Cytotherapy, 2015; 17: 58–67

International Society for Cellular Therapy ISCT®

CrossMark

Improving umbilical cord blood processing to increase total nucleated cell count yield and reduce cord input wastage by managing the consequences of input variation

MAY WIN NAING[1], DANIEL A. GIBSON[2], PAUL HOURD[1], SUSANA G. GOMEZ[2], ROGER B.V. HORTON[2], JOEL SEGAL[3] & DAVID J. WILLIAMS[1]

[1]Centre for Biological Engineering, Healthcare Engineering Research Group, Loughborough University, Loughborough, United Kingdom, [2]Anthony Nolan Cell Therapy Centre, Nottingham, United Kingdom, and [3]Manufacturing Research Division, Faculty of Engineering, University of Nottingham, Nottingham, United Kingdom

Abstract

Background aims. With the rising use of umbilical cord blood (UCB) as an alternative source of hematopoietic stem cells, storage inventories of UCB have grown, giving rise to genetically diverse inventories globally. In the absence of reliable markers such as CD34 or counts of colony-forming units, total nucleated cell (TNC) counts are often used as an indicator of potency, and transplant centers worldwide often select units with the largest counts of TNC. As a result, cord blood banks are driven to increase the quality of stored inventories by increasing the TNC count of products stored. However, these banks face challenges in recovering consistent levels of TNC with the use of the standard protocols of automated umbilical cord processing systems, particularly in the presence of input variation both of cord blood volume and TNC count, in which it is currently not possible to process larger but useable UCB units with consequent losses in TNC. *Methods.* This report addresses the challenge of recovering consistently high TNC yields in volume reduction by proposing and validating an alternative protocol capable of processing a larger range of units more reliably. *Results.* This work demonstrates improvements in plastic ware and tubing sets and in the recovery process protocol with consequent productivity gains in TNC yield and a reduction in standard deviation. *Conclusions.* This work could pave the way for cord blood banks to improve UCB processing and increase efficiency through higher yields and lower costs.

Key Words: *separation, Sepax, umbilical cord blood processing, volume reduction*

Introduction

With the rising use of umbilical cord blood (UCB) as a source of hematopoietic stem cells (HSC), storage inventories of have grown, giving rise to vast numbers of genetically diverse UCB units to provide an alternative when an adult peripheral blood stem cell or bone marrow donor cannot be found. This alternate product is "off the shelf" and is pre-established as "fit for use" through process control and qualification. Querol *et al.* [1] illustrate that UCB users rely on the rigorous assessment carried out in banks to avoid poor engraftment after thaw and that correlated attributes such as total nucleated cell (TNC) count is used as a surrogate of graft potency in the absence of a better marker such as CD34, colony-forming units (CFU) or cellular viabilities because of the ease of standardization across all banks. As is accepted in bone marrow transplantation, the hematopoietic potential of UCB is proportional to the TNC count and thus correlates to transplantation end points [2,3]. It is challenging for public cord blood banks to remain economically viable because UCB utilization rates are relatively low compared with inventory size, being reported as between 3–4% across World Marrow Donor Association and the US National Marrow Donor Program registries [4]. Howard *et al.* [5] analyzed the cost-effectiveness of banks on the basis of inventory size, which serves to illustrate the requirement of the niche that UCB transplantation currently populates. Therefore, despite that the majority of units will never be utilized, the statistical relevance of such inventories is required to overcome the current unmet need as a result of human leukocyte antigen

Correspondence: **David J. Williams**, OBE FREng BSc PhD DEng CEng FIMechE, Centre for Biological Engineering, Healthcare Engineering Research Group, Loughborough University, Loughborough, UK LE11 3TU. E-mail: D.J.Williams2@lboro.ac.uk

(Received 18 June 2014; accepted 12 September 2014)

http://dx.doi.org/10.1016/j.jcyt.2014.09.003

ISSN 1465-3249

(HLA) disparity. Querol *et al.* [6] further examined this, but, in addition to HLA disparity, also further discriminated with respect to UCB quality, again using TNC count as a surrogate marker and illustrating that public banking programs should focus on increasing the quality of banked inventories to mitigate the inventory size to remain statistically relevant while increasing the utilization rate.

When selecting UCB units, transplant centers will facilitate national registries to perform the necessary searches that are typically carried out with the use of complicated algorithms. The Anthony Nolan search algorithm for a basic UCB unit selection first looks to create a list of units that are greater than 2.5×10^7 TNC/kg for a single or 1.5×10^7 TNC/kg for a double, ranked initially by 6/6, 5/6 and finally 4/6 HLA-matched. Howard *et al.* explain that UCB utilization is sensitive to the selection process, whereas Lee *et al.* [4] describe the need to gain the transplant physicians' trust; both illustrate that the individual preferences of transplant centers also have an impact on unit selection. Units will be ranked by HLA (first HLA-C matching at antigen level, double mismatches at the same locus on HLA class I, high-resolution matching in HLA class I loci and mother's HLA typing: Non Inherited Maternal Antigen (NIMA) match to prefer and Inherited Paternal Antigen (IPA) mismatch to be avoided), CD34 dose, CFU/viability, red blood cell (RBC) depletion during cell processing and finally, ethnicity. Such algorithms put the greatest onus on TNC dose above any other cellular attribute. Barker *et al.* [7] concluded that TNC count is of critical importance in predicting graft success alongside HLA disparity and that increasing TNC dose can mitigate the disadvantages of a greater HLA disparity at 5/6 level match.

Despite the fact that the only accepted *in vitro* measure for engraftment is a CFU assay [2] and that markers such as CD34+ have proven to be predictive of engraftment [8], these factors are not weighted highly in selection algorithms; as a result of this, UCBs are driven to increase the quality of stored inventories by increasing the TNC counts.

This is challenging in the presence of process input variation as represented by differences in the blood volume of collected UCBs and TNC count. In some cases collected UCBs and consequent TNC counts are too large to be processed with current protocols.

