International Society for Cellular Therapy
ISCT

TELEGRAFT

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A QUARTERLY NEWSLETTER

ISCT 15th

Annual Meeting to Feature New Technical Applications Track!

May 3-6, 2009
Sheraton San Diego Hotel and Marina
San Diego, California

You asked for it – and we responded! ISCT members have been looking for more technical and applied content at the annual meeting. A survey of attendees at the 2007 Sydney meeting showed that **83% of respondents** were interested in multiple tracks at the annual meeting.

ISCT Leadership and the San Diego planning committee is pleased to announce that the 2009 annual meeting will have a **Technical Applications Track**.

For those looking for educational opportunities and sessions with high relevance to everyday laboratory and manufacturing practices, the 2009 Annual Meeting in San Diego with the **Technical Applications Track** are sure to meet your needs.

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NEW!
Technical Applications Track
14 Sessions Feature:
• validation and qualification • environmental monitoring • methods for thawing cord blood products • raw material management • other technical, quality and regulatory issues

especially Donna Regan have outdone themselves by getting three individuals – Donald Hudspeth, Roger Mrowiec and Koen Theunissen – to summarize their experiences with three different cord blood processing devices. This piece should not be missed, even if you currently do not process cord blood products.

Finally, I hope everyone finds time to truly enjoy this holiday season, even if you are on–call or tending to patients, to embrace family and friends, to reflect on your blessings, and to rejoice and rejuvenate.

A good holiday is one spent among people whose notions of time are vaguer than yours.

J.B. Priestly, British writer

The time you think you're missing, misses you too.

Ymber Delecto §

§ Honorable mention in the next issue to anyone who can tell me who Ymber Delecto is. This "persona" has quotes and t–shirts throughout the internet, but nowhere is there a description of who they are. The more I look at the name, the more it looks like an anagram, in the spirit of Tom Morvolo Riddle of Harry Potter fame.

TECH TALK

Review of New Devices for Processing of Umbilical Cord Blood

Donna Regan, MT(ASCP)SBB
Cardinal Glennon Children's Hospital

There are a variety of ways to effectively manipulate cord blood donations prior to cryopreservation. For some programs, a method may be selected based on practicalities such as space, staffing, or economy. For others a method may be chosen in order to reach a desired endpoint, such as the product volume, cell recovery, or isolation of a particular cell population. Large scale programs may prefer automation, while smaller operations are more impacted by budget. The chosen method must meet the goals of the program and be optimal for the product.

Cord blood processing has traditionally been performed by manual methods that require only a centrifuge, plasma expresser, and controlled rate freezer. Recently, some newer methods have been developed to address efficiency, automation, and standardization. This edition of tech talk presents the technologist's perspective on three of these methods and is based on first hand experience from the field. We're grateful for submissions from Donald Hudspeth of Cryobanks International, Roger Mrowiec of the New Jersey Cord Blood Bank and The Elie Katz Umbilical Cord Blood Program, and Koen Theunissen of the Navelstrengbloedbank in Leuven Belgium. Be on the lookout for clinical data that is soon to be published in a journal or at a meeting near you!

Part 1. Evaluation of PrepaCyte-CB® as an alternative to hetastarch methods for processing umbilical cord blood samples

Donald Hudspeth, BSCLS, MT(ASCP)
Cryobanks International, Inc., Altamonte Springs, Florida

As the manager of a stem cell processing center, I am always looking for ways to control costs while maintaining, if not improving, the production of the highest quality stem cell products for transplantation. Historically, the preferred methodology for cord blood processing has been a manual density gradient separation using hetastarch. Recently, some companies have introduced mechanical variations of this methodology. Alternatively, BioE, Inc. has developed a completely new platform that replaces hetastarch and density gradient processing altogether, without additional instrumentation (read: no capital equipment budget).

Background

We became familiar with BioE's PrepaCyte-CB® Cord Blood Processing System in early 2006 when we agreed to participate in the company's multi-center comparative in-vitro study, in which 155 cord blood units were processed using either PrepaCyte-CB or a hetastarch-based method (modified Rubinstein method[1]). The combined study results from the three sites showed that PrepaCyte-CB was superior to hetastarch in a number of ways. Most importantly, the TNC recovery was significantly improved from the hetastarch control group. We also saw greater yields of CD34+ cells and CFUs, compared to the hetastarch methods tested.

