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Multicenter Cell Processing for Cardiovascular Regenerative Medicine Applications - The Cardiovascular Cell Therapy Research Network (CCTRn) Experience

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Abstract

Background Aims—Multi-center cellular therapy clinical trials require the establishment and implementation of standardized cell processing protocols and associated quality control mechanisms. The aims here were to develop such an infrastructure in support of the Cardiovascular Cell Therapy Research Network (CCTRn) and to report on the results of processing for the first 60 patients.

Methods—Standardized cell preparations, consisting of autologous bone marrow mononuclear cells, prepared using the Sepax device were manufactured at each of the five processing facilities that supported the clinical treatment centers. Processing staff underwent centralized training that included proficiency evaluation. Quality was subsequently monitored by a central quality control program that included product evaluation by the CCTRn biorepositories.

Results—Data from the first 60 procedures demonstrate that uniform products, that met all release criteria, could be manufactured at all five sites within 7 hours of receipt of the bone marrow. Uniformity was facilitated by use of the automated systems (the Sepax for processing and the Endosafe device for endotoxin testing), standardized procedures and centralized quality control.

Conclusions—Complex multicenter cell therapy and regenerative medicine protocols can, where necessary, successfully utilize local processing facilities once an effective infrastructure is in place to provide training, and quality control.

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¹This dose was subsequently changed to 1.0×10^8 ABMMC in an FDA-approved protocol amendment.

Keywords

cell therapy; regenerative medicine; multicenter trials; cardiac cell therapy; quality control; Sepax

Introduction

The use of pluripotent progenitor cells to mediate the repair of tissue damage has excited the interest of the medical community. This is particularly true in cardiology where a number of clinical studies have indicated that improvements in cardiac function can be achieved by infusion or intracardiac injection of autologous bone marrow cells [1–4]. Early studies used a variety of effector cell populations, were often not blinded, and have used diverse criteria to evaluate efficacy. The National Heart Lung and Blood Institute (NHLBI), therefore, established the Cardiovascular Cell Therapy Research Network (CCTRn) [5]. The Network builds on contemporary findings of the cell therapy basic science community, translating newly acquired information to the cardiac clinical setting in Phase I/II studies. CCTRn consists of five clinical research centers (at the University of Florida, Minneapolis Heart Institute Foundation / University of Minnesota, Vanderbilt University, Cleveland Clinic Foundation and Texas Heart Institute). Associated with each center is a cell processing facility. These are physically located at the first three clinical centers, and at University Hospitals Case Medical Center (for the Cleveland Clinic) and at the Center for Cell and Gene Therapy at Baylor College of Medicine, Houston (for Texas Heart Institute). Additional components are a data coordinating center (DCC) (University of Texas School of Public Health), which provides trial management and data analysis, a cell processing quality control center and six core laboratories. Together, these Network components provide standardization of cell therapy preparation and endpoint measurements. All clinical centers participate in the selection and design of Network protocols that are also reviewed by an independent Protocol Review Committee (PRC) and a Gene and Cell Therapies Data Safety and Monitoring Board (DSMB) under the overview of the NHLBI. Each clinical center has independent Institutional Review Boards (IRB) approvals. By recruiting from multiple centers the Network accelerates the speed with which its studies can be completed, increases the applicability of study findings, and improves the dissemination of its findings to influence public health.

The CCTRn has initiated three clinical studies under IND approval for the treatment of heart failure and myocardial infarction. In these protocols the cell populations to be used consist of autologous bone marrow mononuclear cells (ABMMC) enriched using the Sepax device (Biosafe SA, Geneva, Switzerland). This paper describes the establishment of the cell processing network, training of the personnel, validation of the procedure and characterization of the first sixty clinical products.

Materials and Methods

Clinical Protocols

The TIME protocol (Transplantation in Myocardial Infarction Evaluation) is a randomized, Phase II, double blind-placebo-controlled trial to assess the effect on global and regional left ventricular function of the administration of 1.5×10^8 ABMMC infused via a 3.5 Fr infusion catheter in the left coronary artery at 3 or 7 days following acute myocardial infarction (MI). The study involves 120 subjects who have moderate to large myocardial infarctions, no prior history of coronary artery bypass grafting or myocardial infarction that resulted in left ventricular dysfunction and with an initial ejection fraction of $\geq 45\%$. The primary endpoints are global left and regional left ventricular ejection fraction [6]. In the Late-TIME protocol, involving 87 subjects, the cells are administered 2–3 weeks post MI.