There are 2 methods of increasing the TNC count of products, the first being increasing the total number of TNC collected during the procurement of the UCB. This has proven to be highly dependent on the competency of the individual collecting the blood. For this reason, the Anthony Nolan Cell Therapy Centre (ANCTC) adopted a system of highly trained collectors. The level of "uncollected" blood in the placenta is not known, and therefore any improvements in the procurement would need to be evaluated. The second method, the focus of this investigation, is to reduce the TNC loss by improved processing and reduction in wastage of collected UCBs. No system recovers 100% of the TNC. The ANCTC has implemented the Sepax System (Biosafe SA, Eysins, Switzerland) which is well suited to UCB processing. The system is capable of high recoveries [9] while being a functionally closed processing system. All aspects of the separation phases are automated and therefore not subject to user variability. The ANCTC opted for a non-hydroxyethyl starch procedure to better control the process. By not adding anything during the process (other than the cryoprotectant), the risk of contamination is minimized, the processing costs are reduced and the risk of undesirable side effects is eliminated [10]. Initial data showed that processing with and without non-hydroxyethyl starch was comparable with well-controlled units (shipment time and temperature) (unpublished data); the ANCTC already has stringent procedures controlling this. However, it was observed that the yields of TNC recovered after processing tended to be lower for larger UCB units, for example, those with higher TNC counts before processing (Figure 1).

Figure 1 shows the correlation of median yields and ranges of recovered TNC with respect to the range of TNC count before processing. At the ANCTC, Only UCB units with TNC count of at least 1200×10^6 are processed, whereas UCB units with a TNC count of at least 1500×10^6 are defined as large UCB units. It has been observed that as the range of TNC increases, the recovered percentage of TNC decreases. Moreover, the maximum and minimum percentages of recovered TNC range widely, from as low as approximately 40% to as high as nearly 120%.

This is inhibitory to the effectiveness of a cord blood bank. Despite the quality of the input product, the final product is consequently limited to a particular repertoire of potential recipients. An increase in transplant-related mortality rate has been observed in children transplanted with a low UCB dose [11]. Assuming a minimal cell dose of $>3 \times 10^7$/kg, an average final TNC count of 120×10^7 would be suitable for a patient weighing approximately 40 kg, typically a prepubescent child according to the Royal College of Paediatrics and Child Health. High losses of TNC during processing would mean that the final cell dose is insufficient and consequently, potentially ineffective, for many of the patients. The impact of this is somewhat reduced by the potential for double UCB transplants, but, as the processing facility with responsibility for the quality of the products stored as detailed previously, it is imperative to strive to improve the processing system to maximize the yield

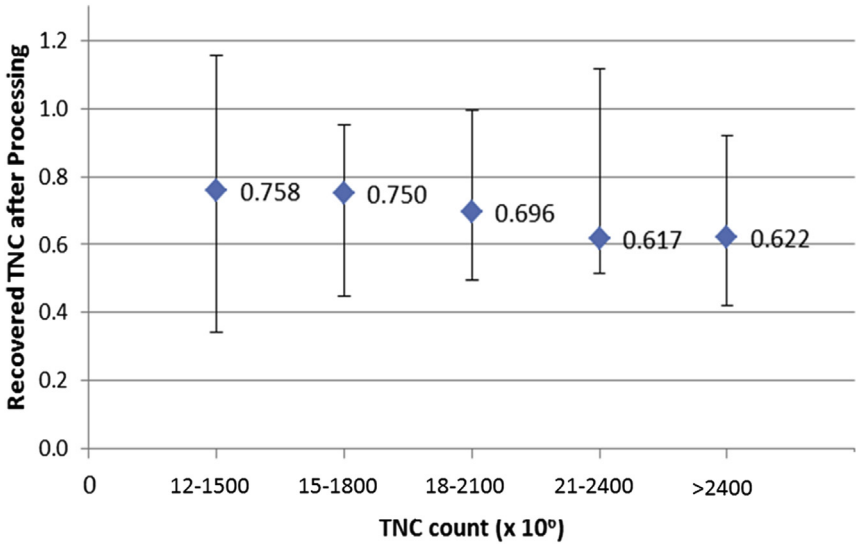

Figure 1. Median TNC yield for different ranges of TNC with the use of a protocol on Sepax.

from every unit. Given that UCB selection criteria stipulate a minimum cell dose per kilogram, reducing cellular attrition during collection can radically change the repertoire of patients for whom the collected UCB is suitable. This is particularly relevant for large units, defined by the ANCTC as UCB units with TNC count $>1500 \times 10^6$ cells, which have recovery rates of $\leq 75\%$ (Figure 1).

Variability in incoming UCB units

One of the major challenges in processing of biological materials for clinical applications is maintaining quality standards in the face of large variations in the input to the process. In the case of processing UCB for banking, banks must contend with the variability of incoming UCB units in terms of volumes as well as TNC counts. An analysis of the 1839 UCB units collected by the ANCTC between December 2008 and June 2012 shows that there is a wide distribution of the UCB units collected in terms of TNC counts and volume (Figure 2A,B). The mean TNC count was 1086×10^6 cells, with a standard deviation of 651×10^6 cells; the mean volume was 93.6 mL, with a standard deviation of 13.9 mL. There may be a relationship between volume of the UCB unit and the number of TNC within the unit (Figure 2C).

Hematocrit of the samples ranged from approximately 10–50%, with a mean value of 36.3% and a standard deviation of 5.15%. There are also many variations in other factors such as platelet counts, proportion of CD34+ cells to TNC, proportions of mononuclear cells (MNC) to TNC, and number of nucleated red blood cells. All or a combination of some of these factors may contribute to processing outcomes.