Tech Talk continued >

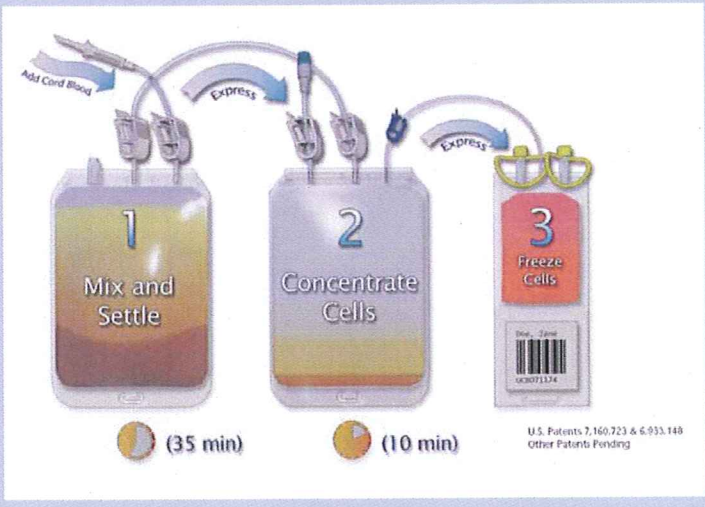

Figure 1. PrepaCyte-CB® Cord Blood Processing System

Product Information and Procedure

PrepaCyte-CB is a cord blood processing system consisting of three interconnected bags with multiple needle-less access ports (Figure 1). The first bag contains 150 mL of the PrepaCyte-CB reagent, the second bag is for centrifuging/cell separation and the third bag is the freezing bag (with multiple freezing bag options). The cord blood bag is connected to the processing set and the cord blood is drained into bag #1. After a short mixing period bag #1 is hung on a plasma expressor for 30 minutes undisturbed. After settling, the leuko-rich plasma is expressed into bag #2. The bag set is spun for 10 minutes and then the leuko-poor plasma is expressed back into bag #1, leaving behind a small volume of highly concentrated cells. These cells are resuspended, counted, tested, and then transferred to bag #3 for cryopreservation. It is really that simple, and the recoveries we get are outstanding. Another added benefit is that there are basically no RBCs left in the final product. In our previous hetastarch process we had to include a portion of the red cell layer to maximize TNC recovery, but with PrepaCyte-CB there is no gradient. The white cells are dispersed throughout the plasma so there is no need to add 10 or 15 grams of post-interface red cells into the second bag or to perform a second starch process. It is so user-friendly and intuitive that very little training was needed for my staff to become proficient. It is also safer for us to use compared to our previous process due to the integrally attached bags. Also, PrepaCyte-CB's process leaves the stem and progenitor cells unaffected by the processing.

Results of Multi-Center Comparative Study

What really intrigued us was the fact that each tech at each clinical site raised their recovery averages using PrepaCyte-CB, independent of processing experience or calculation methods. Also, as shown in the Table 1, most of the important cord blood selection criteria used by transplant centers are improved using PrepaCyte-CB versus hetastarch.

Table 1. Summary of Data from Multi-Center Study*

Clinical Study Data (Post-Processing)	Hetastarch (30)	PrepaCyte-CB (125)	Difference
%TNC recovery	79.1 ± 10.4	85.2 ± 7.3	6.1
%WBC recovery (nRBC-adjusted)	79.3 ± 11.0	87.4 ± 8.5	8.1
% Mononuclear cell recovery	83.6 ± 12.3	85.8 ± 12.7	2.2
% CD34+ cell recovery	81.1 ± 16.9	86.9 ± 26.5	5.8
% CFU-GM recovery	86.3 ± 36.1	119 ± 61.5	32.7
% Red cell depletion	79.1 ± 8.4	98.5 ± 0.6	19.4

**results are reported as mean +/- 1SD*

After participating in the study, we realized the benefits of PrepaCyte-CB and began planning to adopt this methodology. Our techs that processed these samples for the clinical trial frequently commented on the ease of use, and we also realized an unexpected time savings benefit. This led us to perform a secondary validation as a final evaluation of PrepaCyte-CB.