The FOCUS protocol is a blinded, placebo-controlled study of 87 patients to assess the effect of 1×10^8 ABMMC delivered transendocardially via NOGA XP catheter and Myostar mapping to subjects with cardiac artery disease, left ventricular dysfunction and limiting heart failure and/or angina. The primary endpoints are changes in myocardial oxygen consumption, left ventricular end systolic volume and reduction in perfusion defects.

Cell Processing

Approximately 100ml of autologous bone marrow was collected under conscious sedation, and with informed consent, from the posterior iliac crest, using preservative-free heparin as anticoagulant (final heparin concentration 10–25 units/ml marrow). The marrow was transported to the cell processing laboratory at ambient temperature in validated coolers where it was processed on the Sepax device using the density gradient program. Prior to separation, the marrow was filtered (170–200 μ m pore size) and samples were taken for sterility testing (aerobic and anaerobic), using the Bactec blood culture system (Becton Dickinson, Franklin Lakes, NJ) with extended incubation for 14 days, cell count (by automated blood analyzer – Sysmex or Coulter) and three part differential (manual, by automated counter or by flow, depending on processing center). Marrow was diluted to a maximum volume of 120ml (minimum processing volume required is 30ml) in saline containing 5% human serum albumin (saline/HSA), and loaded onto the Sepax for processing.

The Sepax cell processing system uses a rotating syringe technology that facilitates separation through rotation of the syringe chamber and component transfer by displacement of the syringe piston. This is accomplished using a functionally closed and sterile disposable set. In the density gradient procedure the disposable kit is installed into the device and the inlet line primed with marrow. The density medium (Ficoll-Paque Premium, GE Healthcare Bio-Sciences AB, Björkgatan, Sweden) is introduced automatically into the device where the separation is accomplished. The mononuclear cells are retained in the separation chamber, washed three times and then expelled into the final product bag. This procedure has been demonstrated to produce a product comparable or superior in terms of purity and yield to a manual density gradient separation procedure [7]. The Sepax devices used in these studies are not currently approved in the United States for this indication and were used under IND approval (the newer SepaxRM device, however, has received 510K exemption from the FDA for density gradient separation of marrow).

At the completion of the separation, samples were taken for cell count, viability, three part differential, colony forming unit – granulocyte, monocyte (CFU-GM) using the Methocult system (StemCell Technologies, Vancouver, BC), and the cells were adjusted to the required concentration and volume. Samples were then taken for aerobic and anaerobic sterility by Bactec, release sterility by Gram stain, release Endotoxin testing by LAL using the Endosafe device [8] (Charles River, Wilmington, MA), and flow cytometric analysis.

For the TIME and Late-TIME protocols the target cell dose was 1.5×10^8 ABMMC in 30ml saline/HSA packaged in a 150ml transfer pack. The placebo consisted of an equal volume of saline/HSA containing 100 μ l of autologous whole blood to provide a placebo with the same visual appearance as the marrow product. For the FOCUS protocol, in order to conserve cells, the product from the Sepax was sampled for endotoxin, sterility, colony formation and flow cytometry prior to adjustment of the cell concentration. The cells were then adjusted to a target concentration 3.33×10^7 ABMMC/ml in a final volume of 3.2ml. One milliliter of this suspension was then aseptically transferred in a sterile field to each of three 1ml syringes, which were capped and packaged in a sterile plastic bag that was transferred to a second sterile bag for transportation to the cardiac catheterization facility. The remaining cells were used to perform a Gram stain for release testing. Cells for administration were

transported at ambient temperature in coolers validated to maintain the cells within the appropriate temperature range for double the anticipated transport time.

Release specifications consisted of the required number and concentration of cells (acceptable ranges were 1.5×10^8 for TIME and Late-TIME protocols and $0.8 - 1.1 \times 10^8$ for the FOCUS protocol¹), negative Gram stain, Endotoxin dose of <5.0 EU/kg and viability 70% by Trypan blue exclusion. Additional testing consisted of Bactec aerobic and anaerobic sterility testing (specification: negative at 14 days), colony formation (specification: growth), and flow cytometry for CD34 (using ISHAGE gating) [9], CD45, CD133 and viability by 7-AAD (specification: report results). Briefly, for flow cytometric analysis cells in 100 μ l of buffer were stained with 100 μ l of a saturating concentration of the antibody using a lyse-wash protocol. Two hundred and fifty thousand to five hundred thousand events were acquired and progenitor cells analyzed using an analysis template based upon ISHAGE gating strategy.