As seen in Figure 1, as the size of the UCB unit measured in TNC increases, the yields drop, with units of UCB 1800×10^6 faring poorly after processing with yields of <70%. Taking these factors into consideration, this work seeks to test the theory that 21 mL (a standard volume for volume-reduced UCB) is the optimal final volume for all UCB units regardless of the initial number of TNC or initial collected volume. Previously, Papassavas *et al.* [12] have shown that with the use of the Sepax system, splitting large units of UCB into 2 smaller volume units before processing increased TNC, mononuclear and CD34+ recoveries. Solves *et al.* [13] also showed that in systems other than Sepax, the increase in volume of buffy coat collected increased the yield of TNC.

It has been hypothesized that units of UCB centrifuged at similar speeds and durations (adjusted for volume as in the protocols used in Sepax) will produce different volumes of packed TNC, depending on the actual initial number of TNC, that is, a unit with a higher number of TNC should result in a higher volume of buffy coat. Coupled with the results shown in Figure 1, it has been hypothesized that for larger UCB units ($>1500 \times 10^6$ TNC), the yield of TNC can be improved by increasing the final buffy coat volume collected. The ANCTC is already in operation with established protocols in line with internationally accepted standards for UCB banking. Hence, it is imperative that any new protocols must be minimally disruptive to standard operating procedures and must not deviate from already-established measures for quality control. To do this in line with these current protocols, it has been proposed to collect 2 volumes of 21 mL each from each UCB unit. However, initial experiments showed that

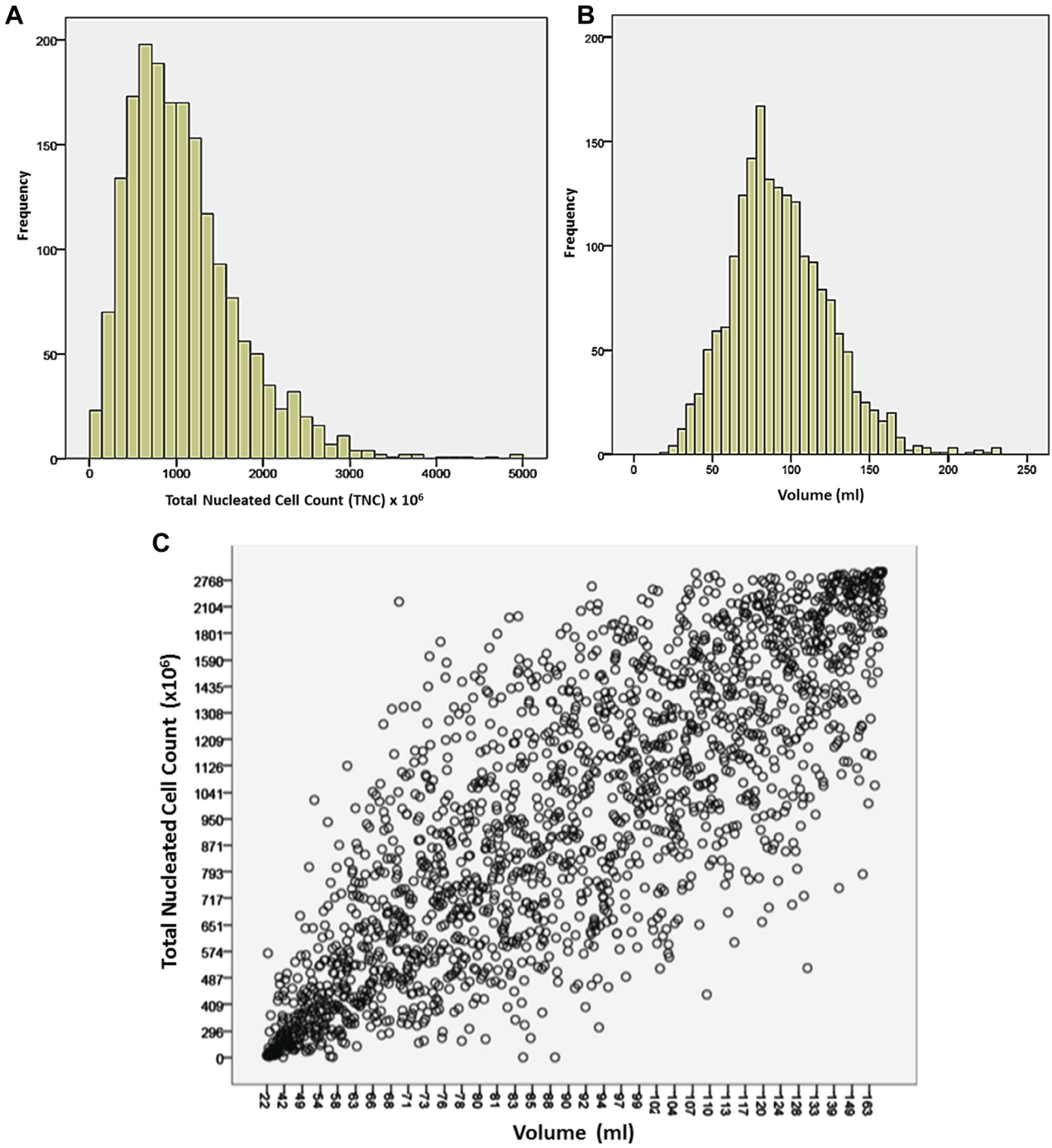

Figure 2. Histograms show distribution of (A) TNC counts of the incoming UCB units, (B) volume of the incoming UCB units and (C) relationship between volume and TNC count; $n = 1839$.

collecting a total of 42 mL does not accommodate buffy coat separation well because the volume itself is too large for effective removal of RBCs. After this, a series of in-house experiments further indicated that 30 mL may be a more optimal volume for buffy coat collection. Hence, it is proposed that 30 mL is collected through the use of the system, and the unit is topped up to 42 mL by use of the volume of plasma collected during the buffy coat separation.

