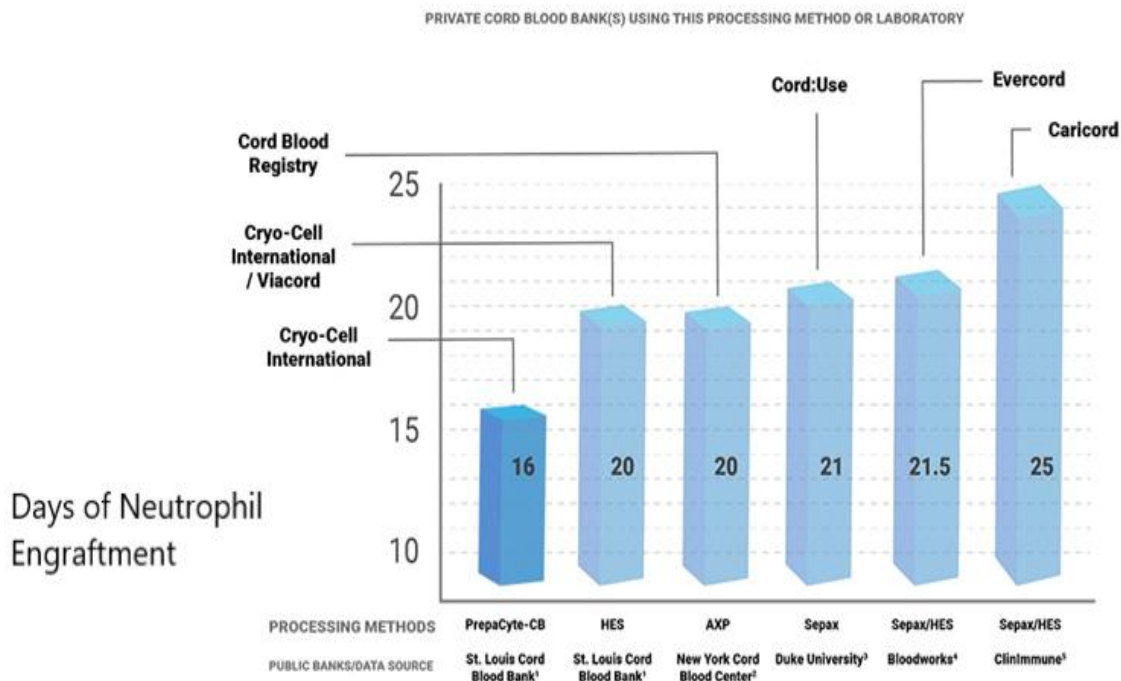


Figure 2: Comparison of Days for Neutrophil Engraftment: PrepaCyte-CB Versus Five Other Processing Methods. Source²⁵

3.3 Percentage Recovery of Colony-Forming Unit and Red Blood Cell from PrepaCyte-CB and Three Other Umbilical Cord Blood Processing Methods

CryoCell International,²⁵ also compared the percentage recovery of CFU and RBC from PrepaCyte-CB and three other UCB Processing Methods. PrepaCyte-CB has the ability to recover more viable stem cells than any other UCB processing methods. St. Louis Cord Blood Bank did a comparison, PrepaCyte-CB has the ability to recover the most percentage of colony

forming units, producing 51 % more than Hetastarch method and 70 % more than AutoXpress (AXP) method. PrepaCyte-CB reduces RBC as high as 99 %. Fewer RBCs post-processing signifies lesser toxic side effects and low chances of contamination. AutoXpress has been noted to cause reduction in RBC by up to 70 %, Hydroxyl ethyl starch can reduce its volume concentration up to 82 %, and Sepax reduces its red blood cells by 84.7 % (Table 2).