In addition, surplus cells were shipped overnight to the biorepositories at the Universities of Florida (for colony and functional assays) and Minnesota (for banking and extensive flow cytometric analysis). Samples were packed in tubes wrapped in insulating material in commercial cell shipping packaging (EXAKT Technologies, Oklahoma City, OK) containing insulated frozen (-20°C) cold packs. These conditions were validated to maintain cell viability over a range of shipping times and external temperatures.

Results

Training Program

Since centralized processing is not feasible for protocols involving administration (at geographically disparate sites) of fresh processed cells within 12 hours of collection (a window that was set in the IND), it was determined that cells should be prepared in facilities close to each clinical site. To reduce potential variability in the product, the Sepax automated cell separation system was selected for cell preparation (described above).

Each center sent two cell processing staff for centralized training at the MD Anderson Cancer Center (MDACC) Blood and Marrow Transplant GMP Facility in Houston. The training program consisted of each processor preparing a cell product and submitting samples to the testing laboratories at MDACC for analysis. Normal bone marrows were purchased from Lonza Walkersville Inc. (Walkersville, MD) and pooled for processing, so that in each training run all five centers processed the same material. Each processor performed two separate procedures under the observation of representatives from Biosafe. The second representative from each center was then trained in the same way on the same Sepax device that was used by the first technologist from that processing center. Release testing was performed at MDACC. The results from this study are shown in Table 1A.

The Sepax devices used by each center at the training program in Houston were then shipped to that center for completion of training and subsequent use in the clinical trials. In the third phase of training marrow, harvested by Lonza, was split into two samples each of which was sent to a different center for processing and release testing. The results are shown in Table 1B. The somewhat lower TNC recoveries by some of the groups on site were attributed to a larger than usual dilution of the marrow before processing on the Sepax.

Overall, the results from the training runs demonstrate that all products met the release criteria, and the processors were certified as trained to perform the procedure.

Quality Control

For the purposes of the trial a centralized quality control group (the CPQC) was established with oversight of processing and product quality. The CPQC developed the formal standard operating procedures and batch records for the TIME and Late-TIME protocols, and for the FOCUS protocol which was initiated subsequently. These were incorporated into a Laboratory Manual which also contained information on reagents, materials, sample shipment, training of additional staff, emergency contact information, protocol updates and ancillary research studies. The manual also contained instructions for randomization and entry of results using the CCTRN computer system. Randomization occurred after preparation of the cellular therapy product and completion of release testing. Results were entered into the CCTRN system which then allocated the intended recipient to receive either the product or placebo. In the case that the patient was randomized to the placebo arm, the cells were used to perform the approved ancillary research studies and the remainder shipped to the biorepositories. For patients on the active treatment arm an algorithm was developed to divide the excess cells between the two biorepositories and ancillary research studies.

Since these were to be blinded studies, recipients on the placebo arm were initially supposed to receive a saline/HSA-only placebo. The CPQC determined that TIME/Late-TIME placebo products were distinguishable from cellular products due to their lack of color and turbidity. This was overcome by adding 100 μ l autologous peripheral blood to the saline/HSA as described above. This was found to produce a product visibly indistinguishable from the cellular product. This change to the protocol was approved by the FDA and the clinical IRBs.

The CPQC also developed and validated the conditions for shipping excess cells to the biorepositories (described in Materials and Methods). An analysis of the first sixty shipments showed that cells were received at the biorepository in Minneapolis at an average time of 10.18am $\pm$ 42 min (n=29 - excludes cells received from the Minneapolis cell processing center); and at the Florida repository at 10.20am $\pm$ 91 minutes (n=47 - excludes cells processed by the University of Florida cell processing center) the day after shipment by Federal Express Priority Overnight service. The mean cell viability in Minneapolis was 93.3% $\pm$ 3.9% (n=57) as measured by ViaCount (Guava Technologies, Hayward, CA) and 86.5% $\pm$ 8.6% (n=53) in Florida as measured by Trypan blue exclusion.

Quality Control staff also site visited each processing laboratory after they had performed several separations to review performance and address any issues. All batch records were centrally reviewed by the CPQC and the staff were available, together with representatives from Biosafe, during each separation procedure to address any technical problems.