Results of Secondary Cryobanks Validation

Our internal validation consisted of an additional 50 umbilical cord blood samples to be processed using PrepaCyte-CB, tested, stored, thawed, and re-tested. We compared the results of the last 150 hetastarch processed samples to the first 100 banked donation samples processed with PrepaCyte-CB. Table 2 shows similar results to the combined clinical trial data, as well as a significant time savings using the PrepaCyte-CB method.

Table 2. Summary of Data from Internal Validation*

Validation Data (Post-Processing)	Hetastarch (150)	PrepaCyte-CB (100)	Difference
Average UCB volume (mL)	107.62 ± 26.2	101.23 ± 25.4	-6.4
Average Pre Process TNCC (x10e8)	15.07 ± 5.88	13.89 ± 4.76	-1.2
TNC recovery (%)	79.86 ± 12.68	88.12 ± 6.86	8.3
Avg. final CD34+ count (x10e6)	2.831 ± 2.15	5.262 ± 4.14	2.4
Red cell depletion (%)	75.73 ± 9.8	98.81 ± 0.5	23.1
Average time to process one sample	3 hrs 2 min	2 hrs 12 min	-50 min

**results are reported as mean +/- 1SD*

Financial Benefit

We determined that the PrepaCyte-CB cord blood processing set is a value for our operation. The processing sets are very competitively priced so disposable costs were easy to manage. More importantly, no additional capital equipment is needed except for a standard laboratory centrifuge, which we already used for the hetastarch method. Additionally, the time savings of 50 minutes per unit allowed us to maximize the work flow efficiency of our laboratory staff. In times of high demand and low supply of qualified laboratorians, this was a greatly appreciated additional benefit!

Tech Talk continued >

We can now process more umbilical cord blood samples with less staff (and less payroll)!

Evaluation

As a quality focused public and private bank, it was imperative that any new processing system at least maintain, if not improve, recovery of the TNC/MNC/CD34+ counts when compared to our existing hetastarch method results. Our Medical Directors and Scientific and Medical Board of Advisors (SAMBA) agreed that red cell reduction was also a benefit to be explored. Additionally, the system needed to be safe, reliable, recoverable, and batchable (no need to wait until completion to begin the next sample) to maximize efficiency. Cost concerns are always a factor in making decisions to change, so the product had to have a tangible value for the increased performance. Since 1995 when Cryobanks first developed its stem cell processing laboratory, it has been very important to us and our mission to process cord blood units in a safe, reliable fashion while routinely achieving the best TNC recovery possible.

Following the clinical trial and internal validation, Cryobanks adopted PrepaCyte-CB as a processing system in May 2008. In the clinical study and in our subsequent validation PrepaCyte-CB has consistently provided high quality stem cell products. Additional benefits of efficiency and increased staff utilization made switching to PrepaCyte-CB an easy decision to make.

Finally, looking at ethnicity as a factor in TNC recovery and CD34+ cell counts yielded an additional benefit not originally explored. While TNC recovery in Hispanic and Mixed/Other minority categories remained almost identical whether processed with hetastarch or PrepaCyte-CB, the Black and Asian/Pacific Islander minorities showed a marked improvement in TNC recovery (+12.87% and +7.97%, respectively) when processed with PrepaCyte-CB. This higher recovery has led to more minority samples being qualified for transplant and/or funding through government sponsored programs.

Conclusion

Cryobanks International, Inc., located in Altamonte Springs, Florida has fully converted to processing all cord blood units with BioE's PrepaCyte-CB. Our company's mission is to provide the highest-quality umbilical cord blood stem cell processing and storage for both private family use and public donation purposes. We believe the PrepaCyte technology allows us to process cord blood units in a safe, reliable manner, consistently achieving the best TNC recovery possible, while helping us manage costs effectively.