Table 2: Percentage Recovery of CFU and RBC from Different Umbilical Cord Blood Processing Methods

	PrepaCyte-CB	Sepax	HES	AXP
CFU Recovery (%)	80.20	62.70	52.90	47.00
RBC Depletion (%)	99.00	84.70	82.00	70.00

Source²⁵

3.4 Comparison of the Major Clinical Processing Techniques

In a study that was carried out to make comparison between the major laboratory processing techniques for stem cells from cord blood, using the following separation methods: plasma depletion, Hetastarch, density gradient, PrepaCyte-CB and Sepax; were evaluated⁹. The findings showed that Sepax depletion produces higher recovery of cells that are nucleated (Figure 3A), which is very crucial for a successful engraftment. After the exclusion of granulocytes recovery, Sepax was still the highest at 78.8 % when compared to others (Figure 3B)⁹.

The workers⁹ who carried out the comparisons also examined the effect that the initial volume of cord blood had on the recovery of cells that are nucleated. When recovery is done using Sepax, it reduces as the size of processed units increases. Density gradient, Hetastarch and plasma depletion separation were likewise so affected, but PrepaCyte-CB was not affected at all by initial volume of collected unit, and recovery of both total nucleated cells and CD34+ progenitor cells was as efficient with smaller volumes the same way it was when compared with larger units. Density gradient separation indicated reverse correlation: as umbilical cord blood volume increases, so does recovery. Interestingly, it does not compare fairly to other methods as maximum volume processed using density gradient was 90ml, while other methods that were tested routinely has units of over 100ml which is essential to promote good engraftment³³. The

results of the study⁹ pointed out that Sepax, produces the best recovery of total nucleated cells, with PrepaCyte-CB and plasma depletion following close behind.

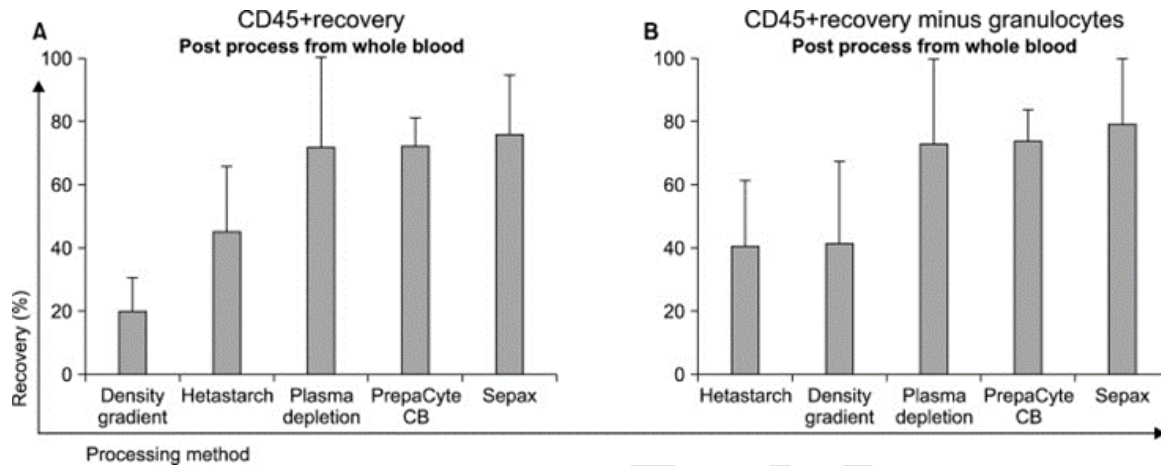


Figure 3: Rate of Recovery of Umbilical Cord Blood Nucleated Cells.

Source⁹

As part of the same comparison the workers⁹ also studied the recovery of haemopoietic stem cells (HSC) of different (three) developmental stages: early-stage (CD34-/CD133+); mid-stage (CD34+/CD133+) and late-stage (CD34+/CD133-). Results showed PrepaCyte-CB as the best methodology for obtaining optimum haemopoietic stem cell numbers from all three developmental stages (Figure 4A, 4B, and 4C), again it is worthy of note to state that Sepax recovery for CD34+ and total nucleated cell diminishes as the volume of umbilical cord blood increases. The advantage of Sepax is its fully automated system which makes provision for mass processing of samples, suitable for very large cord blood banks.