Processing Results

As of July 2009, 60 ABMMC products had been prepared (47 for TIME and Late-TIME and 13 for FOCUS). Figure 1 shows the characteristics of the 60 products obtained from the Sepax after loading a mean volume of 98.1ml of BM ($\pm$ 17.3, median 100.6ml). Data shown include the volume of marrow loaded onto the device, the total nucleated cell (TNC) and red cell content (TRC) of the Sepax product, the percent recovery of TNC (22.27% $\pm$ 6.16, median 22.43), percent depletion of red cells (100% $\pm$ 0.00, median 100%) and a 3 part differential analysis of the product. The differential was performed on an automated cell counter at 3 centers, by flow cytometry at one and manually at the last center. The average processing time on the Sepax device was 89 minutes for the TIME/Late-time products and 93 minutes for the FOCUS products. There were very few technical problems with the separation procedure. In one center there was a problem with the optical sensor in the Sepax,

which resulted in the cells having to be recovered manually from the device. These cells failed to grow in the CFUGM assay in which only a very small number could be plated. At one center loading of the density gradient material was prolonged, the procedure was stopped and the marrow processed on a different Sepax unit. At a third center the initial marrow volume was entered incorrectly into the device resulting in the Sepax waiting for additional material. The emergency stop procedure did not function correctly and the cells had to be recovered from the device and processed manually. The run data from the devices were downloaded and transferred to Biosafe, who worked with each center to determine the cause of the problems. Subsequently the device software was updated and a new operator's manual was produced. Since then there have been no device issues. These data were then analyzed by processing group to determine whether there were significant differences in the results based on location. The results shown in Figure 2 demonstrate that comparable products were obtained from the Sepax at all centers. Progenitor cell recovery from the Sepax was measured at one of the centers. The results, Figure 3, show the total numbers and percent recoveries of CD34 and CD133-positive cells obtained after separation. The mean recovery of CD34-positive cells was $59.0\% \pm 12.6$ SD (n=14) and $62.6\% \pm 11.8$ SD (n=14) for CD133-positive cells.

The Sepax products were then further processed to adjust the concentration and volume and to perform the required release and additional testing.

The randomization was then carried out and the product or placebo was transported to the clinical center. The overall time from receipt in the processing laboratory to return to the clinical center was 6 hours 58 minutes for TIME/Late TIME products (n=47) and 6 hours 59 minutes for the FOCUS products (n=13).

The target cell dose (1.5×10^8 ABMMC) was achieved in 42/47 (89.4%) TIME/Late-TIME products. Of the remaining products, 4 contained a mean of 1.38×10^8 cells (range $1.30 - 1.44 \times 10^8$), the fifth contained only 3.60×10^6 cells, due to a problem with the optical sensor in the Sepax described above. This resulted in the cells having to be recovered manually from the device and washed on a cell separator. For the FOCUS protocol the target cell dose (1.0×10^8 ABMMC) was achieved in 10/13 products (76.9%), the remaining three contained $8.00 - 9.96 \times 10^7$ cells. All final products met the release criteria with respect to Gram stain, viability ($98.0\% \pm 1.56$ SD, median 98.0 by Trypan blue exclusion and $96.1\% \pm 4.25$ SD, median 97.7 by 7-AAD staining), and endotoxin level (Figure 4). Additional test results (Bactec aerobic and anaerobic sterility) and CFU growth were also acceptable, with the exception of failure of one product to grow CFU (the product that was recovered manually from the Sepax and for which very few cells were available for plating).

Characteristics of the final products are shown in Figures 5 and 6 for the TIME/Late TIME and FOCUS products respectively. The CD34 and CD133 numbers reported are based upon flow data reported by each of the cell processing centers. In a pre-trial sample exchange there was generally good concordance in the flow data reported by the centers. Also shown is the same information excerpted from more comprehensive central flow analysis performed at the Minnesota biorepository. These results, although somewhat higher, are not significantly different from those obtained at the processing sites. The relevance of these particular progenitor subsets in cardiac repair is unknown and this issue may be elucidated by the more detailed analyses ongoing at the biorepositories.