Tech Talk continued >

Part 2. Sepax – Automated UCB Processing

Zbigniew (Roger) Mrowiec, PhD
New Jersey Cord Blood Bank and The Elie Katz Umbilical Cord Blood Program, Allendale, NJ

Processing umbilical cord blood (UCB) to concentrate the buffy-coat fraction rich in hematopoetic stem cells, has been made a routine process over the last decade. Public and private UCB banking facilities have opened around the world, in hopes of providing stem cells for cellular transplants into patients with life-threatening illnesses. Many of these diseases we are all readily familiar with and many more disease states are currently being investigated.

One of the acceptable methods employed in the process of concentrating the buffy-coat fraction has been to use hydroxyl ethyl starch (HES) to enhance RBC sedimentation and to play a role in maintaining cell viability post-cryopreservation. The vast majority of public and private cord blood processing banks in the United States utilize this particular methodology.

This manual process, in the hands of an experienced lab technician, can maximize the recovery of total nucleated cells (TNCs) and CD34+ cells which they can then 'bank' or cryopreserve. Unfortunately, the down side to this methodology has two primary concerns. One concern is 'technician to technician variability' and the other is that this process is an 'open' system. As federal and international regulations on cellular therapy become increasingly stringent, open systems may be viewed as lacking control for maintaining sterility and cell recoveries.

Cell therapy laboratories around the world have looked to automating processes in efforts to keep systems closed and to be more compliant with current Good Manufacturing Practices (GMP). One process where automating to a closed, sterile environment has been cleared by the FDA is the UCB-HES protocol. In late 2006 the FDA cleared a new type of cell separation system for fast, reproducible and automated processing of UCB units which has been available for purchase since early 2007. The device approved, the Sepax cell separation system, manufactured by Biosafe in Switzerland, became readily available to the US marketplace, and is distributed exclusively by Genesis BPS of Hackensack, NJ.

Briefly, the Sepax and the UCB-HES protocol is specifically designed for routine processing of umbilical cord blood using HES, in a closed, sterile environment which utilizes single-use disposable kits. The protocol allows for a volume reduction of the input UCB unit to a predetermined, user defined, fixed volume of 20 – 50 mls of buffy coat. The fixed volume is adjustable by 0.1 ml increments. Through optical source and digital image sensors in the separation pit, precise volume management is maintained and an in-line optical sensor allows for cell separation control. Each unit of UCB separated on the Sepax takes approximately 30 minutes. The user has full traceability and a data record of each run on a memory card interface, containing protocol files and patient data. The Sepax also provides for a patient data print out following each unit that is processed. Increased data management capability allows the user to remove the memory card interface and permits the data to be transferred to a computer. This memory card interface also allows for ease of installing software updates. And for even greater control, Biosafe has just introduced SepaxNet, which is a proprietary hardware and software management system which controls data on up to 20 machines. The SepaxNet runs hardwired or wirelessly.

When using the Sepax, once the UCB unit has been spiked or sterile welded to the disposable kit and mounted on the machine, no operator intervention is required for the duration of the separation process. Built-in safeguards include an automatic purge recovery system should anything transpire that would require the cord to be reprocessed. Because it is a closed system, once the UCB is purged backed into the original UCB bag, the unit can be re-processed on the same disposable kit.