For this study, the Sepax 1 was used for the majority of the dataset. Part-way through the study, Biosafe provided the use of a Sepax 2 for the purposes of evaluating process optimization. The Sepax 2 is functionally the same as the Sepax 1, with changes to the user interface and a better module for traceability. A small sample set was processed on the Sepax 2 according to the same process methodologies to confirm this.

For validation purposes, 30 UCB units with TNC counts ranging from 735×10^6 to 2344×10^6 were processed over a period of 6 months between June 2012 and December 2012 with the use of this new setup and protocol.

Methods

Because the purpose of the improvement project was to allow for rapid implementation on successful validation, it was crucial that as far as possible, the experimental protocols follow the clinical unit processing protocol. Hence, in selecting the UCB unit for the validation exercise, the following criteria were used in accordance with ANCTC standards: (i) input TNC must be >600 and (ii) UCB units must be <33 hours old at the time of processing.

Because the aim of this work was to recover a higher percentage of TNC from large units, wherever possible, units with counts $>1500 \times 10^6$ TNC were used. The units used for this validation have been rejected for clinical use because they fell under exclusion criteria such as the mother having undergone hormone treatments. All units used had been consented for research before the validation experiments. To comply with the current regulatory framework for cord blood banks, all processing was carried out in C-grade Good Manufacturing Practice facilities and quality control tests were performed in class II laboratory facilities according to the current standard operations procedures for processing of UCB for clinical use within the ANCTC.

Initial counts

All UCB units to be used in the validation were processed under the same protocol for clinical units for reception and initial sampling. The UCB unit was weighed and passed into the processing room, where a sample bubble was attached to the UCB unit by means of the total containment device. The unit was then placed onto the shaker for 10 min before a sample calibrator (calibrated for 1mL) was used to transfer 1 mL of UCB to the sample bubble. The sample bubble was then sealed with the use of a tube sealer.

The sample was transferred to the quality control laboratory, where the cell count for TNC was performed with the use of a Sysmex XE-2100 Automated Hematology System (Sysmex UK Ltd, Milton Keynes, UK). The following parameters were recorded into the database: bar code, date, time of reception, weight, volume, TNC count, percentages of neutrophils, leukocytes and monocytes, number of nucleated red blood cells and hematocrit.

UCB volume reduction with the use of the Sepax system

Before volume reduction with the use of the Sepax system, another sample of approximately 0.5 mL was taken as a control and stored in the processing room. Because the volume to be collected (30 mL) exceeded the fill volume of the final bag on in the manufacturer's kit (maximum volume = 25 mL), the Sepax single-use kit CS-540.4 (Biosafe SA, Eysins, Switzerland) was modified by removing the final bag and substituting with an Eva double kit (Macopharma, Twickenham, UK) (Figure 3). The Eva double kit has 2 bags with a fill volume of up to 30 mL each. The Eva double kit has individual clamps on the tubing leading to each bag and hence makes it possible to collect and analyze the different volumes collected during the 2 separate rounds of buffy coat collections according to Sepax protocol.

The 2 Eva bags were labeled as buffy coat bag 1 (BC1) and buffy coat bag 2 (BC2). The modified kit was mounted into the system according to standard operating procedure, in accordance with the instructions in the manufacturer's manual. On Sepax, the level of sensitivity was set to 3 (high) and the volume of buffy coat to be collected was set to 30 mL, as mentioned previously. All other settings remained unchanged. Checks were performed to ensure that all connections were secure and that there were no leakages. Once the initial vacuuming of the kit was done by the system, the clamp on BC2 was closed off. As a result, the extracted buffy coat after the first sedimentation flowed only into BC1. During the second phase of sedimentation, the clamp of BC1 was closed off and that of BC2 was opened such that the buffy coat from the second separation only flowed into BC2.

Once the run was completed, the kit was removed according to Sepax protocol. Each bag (plasma bag, RBC bag, BC1 and BC2) was sealed and placed on the shaker for at least 10 min to ensure that contents were homogeneous. The RBC bag, BC1 and BC2 were weighed, and weights were recorded. In a biosafety cabinet, 0.5 mL of each sample from all of the 4 bags were taken and transferred into sample tubes. The control sample (taken before processing) was also transferred to a sample tube. The contents of BC1 and BC2 were combined, and the weight was recorded. A 1-mL sample was taken from this bag as the final sample. All the sample tubes were transferred to the quality control laboratory for analysis.

Quality control analysis

Table I summarizes the quality control analysis carried out on each UCB. TNC counts were performed on all samples including control samples and samples drawn

