Automated Separation of Cord Blood MNC Fraction in a Closed System: ThermoGenesis AXP™ System

John Chapman, Ph.D., Dennis Marr, Ph.D., Bruce Baker, M.S., Karen Kryston, M.T., ThermoGenesis Corp., Rancho Cordova, CA USA

BACKGROUND: Current good tissue practices (cGTP) are necessary in the cord blood processing laboratory to ensure uniform product quality and to comply with FDA regulations. One critical step in the processing of cord blood is to reduce its volume to a uniform of 20 ml so as to standardize the freezing rate, essential to maintain stem cell viability, and to maximize the number of frozen units that can be stored frozen in a limited space. To facilitate this step of cord blood processing in compliance with cGTP, ThermoGenesis has developed the AutoXpress™ System consisting of a microprocessor-controlled device and a new disposable, closed blood bag set (FIGURE 1). The AXP™ fits standard refrigerated blood bank centrifuge buckets. Cord blood, transferred to the bag set, is centrifuged. A mononuclear cell (MNC) fraction is separated and the AXP™ device automatically delivers it to a freezing bag designed for optimal space-saving storage. Excess red cells (RBC) and plasma are removed to connected sterile bags. With the AXP™ system, up to six units of cord blood can be processed at one time with a standard blood bank centrifuge. MNC separation does not require hetastarch.

MATERIALS & METHODS:

AXP™ Device and Docking Station

The AXP™ Device is self-powered and microprocessor-controlled. It contains compartments for housing the disposable blood processing set, and flow control optical sensors to achieve the separation of a concentrated MNC fraction of uniform volume (nominally 20 ml) while the bulk of red blood cell mass and plasma are each separated automatically during the centrifugation run. The device is powered by a NiMH battery that is recharged concurrent with data downloading in a docking station.

AXP™ Disposable

The disposable for use in the AXP™ is a closed blood processing set with a sterile, non-pyrogenic fluid path that is easily loaded with cord blood and loaded into the AXP™ device. The disposable set includes:

- Processing/plasma bag with sterile docking input line and clot filter
- RBC bag
- Freezing bag total capacity: 25 ml
- Cryoprotectant line with sterile filter
- Integrated sampling capability

Figure 1

AXP™ XpressTRAK™ Software

The AXP™ is designed to capture essential data for quality assurance and compliance with cGTP. AXP™ software tracks and documents each cord blood unit's separation data during and after centrifugation. Other data fields (Blood unit ID, User ID, Centrifuge ID, and Processing Bag lot number/expiration date) are entered via a keyboard or a wireless scanner. The XpressTRAK™ software produces a computer report of the processing cycle and stores in a searchable, sortable database. Records can be downloaded and printed. Additional features of the software include the ability to modify an ISBT 128 barcode to identify a split-bag sample, to run QC testing on AXP™ devices, assist with troubleshooting and prepare reports on device history.

STUDY DESIGN: A study was performed to test whether cord blood hematopoietic progenitor cells (the CD34 marker cell and colony-forming unit (CFU) counts being used as indices) could be recovered with high efficiency using the AXP™ system. Twenty-three consecutive cord blood units were processed with the AXP™. The blood volume, hematocrit, total nucleated cells (TNC), mononuclear cells (MNC), CD34+ cells and colony-forming units (CFU) were determined in samples from cord blood units before and after AXP™ separation.

RESULTS: Results are presented as the mean ± S.D. (N=23) for all values. The AXP™ process achieved mononuclear fraction volumes of 19.7 ± 0.3 ml with a final average hematocrit of 29.8 ± 2.6% (TABLE 1). The recovery was 98.2 ± 8.0% (CD34+ cells), 94.6 ± 7.0% (CFU), 97.9 ± 4.9% (MNC) and 84.8 ± 9.2% (TNC) (TABLE 2 and FIGURE 2). Less than 1% of TNC were found in the excess plasma bag. Fifteen percent of TNC were lost and recovered in the red cell bag, (TABLE 3) and were mostly granulocytes. Less than 0.5% of the CD34+ cells were found in the RBC bag.