The Sepax cell separation technology has performed well over 120,000

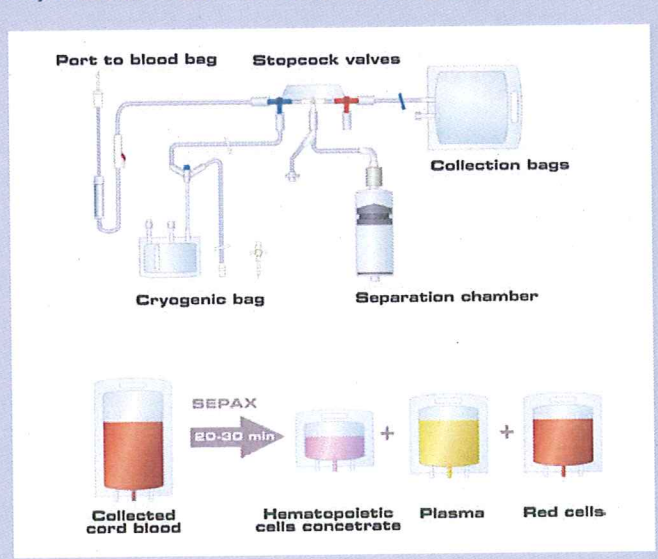

Figure 2. Sepax System

Tech Talk continued >

separations world-wide with many of those banked cells used in successful cellular transplants. Studies have been performed within centers to compare manual processing against the Sepax system as well as comparing Sepax to other automated systems. It has been repeatedly shown that Sepax not only matches well-honed manual techniques, but it has been found to increase recovery of TNCs, MNCs and CD34+ cells and has the advantage of having less RBC contamination. Furthermore, post-thaw viability and CFUs were as robust as the initial recoveries. If one compares data of the Sepax to another automated UCB separation process, Sepax edges out the competition with simplicity of use, a larger separation chamber and far less time to process each umbilical cord blood unit. It has been demonstrated that Sepax has the capability to have the best TNC and CD34+ recoveries and statistically significant less RBC contamination when compared to manual methods.

Though we have not seen the last of automating cellular processing, automated UCB-HES protocols can now be performed confidently. Though we all recognize that various methods may provide a similar outcome, automation provides for more consistent, reproducible results and reduces the risk of operator error. Decisions on automating this process will be slightly different for every center that processes UCB units, but common factors will include the size of the center's throughput, consideration of labor and, perhaps most importantly, the ease of use.

Part 3. AXP – Automated UCB Processing

Koen Theunissen, MD
Navelstrengbloedbank, Leuven, Belgium

Since 1988, when the first hematopoietic stem cell transplantation (HSCT) was carried out using umbilical cord blood (UCB) derived progenitors, the use of this stem cell source has become quite more widespread as an alternative to matched unrelated or haplo-identical donors. This is not only the case in the pediatric, but also in the setting of adult HSCT. In parallel, and to allow these evolutions to happen, cord blood banks have been established throughout the Western world, with the aim of providing the transplant community with the needed diversity of HLA typed UCB units. In order to allow storage of large amounts of units in an economically feasible way, volume reduction was put in place, as was first described by Pablo Rubinstein from the New York Cord Blood bank[1]. Since the success of the procedure is largely dependent on the number of stem cells delivered to the patient per kilogram body weight, and their number in most units is limited, processing requires not only adequate volume reduction but also maximal recovery of these stem cells. A convenient but imperfect indicator of stem cell recovery is measurement of total nucleated white blood cells recovered.

Recently we at the Leuvense Navelstrengbloedbank in Leuven, Belgium had the privilege of evaluating the new AXP AutoXpress™ Platform that was introduced by the Thermogenesis Corporation (Rancho Cordova, CA, US). This technology consists of microprocessor-controlled sub-devices, docking stations, and special design disposable blood bag sets for closed processing. The overall device is sized to fit standard blood bank centrifuge cups. It is a microprocessor-driven, mechanical instrument with light-sensitive sensors that optimize the operation of a three-way valve at the intersection of the tubing linking the three bags that make up the bag set. The valve is operated externally and individually for each cord blood unit