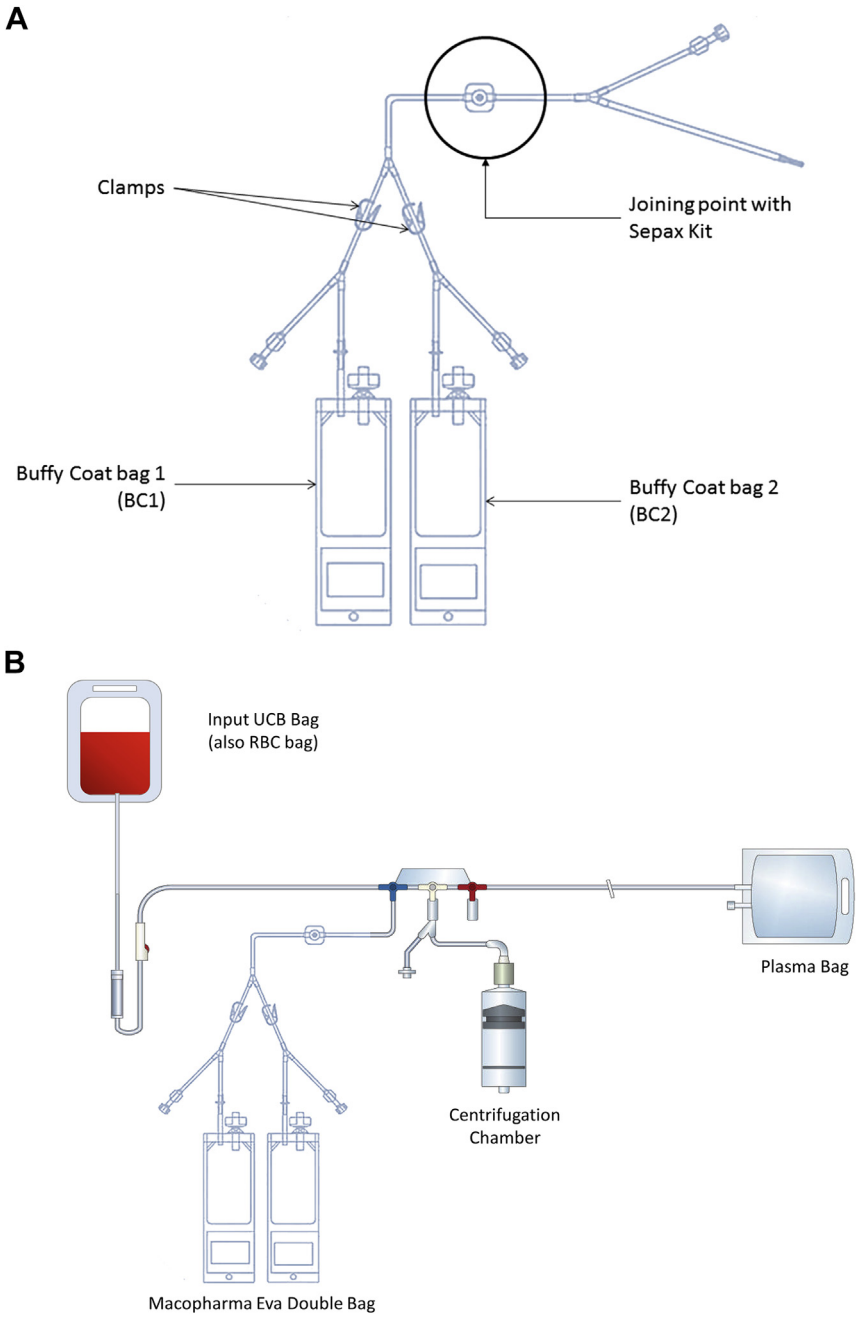

Figure 3. (A) Macopharma Eva double kit and (B) Sepax single-use kit CS504.1 attached to Macopharma Eva double kit.

from plasma bags. Flow cytometry analysis and CFU assays, currently the only accepted in vitro measure for engraftment [2], were only performed for control samples and final samples.

Flow cytometry protocol (fluorescence-activated cell sorting)

With the use of the values from the TNC count, the volume of blood necessary to add 0.6×10^6 cells to a fluorescence-activated cell sorting (FACS) tube was calculated. If the total RBC count in the tube exceeded $175{,}000 \times 10^3$, the calculated volume of blood was reduced accordingly to ensure that complete lysis of red cells occurred in the assay. Each TruCOUNT tube was labeled with the sample identification and checked to ensure that the microbead pellet was intact and contained within the metal retainer at the bottom of the tube. In a biosafety cabinet, the volume of cells required was added onto the side of the tube just above the retainer and topped up to 50 µL with FACS buffer; 50 µL of the fluorescein isothiocyanate antibody cocktail was added to each tube. The tubes were

Table I. Summary of quality control analysis for validation units.

	Sysmex (TNC)	FACS (CD45/CD34)	CFU assay
Initial quality control	Yes	–	–
Control	Yes	Yes	Yes
Buffy coat 1 (BC1)	Yes	–	–
Buffy coat 2 (BC2)	Yes	–	–
Final	Yes	Yes	Yes
RBC bag	Yes	–	–
Plasma bag	Yes	–	–

incubated for 15 min in the dark. Lysis solution was prepared by dilution of Pharm lyse with distilled water in a centrifugal tube; 900 μL of the lysis solution was added to the tubes and vortexed gently to allow mixing. Because of the higher hematocrit of the samples, the tubes were incubated for 10 min at 37°C. Subsequently, 5 μL of 7-aminoactinomycin D (7-AAD) was added to the tube, mixed gently and allowed to incubate for at least 2 min before being run on the cytometer.

The FACS results from the control sample and the final sample were compared to ensure that the volume reduction process does not adversely affect the viability of the CD34+ cells. Within each sample, the CD45 readings from the FACS results were also compared against TNC readings to check that sampling was consistent and as a precaution against systematic errors such as machine malfunctions. The counts were repeated if the difference between the TNC reading and the FACS reading was more than ±20%.

CFU protocol

MethoCult GF (STEMCELL Technologies, Grenoble, France) was aliquoted into 3-mL tubes and stored in the refrigerator. Before starting the assay, the required number of MethoCultGF aliquots were removed from the refrigerator and allowed to stand at room temperature. The FACS results were entered into the database and used to calculate the volume of the sample required in microliters for addition to a 3-mL aliquot of MethoCultGF such that the final cell density was approximately 150 CD 34+ cells per dish.

In a biosafety cabinet, the calculated volume of the blood sample cell suspension was pitpetted into the 3-mL tube of MethoCult GF. The sample volume in each aliquot was subsequently made up to 300 μL with Iscove's Modified Dulbecco's Medium + 2% fetal bovine serum. The contents of the tube were vortexed vigorously until the cells were evenly suspended, and the tube was left standing for 10 min to allow all bubbles to rise to the surface. Subsequently, 1.1 mL of the mixture was drawn with a blunt needle and dispensed into a well of a 6-well plate. Another 1.1 mL from the same tube was placed into a well diagonally opposite to the first well. The plate was rotated and tilted to spread the viscous

Table II. Summary of results of experimental data compared with clinical data.