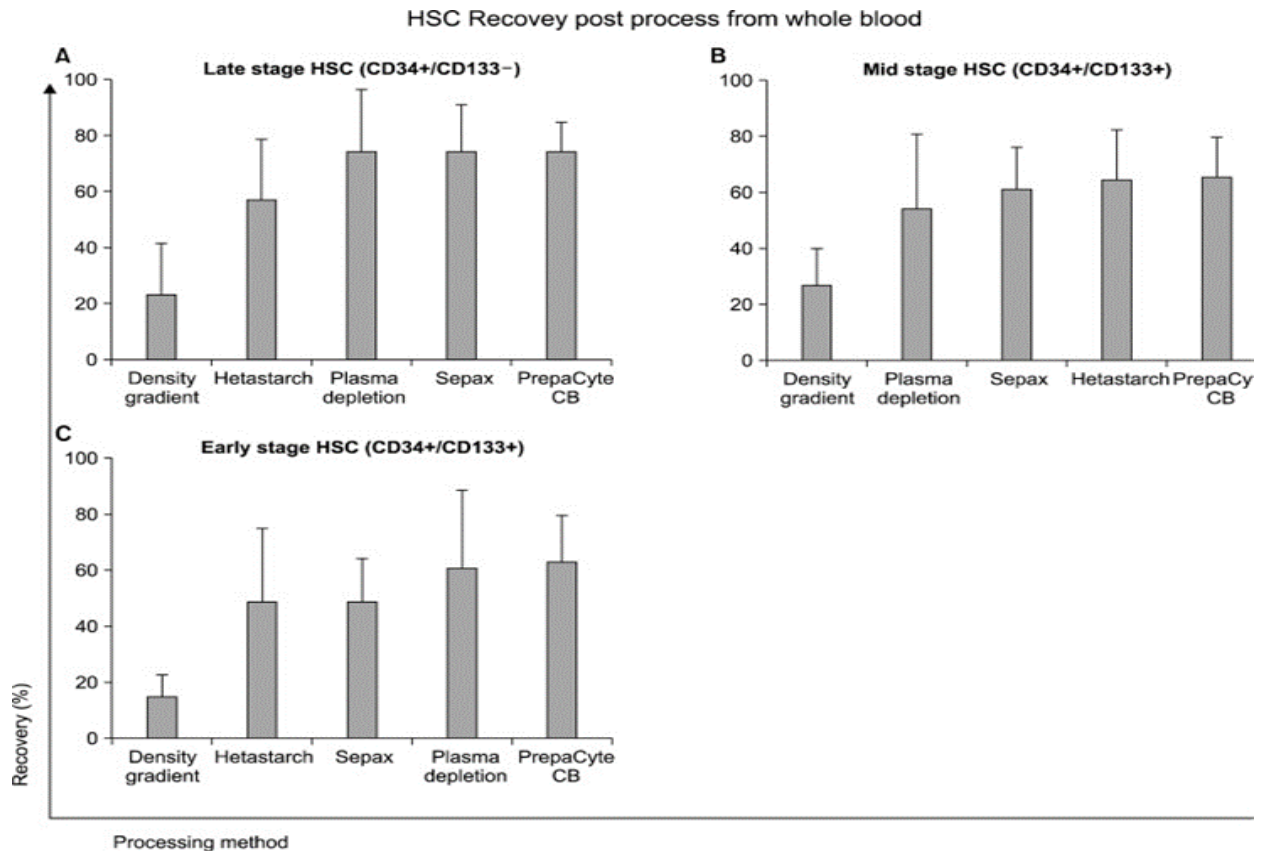


Figure 4: Recovery of HSC of the Different (three) Developmental Stages. Source⁹

Recovery of T and B cells were examined also⁹. In this study⁹ it was found out that there was a more recovery of CD45+/CD3+ T lymphocytes using PrepaCyte-CB which also produced the best outcome for CD10+ B cells as shown in Figure 5A and 5B. So much is not known if these cells play significant role in engraftment when performed in humans but in some mice models, it has shown increase in numbers of transplanted T cells which increase bone marrow reconstitution and ultimately haemopoiesis while also eliminating residual leukaemic disease as seen in the mice where the transplant was done⁹.