Discussion

Regenerative medicine is an area of intensive investigation, based on the observation that the differentiation and de-differentiation of stem cells along tissue-specific pathways can be

induced in the laboratory. One of the most active applications has been in cardiology where a number of trials have been conducted using a variety of marrow cell subpopulations delivered by infusion or intracardiac injection [4]. Early studies of this type often were neither placebo controlled nor blinded, were conducted at a single site, and did not use standardized cell doses. The CCTRN aims to address these issues by using multiple sites to accrue a larger number of patients to standardized blinded studies. In order to provide the cell products for these trials, two approaches were available. In the first, cells could be processed at a central location and shipped to the clinical sites. However, the requirement to administer fresh cells, and the restricted time window available for the protocols (no longer than 12 hours between marrow harvest and product administration), made this impossible. The alternative was to utilize cell processing facilities associated with the clinical sites, or located nearby, and to develop standardized processing and testing procedures. Once this decision was made, the Sepax device was selected to provide a more uniform automated cell separation procedure, since variability in recipient outcome has been attributed to differences in cell quality [10] and processing techniques [11], although this has been disputed [12]. The Sepax device has been used extensively for cord blood processing [13] and the density gradient program has been used to prepare cells for cardiac applications, where it was shown to produce separations comparable or superior to those obtained using manual procedures. An extensive study by Atkas et al using Sepax produced separations similar to those from the CCTRN processors [7] and the product composition. In this earlier study a third wash of the cells in the Sepax was shown to improve platelet depletion and the current Sepax software incorporates this additional wash step.

This comparability to manual techniques was also confirmed by the group at MDACC [13] who conducted the initial training program for the CCTRN cell processors (Tables 1A and B). This program demonstrated that comparable results could be obtained by various individuals processing the same starting marrow, both at the initial training, and when repeating the procedures using the same device in their own facilities. In the subsequent clinical applications the Sepax device generally performed well. There were two technical issues, in response to which Biosafe introduced modifications to both the software and the instruction manual and subsequently there have been no further problems.

The CCTRN trial design required that randomization to receive the cell product or placebo was conducted after the product had been prepared; therefore, product information is available for all sixty intended recipients, regardless of whether they ultimately received a placebo. The final products all met release criteria and the use of Gram staining and the Endosafe device for endotoxin testing [8] reduced the turnaround time for testing to about one hour. FOCUS product preparation was more involved since the product had to be drawn into syringes that were to be handled in a sterile field in the catheterization laboratory.

It was recognized that standardization of additional testing i.e. flow cytometric analysis and colony assays between facilities was more difficult to achieve without extensive validation, and it was decided that these should be handled primarily by the biorepositories in Minnesota (for banking and flow cytometry) and Florida (for colony and functional assays). Basic flow and CFU assays were, however, also performed at the processing centers and these data are included in this report.

The use of centralized testing laboratories required development of careful information dissemination between the processing staff, the trial coordinating center, the CPQC staff and the two biorepositories to alert them to upcoming samples, shipment tracking information, delays etc. This was facilitated through the CPQC who also developed and validated the shipping conditions to address the wide range of temperatures, ranging between Florida in summer and Minnesota in winter. This group also provided centralized quality oversight.

Batch records were reviewed prior to randomization of the patient by quality assurance staff at each site and then sent on to CPQC for formal review. They were also responsible for approving any planned deviations (such as filtering the Sepax product if any fibrinous strands or clumps were detected) and for performing on-site audits of the facilities.

This study has demonstrated the feasibility of conducting a multicenter cardiac cell therapy study involving multiple processing sites. As these types of protocols become more widespread this model can be further developed in cases where it is not possible or desirable to cryopreserve the effector cells for shipment. The study has been facilitated by centralized training and quality control, by the use of a more uniform automated cell processing system and by rapid release testing techniques. The CCTR design has now been extended to include satellite clinical centers up to 128 miles from the main clinical center. This has necessitated validation of transport conditions for shipping the separated cells, but further extends the utility of this system for extending access to cellular therapies.

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Figure 1.

Analysis of Sixty Sepax Clinical Separations

The two columns to the left of the graph show the numbers of total nucleated cells and red cells (TRC) in the product as measured by automated cell counters. The next two columns show these results expressed as percent recoveries of the initial numbers of these cells loaded onto the Sepax. The three columns to the right of the graph show the three part differential on the products as measured by automated cell counter at three centers, by flow cytometry at one center and manually at the last center. All numbers shown are mean $\pm$ 1 standard deviation. Standard deviation on %TRC depletion is too small to show.

Figure 2.

Sepax Processing Results shown by Processing Laboratory

The bars to the left of the graph show the mean numbers of nucleated marrow cells harvested at each clinical center. The bars in the center show the mean percent recovery of nucleated cells in the Sepax products. The bars to the right show the mean percent depletion of red cells in the Sepax products obtained at each center. Error bars show 1 standard deviation.