	Clinical ($n = 333$)	Experimental ($n = 30$)	*P* value
Pre-processing			
Volume (mL)	117.8 ± 26.9	159.4 ± 34.8	<0.001
TNC × 10^6	1769.4 ± 572.2	1816.1 ± 422.2	>0.05
MNC × 10^6	933.6 ± 374.2	1053.8 ± 419.0	>0.05
CD34+ stem cells × 10^3	6384.5 ± 2909.9	6598.3 ± 4570.3	>0.05
Leukocyte %	36.7 ± 3.9	35.4 ± 5.4	>0.05
Monocyte %	10.9 ± 3.3	11.1 ± 2.6	>0.05
Granulocyte %	47.3 ± 8.8	48.1 ± 5.9	>0.05
Hematocrit %	38.8 ± 4.6	39.1 ± 3.4	>0.05
Post-processing			
Final volume (mL)	20.5 ± 0.6	30.4 ± 0.4	Not Applicable
TNC recovery (%)	75.2 ± 15.70	89.3 ± 6.2	<0.001
MNC recovery (%)	80.8 ± 14.2	86.1 ± 6.1	<0.001
CD34+ stem cell recovery (%)	88.2 ± 21.2	97.1 ± 23.7	<0.001
Leukocyte %	39.7 ± 12.4	38.0 ± 6.1	>0.05
Monocyte %	17.3 ± 7.2	12.3 ± 3.2	<0.001
Granulocyte %	37.4 ± 10.7	42.8 ± 7.2	>0.05
Final hematocrit %	37.4 ± 10.2	39.2 ± 9.1	>0.05
Viability (%)	97.2 ± 3.7	95.2 ±8.6	>0.05

Values are expressed as mean ± standard deviation. Hematocrits of experimental validation units are based on 42 mL (30 mL of buffy coat topped up with 12 mL of plasma). Because clonogenic efficiency results are not available for all clinical units, $n = 144$ (clinical units) for this comparison.

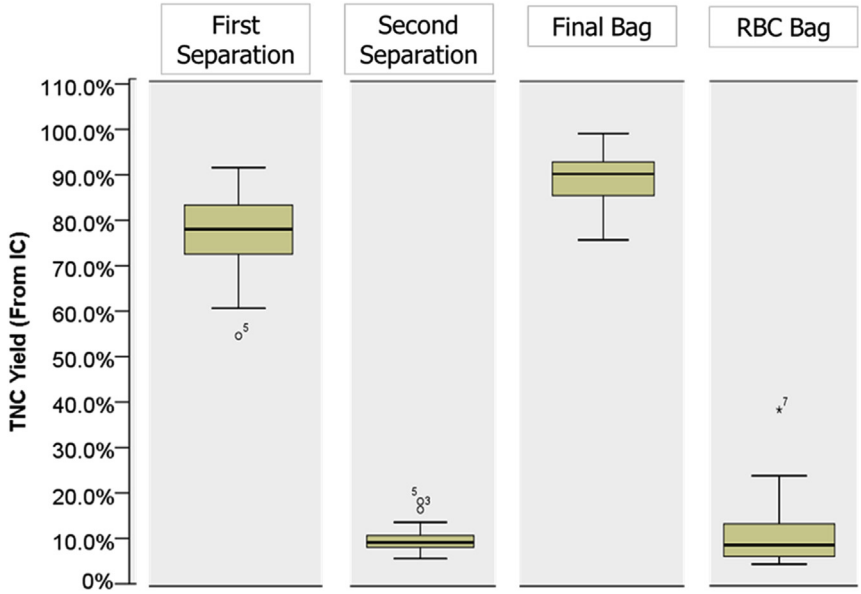

Figure 4. Chart shows TNC content (expressed in percentage of initial count) in the first separation (BC Bag 1), second separation (BC Bag 2), final bag and RBC bag.

mixture evenly across the surface of each dish such that the meniscus attached to the wall of the dish on all sides. Both wells were labeled, and the empty wells in the plate were topped up with sterile water. The plate was incubated for 14 days at 37°C, 5% CO_2 in air and >95% humidity before being removed from the incubator for counting of colonies. The counts were recorded in the database, and the clonogenic efficiency of the unit was calculated.

Results

Results were analyzed and plotted with the use of statistical software (SPSS, version 19; SPSS Inc, Chicago, IL, USA). A total sample of 30 units was processed and measured with TNC counts ranging from 735 × 10^6 to 2344 × 10^6; 20 units were processed with the use of Sepax 1, and 10 additional units were processed with the use of Sepax 2. Table II shows the summary of results comparing clinical data (n = 333) processed up to 2012 and the experimental units processed with the use of the modified double-bag kit (n = 30).

The clinical data set and the experimental dataset were compared by means of a 2-sample t-test. A value of $P \leq 0.05$ was considered significant. As shown in Table II, the mean input volumes for the experimental dataset were significantly higher than for the clinical dataset ($P \leq 0.05$). However, after processing, the recovery rates for TNC, MNCs and CD34+ progenitor cells were all significantly higher than that of the clinical dataset ($P \leq 0.05$). We also noted that the value of the hematocrit in the modified protocol did not significantly differ from that of the current clinical protocol.

Figure 4 shows a graphical presentation of distribution of TNC (in percentages) in 4 bags: first separation (BC1), second separation (BC2), final