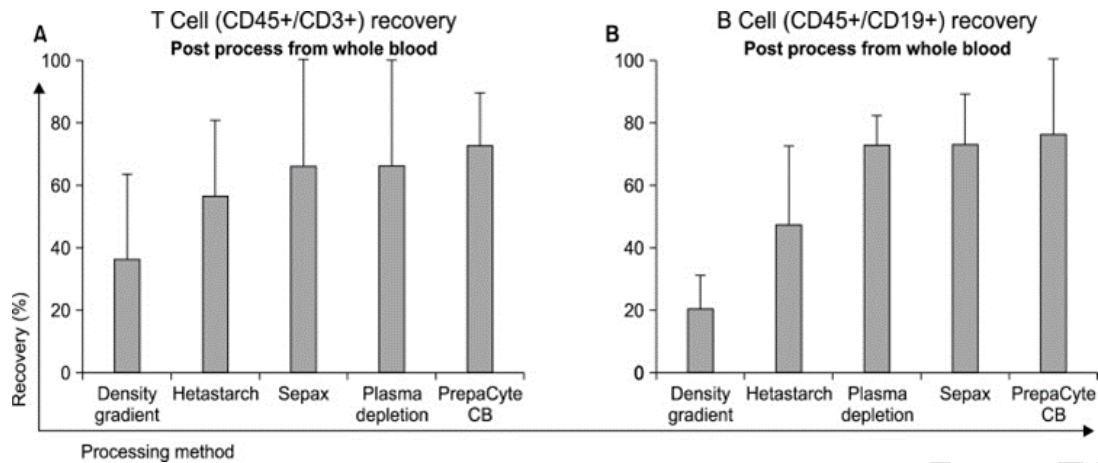


Figure 5: Comparison of T-Cell and B-Cell Recovery Rate for Different UCB Processing Methods. Source⁹

The effectiveness of the different processing methods on cord blood volume reduction was also examined⁹. For RBC removal, density gradient separation was identified as the most effective method. Average number of RBC per ml of whole blood was reported to approximately 2.9×10^6 cells. After processing using density gradient methods, the number of RBCs dropped to approximately 0.03×10^6 cells/ml of blood. This drop was significantly higher than that of PrepaCyte-CB, Sepax, plasma depletion, and Hetastarch (Figure 6A). The same condition applied in the removal of haemoglobin (Figure 6B). In their examination, they assessed if initial collected cord blood volume produced an effect on erythrocyte reduction but their result (data) revealed there was no correlation. Initial collected volume of umbilical cord blood also showed no correlation on red blood cell reduction⁹

Taking a look at the physical size of the unit in terms of volume reduction, it seems to be that plasma depletion is of benefit, as lower volume reduces the space required for storage⁹ and also means low amount of DMSO is added to the sample when preparing it for the process of cryopreservation³⁴. This implies that it could prevent the need to wash the samples prior to infusion (for haemopoietic transplant only) as it has been previously shown that total nucleated cell recovery after cryopreservation is higher without the inclusion of the wash step³⁵. However, if the volume reduction is measured based on the ability to remove red blood cells and

haemoglobin; then it is really a simple/economic density gradient separation which is more efficient.