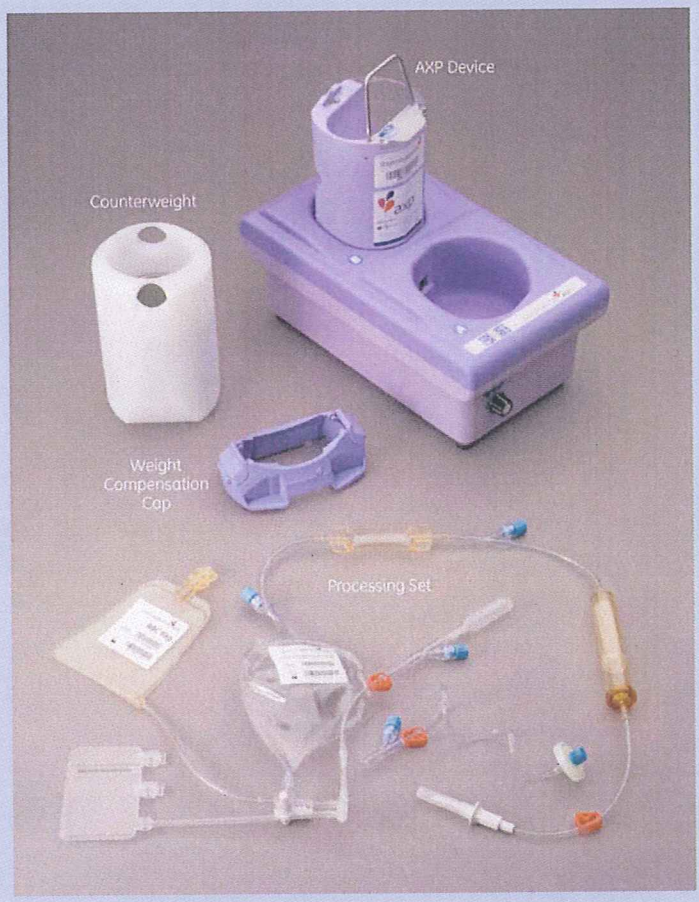

Figure 3. AXP AutoXpress™ Platform

Tech Talk continued >

(CBU). With sensor control the valve's function promotes separating the buffy coat from the erythrocyte bulk and the excess plasma into discrete bags. The bag-set also includes the valve and small sampling segments for withdrawing pre- and post processing samples in a closed mode. The valve and other bag-set components fit into specific areas of the device that also house, store, and protect the bags and specimen sections.

Up to six units of cord blood can be processed at one time with a standard six-bucket blood bank centrifuge. During centrifugation, blood is stratified into red blood cells, buffy coat, and plasma. The device separates these components sequentially into the erythrocyte bag and the freezing bag (buffy coat), while keeping the excess plasma in the processing bag. The mononuclear cell (MNC) product containing the stem cells is concentrated into a uniform volume in the freezing bag, which is ready to be cryoprotected and fully compatible with the BioArchive™ system that was introduced by the same company several years ago to allow for controlled rate freezing and storage of volume reduced UCB units.

The addition of HES to cord blood offers a convenient way to significantly increase TNC recoveries. Due to some concerns about potential allergic reactions and other side effects, many European countries do not allow for HES to be used in patients anymore, and in the near future, clinical grade HES might not be available for cord blood banking. Therefore, a special software version has been designed for the AXP™ device that allows for buffy coat isolation without the addition of HES. We performed 20 volume reduction procedures on this platform, cryopreserved these units for at least 2 weeks, and then thawed them to collect data on viability and recovery of progenitors. The results for MNC, CD34 and CFU recoveries, both right after processing or after freeze and thaw were excellent and at least comparable to those observed with the Sepax™ platform using HES (although no formal head to head comparison was performed). As was expected, a somewhat inferior recovery of TNC was observed, due to the less efficient separation of granulocytes out of the red blood cell layer. Although this should not influence the engraftment characteristics of the cord blood unit, it might be of concern when listing the units in the registries, since selection of UCB units is still based upon TNC, rather than MNC or CD34 counts (unlike bone marrow or peripheral blood progenitor cells).

The XpressTRAK™ software feature of the platform also pleasantly surprised us. This software tracks and documents each cord blood unit's separation data during and after centrifugation. Other data such as cord blood unit ID, centrifuge ID, and processing bag set lot number/expiration date were entered with a bar code scanner. This software produces a computer report of the processing cycle and stores it in a searchable database in the user's computer, thus providing perfect traceability.

In conclusion, it is clear that, upon the development of this platform, Thermogenesis has anticipated and improved upon some of the flaws of the currently available technology. Likewise, it is fair to say that Biosafe has meanwhile developed a closed system disposable set, a comparable traceability software platform and a processing software version that allows for HES-free volume reduction, although the performance of this last application seems to be less convincing. The AXP™ platform offers the opportunity for highly efficient high throughput UCB unit processing, using a standard blood bank centrifuge, and precluding the need for the extra addition of HES.

References

[1] Rubinstein P, Dobrila L, Rosenfield RE et al. Processing and cryopreservation of placental/umbilical cord blood for unrelated bone marrow reconstitution. Proc Natl Acad SciUSA 1995;92:10119–22.