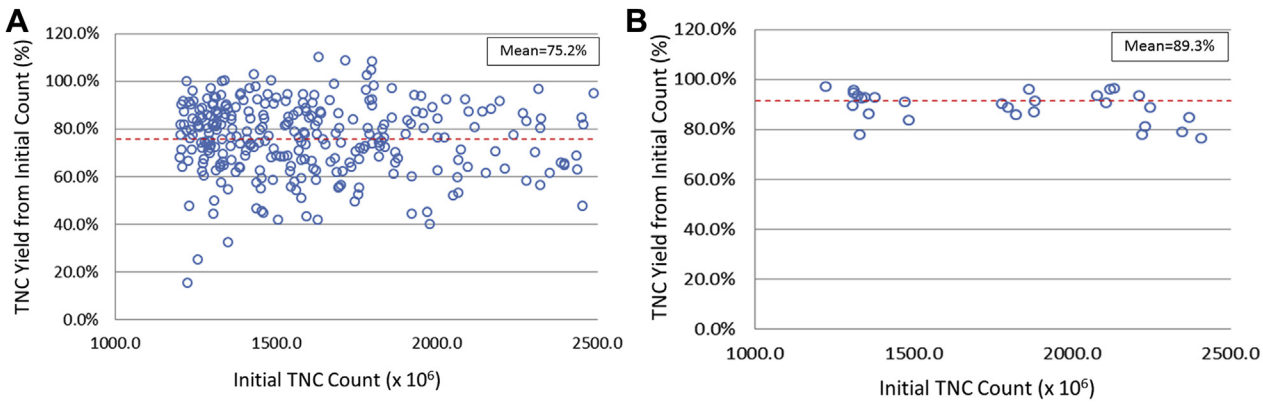

Figure 5. Charts show the yields of (A) clinical units (mean TNC yield = 75.2%) and (B) experimental units (mean TNC yield = 89.3%).

bag (combined) and RBC bag. Results showed that the Sepax protocol recovered a higher percentage of the TNC during the first separation than during the second separation when sensitivity level was set to 3 and the volume of buffy coat was set to 30 mL. For this particular setting, the first separation drew approximately 18−19 mL, whereas the second separation drew approximately 11−12 mL. The TNC counts carried out on plasma bags show that there is little loss of nucleated cells into the plasma bag, indicating effective separation of plasma and buffy coat. However, up to 20% of nucleated cells, mainly neutrophils, were lost to the RBC bag, with a mean loss of approximately 7% for each unit of UCB (Figure 4).

The results of the final bag were checked not only against the values of BC1 and BC2 but also against that of the RBC bag to ensure that the values were consistent.

Comparing the results of this protocol with data collected from clinical units ($n = 333$), in which only 21 mL of buffy coat was collected, this work shows promising results in terms of mean recovery values and standard deviations (Figure 5). The mean TNC recovery from cord blood units with the use of the modified double-bag method was >14% higher and the variability in recoveries 2.5-fold lower (7% compared with 16% coefficient of variation (CV)). There was no significant difference in the results between the experimental units and the clinical units in terms of clonogenic efficiency, which shows that this change in methodology does not adversely affect the clinical efficacy of the cells.

Because the ANCTC had recently acquired one Sepax 2 system from Biosafe, a number of units were also experimented on Sepax 2 with the use of the modified plastic ware and protocol. The same standard operating procedure was followed as with units run on Sepax 1. Because of resource constraints, only a small number of units were run on Sepax 2. Table III shows a detailed comparison between units run on Sepax 1 ($n = 20$) and units run on Sepax 2 ($n = 10$). Each corresponding set of data was compared by means of a 2-tailed unpaired *t*-test.

The results show that in the majority of the parameters, there were no significant differences within Sepax 1 and Sepax 2. There was no indication that the different default preset protocols affected the yield of TNC. Although units were randomly assigned during the experiment, input values for both sets of data were comparable. We noted that during the first separation, a higher buffy coat volume was drawn on Sepax 2 compared with that in Sepax 1. This may be a result of the enhanced sensitivity of the new protocol that Sepax 2 is running. We observed that during the second separation, a smaller volume was drawn to compensate and make up the correct amount of the total volume. As a result, the corresponding hematocrit was significantly higher in the first separation. Inexplicably, the hematocrit in the second separation was also significantly higher for Sepax 2. When the final volume was adjusted to 42 mL with the addition of 12 mL of plasma, however, the hematocrit values were comparable between the 2 systems.

Table III. Comparison between Sepax 1 and Sepax 2.

	Sepax 1 ($n = 20$)	Sepax 2 ($n = 10$)	*P* value
Pre-processing			
Volume (mL)	143.9 ± 33.8	166.6 ± 36.6	>0.05
TNC count × 10^6	1738.9 ± 459.3	1876.0 ± 371.2	>0.05
MNC × 10^6	912.2 ± 267.1	947.2 ± 186.0	>0.05
CD34+ stem cells × 10^3	6342.1 ± 2627.3	6469.2 ± 3403.9	>0.05
Leukocyte %	35.7 ± 6.8	34.9 ± 3.5	>0.05
Monocyte %	11.2 ± 2.2	11.1 ± 2.2	>0.05
Granulocyte %	47.5 ± 6.2	49.2 ± 5.1	>0.05
Hematocrit %	39.6 ± 3.3	38.2 ± 3.3	>0.05
First separation			
Volume 1 (mL)	18.9 ± 1.7	20.0 ± 0.5	<0.05
TNC recovery (%)	77.4 ± 9.6	80.1 ± 8.7	>0.05
MNC recovery (%)	76.3 ± 9.6	75.3 ± 5.4	>0.05
Leukocyte %	39.8 ± 8.2	36.3 ± 5.8	>0.05
Monocyte %	12.5 ± 2.8	12.6 ± 2.7	>0.05
Granulocyte %	38.3 ± 11.7	38.7 ± 11.3	>0.05
Hematocrit %	53.6 ± 9.3	63.1 ± 5.7	<0.05
Second separation			
Volume 2 (mL)	11.4 ± 1.5	10.5 ± 0.3	<0.05
TNC recovery (%)	10.4 ± 3.1	9.5 ± 1.4	>0.05
MNC recovery (%)	6.9 ± 2.1	7.2 ± 1.9	>0.05
Leukocyte %	28.5 ± 9.5	29.3 ± 6.0	>0.05
Monocyte %	7.8 ± 2.9	9.6 ± 1.8	>0.05
Granulocyte %	57.5 ± 11.6	57.0 ± 8.2	>0.05
Hematocrit %	53.6 ± 6.1	60.5 ± 7.8	<0.05
Post-processing			
Final combined volume (mL)	30.3 ± 0.3	30.5 ± 0.5	>0.05
TNC recovery (%)	89.6 ± 6.3	88.8 ± 6.0	>0.05
MNC recovery (%)	87.0 ± 5.8	84.2 ± 8.3	>0.05
CD34+ stem cell recovery (%)	102.5 ± 23.3	86.4 ± 20.7	>0.05
Leukocyte %	39.8 ± 8.2	36.1 ± 5.7	>0.05
Monocyte %	12.5 ± 2.8	12.4 ± 2.8	>0.05
Granulocyte %	38.3 ± 11.7	44.6 ± 7.4	>0.05
Hematocrit % (in 30 mL)	53.6 ± 7.2	61.9 ± 4.4	=0.001
Hematocrit % (corrected; 42 mL)	38.6 ± 5.3	40.4 ± 13.9	>0.05
Viability (%)	96.6 ± 1.9	97.0 ± 1.4	>0.05