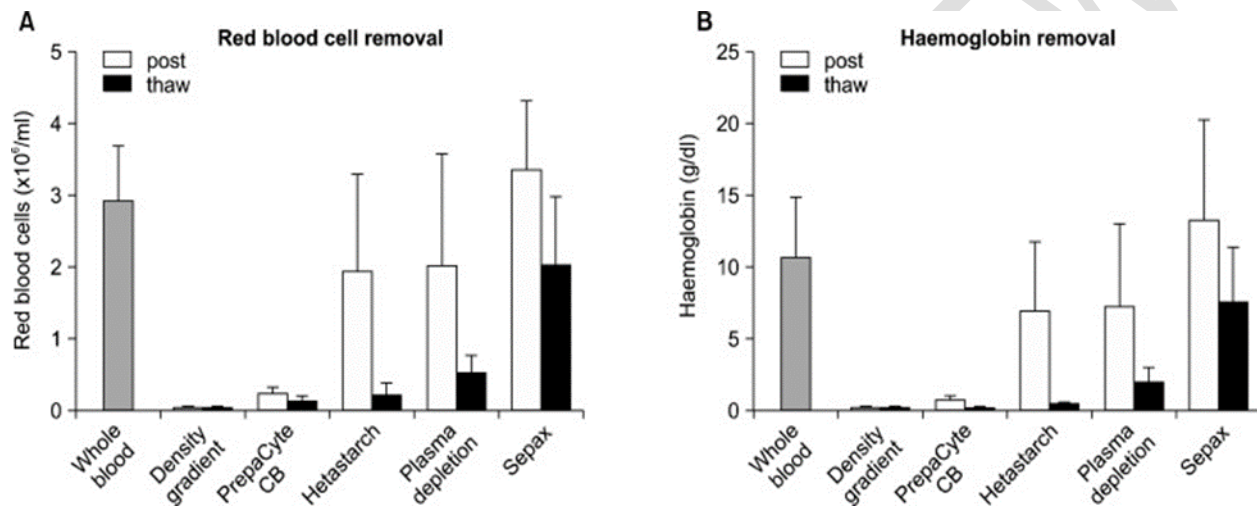


Figure 6: Comparison of Red Blood Cell Removal for Different Umbilical Cord Blood Processing Methods. Source⁹

The colony formation potential of umbilical cord blood units measured by colony forming unit assay using the different laboratory processing techniques was analyzed⁹. Importantly, this is the very critical test, based on the fact that it gives the most possible read-out for potential of the therapeutic usefulness of the cord blood. PrepaCyte-CB was observed to have performed best, not only in post processing, but also after the process of cryopreservation, and subsequently thawing (Figure 7). The importance of post thaw colony forming unit is key for future therapeutic utility of umbilical cord blood units as it is vital to know that they will still be able to engraft even after storage³⁶. This could be based on the fact that PrepaCyte-CB is the second most efficient/reliable method for removing red blood cell. A reduction in red blood cell counts has previously been shown to have effect that are advantageous on CFU³⁷.