Figure 3.

Recovery of Progenitor Cell Populations in Sepax Products

Progenitor cell recovery was measured at one processing center. The bars to the left show the mean numbers of and CD34- and CD133 positive cells in the Sepax products. To the right are the percent recoveries of these cells together with the recovery of total nucleated cells. Error bars show 1 standard deviation.

Figure 4.

Quality Control Test Results on Final Cell Products

Shown are the results of release testing of the final cellular therapy products. All products met the release criteria of viability ≥ 70% by Trypan blue exclusion, sterility by Gram stain, and endotoxin ≤ 5.0 EU/kg. Additional test results for CFU- growth and viability by 7-AAD are shown. CFU growth was seen in all samples plated according to SOP, however, in one case too few cells were plated and colonies were not detected, and in a second the assay was omitted in error.

Figure 5.

Characteristics of Products prepared for the TIME/late Time Protocol

Shown are the mean numbers of nucleated, red and progenitor cells. For the progenitor populations the bars to the left show numbers based on flow cytometric analysis performed at the processing centers; bars to the right show numbers based on analysis performed at the Minnesota biorepository. Error bars show 1 standard deviation.

Figure 6.

Characteristics of Products prepared for the FOCUS Protocol

Shown are the mean numbers of nucleated, red and progenitor cells. For the progenitor populations the bars to the left show numbers based on flow cytometric analysis performed at the processing centers; bars to the right show numbers based on analysis performed at the Minnesota biorepository. Error bars show 1 standard deviation.

Table IA

Results from Central Training Runs on the Sepax Cell Processing System Processing groups are shown in the first column. The first technologist from each center processed the same pooled marrows on the Sepax intended for delivery to their laboratory. The same procedure was repeated by the second processor from that center on additional pooled marrows. Results were analyzed by a centralized laboratory. Data shown are the recoveries of total nucleated cells and CD34-positive cells and the viability as determined by 7-AAD staining and flow cytometry.

	% Nucleated Cell Recovery		% CD34		% Viability by 7-AAD	
Processing Group	Run #1	Run #2	Run #1	Run #2	Run #1	Run#2
Group 1						
Tech 1	47.1	58.3	0.23	0.20	97	96
Tech 2	35.8	36.5	0.91	0.90	92	97
Group 2						
Tech 1	49.8	52.6	0.23	0.20	96	95
Tech 2	48.9	33.1	0.75	0.95	93	97
Group 3						
Tech 1	44.9	56.2	0.22	0.23	97	94
Tech 2	45.8	37.2	0.74	0.90	93	97
Group 4						
Tech 1	49.8	59.1	0.21	0.22	97	95
Tech 2	49.6	34.0	0.65	0.95	93	97
Group 5						
Tech 1	44.8	53.8	0.22	0.19	96	96
Tech 2	45.6	37.1	0.95	0.83	97	97

Table 1B

Results from Training Separations performed in each Cell Processing Laboratory The Sepax devices used for centralized training were shipped to each Processing Laboratory and used to perform two additional training separations. For each training separation bone marrow was split between two processing laboratories as shown in the first column. For the second separation performed subsequently the marrow was split with a different laboratory. Release testing was performed at each center. Release specifications used for these experiments were: Viability 70%, Endotoxin 5.0 EU/kg (values shown are as EU/ml Sepax product, which converts to <5.0EU/kg based on final product volume and average adult body weight), and negative sterility (no organisms) at 14 days by Bactec. Total nucleated cell (TNC) and CD34-positive cell recoveries are also shown.

Processing Group	% TNC Recovery	% CD34	% Viability by 7-AAD	Endotoxin EU/ml	Sterility Aerobic & Anaerobic	CFU Assay
Group 1	15.4	4.59	94.5	<2.0	Pass	Growth
Group 4	16.6	3.29	97.8	<2.0	Pass	Growth
Group 1	15.9	3.07	85.2	<2.0	Pass	Growth
Group 2	25.5	2.35	94.3	<2.0	Pass	Growth
Group 2	25.8	2.72	95.4	<2.0	Pass	Growth
Group 5	13.2	5.09	92.6	<2.0	Pass	Growth
Group 5	11.9	6.75	94.6	<2.0	Pass	Growth
Group 3	17.0	4.65	91.9	<2.0	Pass	Growth
Group 3	22.8	3.29	95.4	<2.0	Pass	Growth
Group 4	17.8	2.87	97.6	<2.0	Pass	Growth