For additional information, contact John Chapman at jchapman@thermogenesis.com.
The AXP™ System is not currently available for sale.

Presented at the International Symposium on Stem Cells: Premises and Promises for Research and Therapeutics
September 2005, Mumbai, India

PROCESSING CORD BLOOD IN THE AXP™ SYSTEM

Collect placental blood into CPD (range 60-200ml)

Spike or sterile dock collection bag to AXP™ processing set and transfer blood to processing bag

Load AXP™ processing set into AXP™ device

Centrifuge at 1,400 x g for 20 minutes to segregate WBC at RBC/plasma interface and 80 x g for 20 minutes to express the RBC to the RBC bag and WBC to freezing bag

Remove bag set from AXP™ device

After separating freezing bag and cryoprotectant line from bag set, introduce 5 ml DMSO/ Dextran 40 into freezing bag

Tube seal and separate freezing bag from cryoprotectant line

TABLE 1: Control of Volume Reduction by AXP™ Processing

Pre-process cord blood volume (ml)	93.4 ± 12.5
Post-process reduced cord blood volume (ml) Target volume = 20 ml	19.7 ± 0.3
Post-process reduced cord blood hematocrit	29.8 ± 2.6

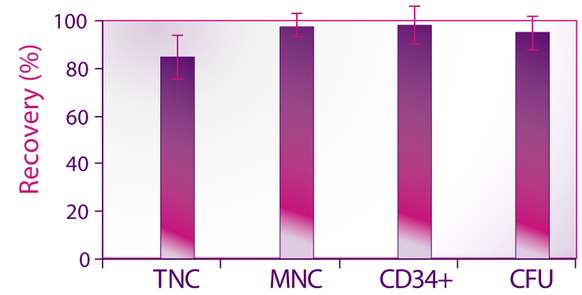
TABLE 2: Nucleated and CFU Cell Content of Post Process Volume Reduced Cord Blood (MNC Fraction)

CELL POPULATION	PRE-PROCESS (CORD BLOOD)	POST- PROCESS (HARVESTED MNC FRACTION)	% RECOVERY
Total Nucleated Cells x 10^6	1150 ± 273.4	969 ± 225.3	84.8 ± 9.2
Total Mononuclear Cells x 10^6	453 ± 134.6	442 ± 126.6	97.9 ± 4.9
Total CD34+ Cells x 10^3 (% Viable)	3364 ± 1594.0 (99.7 ± 0.3)	3256 ± 1412.7 (99.8 ± 0.2)	98.2 ± 8.0 –
CFU x 10^6	2.6 ± 1.1	2.5 ± 1.0	94.6 ± 7.0

TABLE 3: Nucleated Cell Loss (% of Total) in Packed RBC, Plasma Bags

COMPONENTS	TOTAL NUCLEATED CELL LOSS (% OF TOTAL)	MONONUCLEAR CELL LOSS (% OF TOTAL)	CD34+ CELL LOSS (% OF TOTAL)
RBC	15.4 ± 8.4	1.1 ± 1.5	0.3 ± 0.3
Plasma	0.7 ± 0.3	1.4 ± 0.7	–

FIGURE 2: Recovery of Cord Blood Cells in the Mononuclear Cell Fraction by the AXP™ System



CONCLUSIONS:

- The AXP™ cord blood volume reduction process reproducibly separated cord blood mononuclear cells, CD34+ cells and CFU activity with very high efficiency (over 94%).
- The mononuclear cell fraction was obtained in a consistent and uniform volume in a closed system without using hetastarch.
- These results indicate that AXP™ can be effective in meeting the highest quality standards for automated cord blood processing.

ACKNOWLEDGMENT:
The study site for this research was The New York Blood Center. The research was performed by Pablo Rubinstein, M.D. and Ludy Dobrila, Ph.D.