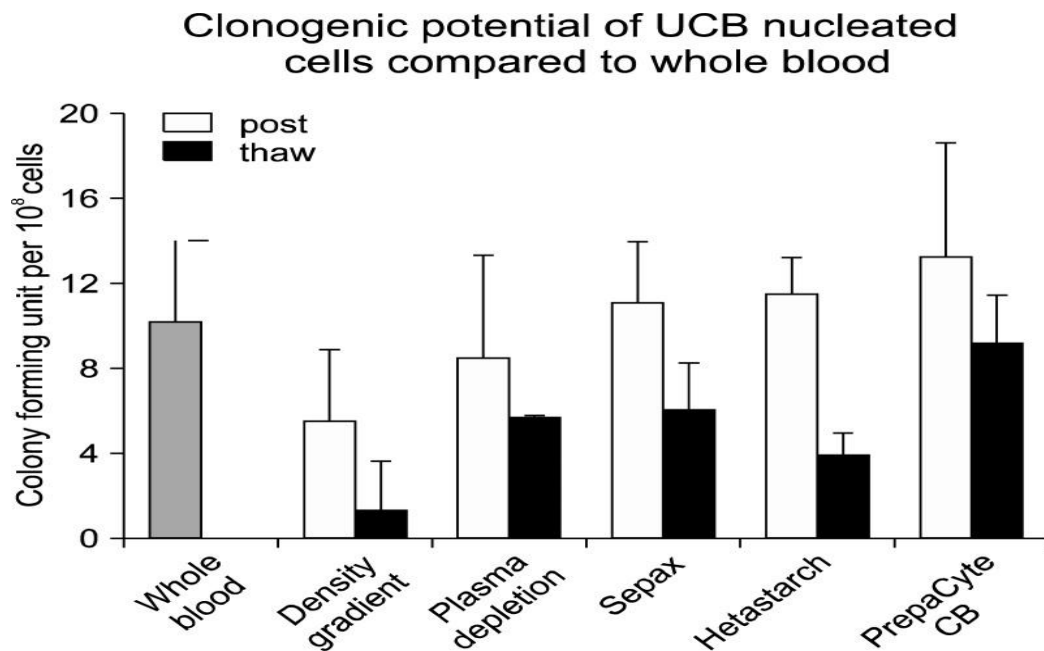


Figure 7: Clonogenic Potential of UCB Units Using the Different Clinical Processing Techniques. Source⁹

Total Nucleated Cells and Mono Nucleated Cells recovery rate of Hespan and Sepax against AXP processing methods were compared³¹. His work showed that both Hespan and Sepax reproducibly recover greater than 95% of the cord blood stem cells in a typical collection and result in a reduced final volume of approximately 20 cc for final storage (Figure 8). AutoXpress allows for greater throughput with fixed personnel numbers (increasing the economy of operations) and is a Food and Drug Administration (FDA)-cleared, functionally closed system which is capable of processing cord blood collections of any volume.

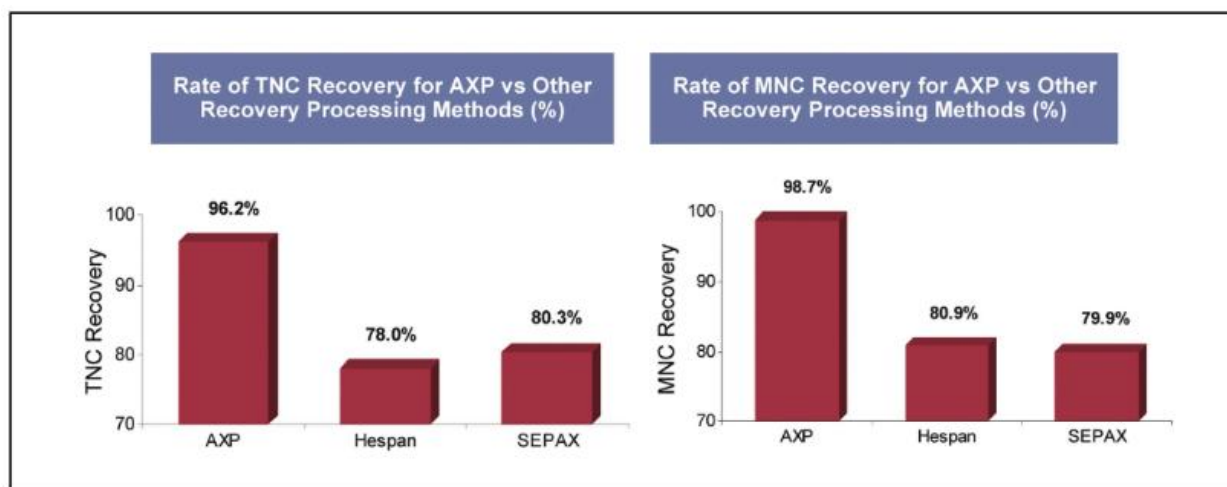


Figure 8: Rate of TNC and Mono Nucleated Cell Recovery for Different Processing Methods.