Conclusions

Many parameters of collected UCB units may vary largely, depending on factors such as race, size of the baby, size of the mother, type of birth and so forth. In

the face of such wide variations, it is a challenging task to reliably and reproducibly process UCB units such that they are not only optimized for storage but also matched to the needs of patients in terms of diversity and size. An ineffective processing method will reduce the pool of UCB units available for patients in need because a smaller-than-expected volume of TNC is recovered; this not only decreases the quality of individual units but eventually may be detrimental both to the bank and to the patients. Because transplant centers regularly pick units with large TNC counts after processing, only the units containing the greatest TNC counts are used, and units with fewer nucleated cells may never be used. Over a long period of time, this will drive up unnecessary costs in terms of storage and facility maintenance, which will in turn be passed on to transplant centers and finally to the patients.

The validation shows that MNC and TNC yields can be increased in a cost-effective manner within the current Sepax protocol by modifying the processing plastic ware and increasing collection volume (30 mL), thus standardizing the yields irrespective of input TNC and/or volume. Furthermore, the yield of CD34+ cells is also maintained and improved. Even though Papassavas *et al.* [6] were successful in splitting large units into 2 bags to increase recovery, the splitting method not only increases time spent per unit but also doubles the consumable costs. With the modified kit and protocol presented in this work, the comparable increase in time and cost are much lower for the similar increases in yields. However, it must be noted that this method probably only applies to large units, and it is not the intention that the collection volume for all UCB should be changed to 30 mL. In conclusion, this work shows that it is possible to gain high consistent yields from across all UCB units during volume reduction without incurring high extra costs and with minimal disruption to the standard operating procedures established within the current regulatory framework.

Acknowledgments

This work was funded via the EPRSC Centre for Innovative Manufacturing in Regenerative Medicine. The authors would like to acknowledge all staff from Anthony Nolan Cell Therapy Centre, Nottingham, United Kingdom.

Disclosure of interests: The authors have no commercial, proprietary, or financial interest in the products or companies described in this article.

References

[1] Querol S, Gomez SG, Pagliuca A, Torrabadella M, Madrigal JA. Quality rather than quantity: the UCB bank dilemma. Bone Marrow transplantation 2010;45:970–8.

[2] Migliaccio AR, Adamson JW, Stevens CE, Dobrila NL, Carrier CM, Rubinstein P. Cell dose and speed of engraftment in placental/umbilical UCB transplantation: graft progenitor cell content is a better predictor than nuclear quantity. Blood 2000;96:2717–22.

[3] Barker JN, Scaradavou A, Stevens C, Rubinstein P. The dose-match interaction in umbilical UCB transplantation: an analysis of the impact of cell dose and HLA-match on the disease-free survival of 989 patients transplanted with single units for hematologic malignancy. Blood (ASH Annual Meeting Abstracts) 2007:110.

[4] Lee YJ, Kim JY, Mun YC, Koo HH. A proposal for improvement in the utilization rate of banked UCB. Blood Res 2013;48(1):5–7.

[5] Howard DH, Meltzer D, Kollman C, Maiers M, Logan B, Gragert L, et al. Use of cost-effectiveness analysis to determine inventory size for a national cord blood bank. Med Decis Making 2008;28(2):243–53.

[6] Querol S, Rubinstein P, Marsh SG, Goldman J, Madrigal JA. UCB banking: 'providing UCB banking for a nation'. British Journal of Haematology 2009;147:227–35.

[7] Barker JN, Scaradavou A, Stevens CE. Combined effect of total nucleated cell dose and HLA match on transplantation outcome in 1061 UCB recipients with hematologic malignancies. Blood 2010;115:1843–9.

[8] Wagner JE, Barker JN, DeFor TE, Baker KS, Blazar BR, Eide C, et al. Transplantation of unrelated donor umbilical UCB in 102 patients with malignant and non malignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 2002;100:1611–8.

[9] Basford C, Forraz N, Habibollah S, Hanger K, McGuckin C. The UCB Separation League Table: a Comparison of the Major Clinical Grade Harvesting Techniques for UCB Stem Cells. International Journal of Stem Cells 2010;3:32–45.

[10] Treib J, Baron JF, Grauer MT, Strauss RG. An international view of hydroxylethyl starches. Intensive Care Med 1999;25:258–68.

[11] Gluckman E, Rocha V. UCB transplantation: state of the art. Haematologica 2009;94:451–4.

[12] Papassavas AC, Gioka V, Chatzistamatiou T, Kokkinos T, Anagnostakis I, Gecka G, et al. A strategy of splitting individual high volume UCB units into two half subunits prior to processing increases the recovery of cells and facilitates ex vivo expansion of the infused haematopoietic progenitor cells in adults. Int J Lab Hematol 2008;30:124–32.

[13] Solves P, Mirabet V, Blanquer A, Delgado-Rosas F, Planelles D, Andrade M, et al. A new automatic device for routine UCB banking: critical analysis of different volume reduction methodologies. Cytotherapy 2009;11:1101–7.