Source³¹

A group of workers² examined the volume reduction methods of processing umbilical cord blood and classified them into zero, first, second and third generations (Table 3). The most common methods used for the processing of cord blood units today seems to be the Hetastarch or HES red blood cell reduction (RCR), whether automated or manual.

Table 3: Technical Comparison made on some of the Most Popular First-generation Cord Blood Processing Techniques and the Proprietary Second and Third Maxcell Cord Blood Processing Technologies

	Nature of Umbilical Cord Blood	Manual or Automated Processing	Red Blood Cell reduction	Plasma depletion
Zero generation	Whole cord blood	Manual	None	None
1st generation Hetastarch	Red blood cell reduced	Manual Sepax or AutoXpress	Yes	Yes
1st generation PrepaCyte-CB	Red blood cell reduced	Manual	Yes	Yes

1 st generation Top & bottom Operations II	Red blood cell reduced	Manual	Yes	Yes
1 st generation Ficoll	Red blood cell reduced	Manual	Yes	Yes
MaxCell (MC) Technologies				
2 nd generation	Plasma depleted	Manual Sepax	NO	YES
3 rd generation	MaxCord Red blood cell reduced + Red blood cell Replete	Manual Sepax AutoXpress	Yes/No	Yes

Source²

These have been called 1st generation cord blood processing techniques. These methods are often mistakenly referred to as red blood cell reduction when all of these techniques retain considerable red blood cells and do not deplete, but only lower the count of RBCs³⁸. Unfortunately, all 1st generation RCR processing methods lose significant numbers of stem cells, nucleated cells, and progenitor cells as measured by CD34+ cells or CFUs counting, with an approximately 25 percent average loss of nucleated cell among various reports^{3, 5,26,39-43}.

4. Conclusion

First problem of cord blood banking is that banking a sufficiently large number of cryoprotected cord blood units requires large amounts of costly storage space in liquid nitrogen. It was as a result of this that volume reduction processing of cord blood units was developed, with its associated decrease of the bulk of the RBC and depletion of plasma.

The five most popular processing techniques are Plasma Depletion, Density Gradient, Hetastarch, PrepaCyte-CB and Automated Centrifugal Machine (Sepax). Most methods involve

centrifugation, sedimentation and/or filtration for reducing the red cell content, plasma volume, or both

Many workers have compared the performance of the different processing techniques in achieving the set goals. PrepaCyte-CB and Hespan record similar success of neutrophil engraftment. PrepaCyte-CB however has faster engraftment time than the four other UCB processing methods. PrepaCyte-CB also recovers more viable stem cells significantly than other method of processing.

In terms of the recovery of nucleated cells crucial for successful engraftment, it was reported that Sepax depletion gives a high recovery of nucleated cells, and this is very crucial for a successful transplantation, but recovery using Sepax has been reported to be reduced as size of unit processed increases. Density gradient, Hetastarch and plasma depletion separation were also affected in this way, however, PrepaCyte-CB was not affected by initial volume of the collected unit, and recovery of both total nucleated cells and CD34+ progenitor cells was as efficient with smaller volumes the same way it was with larger units. Density gradient separation showed a reverse correlation: as umbilical cord blood volume increase, so does recovery.

PrepaCyte-CB is the best processing methodology for optimum haemopoietic stem cell numbers from all the major three developmental stages of stem cells. For the removal of red blood cell, density gradient separation has been found to be the most effective method.

The clonogenic potential of umbilical cord blood units have been measured by the colony forming unit assay using different laboratory processing techniques. PrepaCyte-CB came out as best in this test, not only in post processing but also after the process of cryopreservation and subsequent thawing process. Hespan and Sepax reproducibly recover greater than 95 percent of the cord blood stem cells in a typical collection and result in a reduced final volume of approximately 20 cc for final storage.

It is worthy of note that Sepax and Hespan are often erroneously referred to as red cell depletion when all of these techniques retain considerable red blood cells and still do not deplete, but only reduce the number of red blood cells.

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