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Automated Volume Reduction of Human Cord Blood Using the AXP AutoXpress™ Platform: A Study with HES

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BACKGROUND: The AXP AutoXpress™ Platform (Figure 1) has been developed for automatically processing cord blood stem cells before cryopreservation. The system

Figure 1

consists of a microprocessor-controlled device, docking station, sterile disposable bag set and supporting XpressTRAK™ software. Cord blood is transferred using aseptic technique to the bag set and centrifuged in the device at high and low speeds. Hetastarch (HES) is added through a sterile filter to maintain a functionally-closed system. During the high speed spin, the cells separate into layers according to density. During the low speed spin the device transfers the red blood cells (RBC) into the RBC bag, total nucleated cells (TNC) and mononuclear cells (MNC) into the freezing bag, and retains the plasma in the processing/plasma bag. The product volume in the freezing bag is operator-selected and achieved with an analytical balance built into the AXP™ device. After being mixed with 5 ml cryoprotectant added through the sterile filter, approximately 25 ml cell solution in the freezing bag can be directly frozen at a controlled rate and stored in the robotic BioArchive™ System.

STUDY DESIGN: The study was designed and performed to determine the efficiency of MNC, TNC, CD34+ stem cell and colony formation unit (CFU) recovery with addition of 20% HES before processing. Twenty units of human cord blood with post-collection age less than 48 hrs were used in the study. High speed centrifugation was performed at 1400xg for 20 minutes and low speed spin at 80xg for 10 minutes. Target product volume in the freezing bag was set to 21 ml. In addition to cell recovery, other parameters that were examined included cell viability, red cell reduction, and hematocrit (Hct) of the blood in the freezing bag. To verify the accuracy of the cell recovery data, the initial total cell count was compared to the sum of the individual total cell counts in the product bag, plasma bag and red cell concentrate bag (mass balance analysis).

RESULTS: Results are presented in the following table and graphs.

Table 1: Volume and RBC Reduction post AXP Process

	Pre-AXP process		Post-AXP process		
Sample #	Vol (ml)	Hct (%)	Vol (ml)	Hct (%)	CD34+ cell recovery
1	80.9	32.7	20.8	19.7	92.5
2	88.1	38.3	20.7	28.7	102.3
3	58.6	35.1	21.0	25.7	115.7
4	52.7	36.6	20.8	31.4	100.4
5	54.2	33.9	20.9	30.5	90.7
6	51.3	32.2	21.2	29.3	92.6
7	45.9	38.1	21.0	31.2	110.5
8	59.8	33.4	21.0	30.3	98.6
9	82.9	26.9	21.2	28.4	94.0
10	73.0	41.0	21.2	29.7	115.6
11	56.6	35.7	20.7	31.0	108.4
12	75.3	40.0	20.7	30.5	105.8
13	99.8	35.2	21.0	28.2	112.3
14	117.3	32.0	21.0	28.1	98.2
15	54.7	24.1	21.0	33.6	97.8
16	107.3	32.8	21.0	27.4	94.7
17	106.8	29.5	21.0	28.2	101.1
18	102.9	33.8	20.6	32.1	103.1
19	109.9	31.5	21.0	29.3	104.6
20	141.9	42.0	20.9	26.2	107.1
Mean	81.0	34.2	20.9	29.0	102.3
SD	27.2	4.5	0.2	2.9	7.6

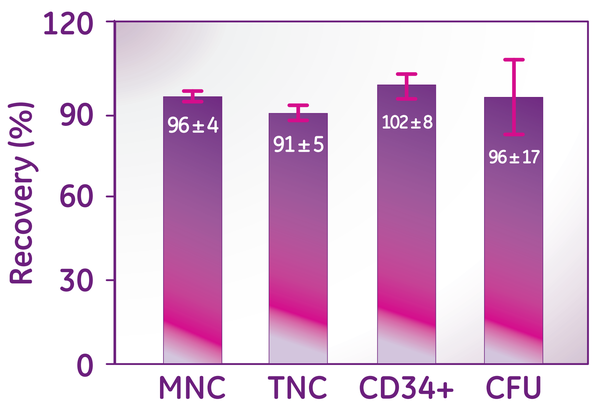


FIGURE 1: The average cell recovery was 96 ± 4% for MNC, 91 ± 5% for TNC, 102 ± 8% for CD34+ cells and 96 ± 17% for CFU. Cell count was measured by Sysmex 2100. CD34 cell was quantified by flow cytometry with Beckman Coulter's Stem-kit™. CFU assay was performed with Stemcell Technologies' MethoCult®.

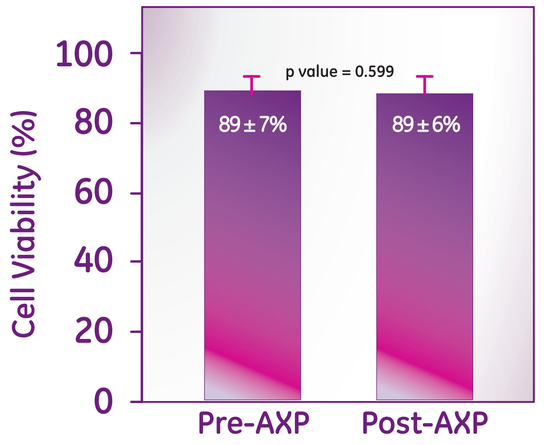


FIGURE 2: The AXP process did not affect cell viability since there was no significant difference of cell viability between pre-AXP and post-AXP samples (p value = 0.599). Cell viability was determined by flow cytometry with Stem-Kit which utilized 7-AAD method.

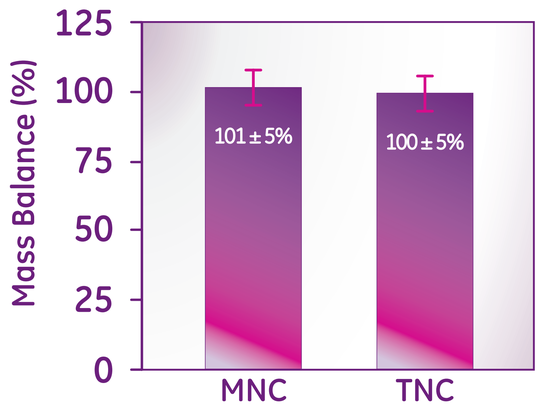


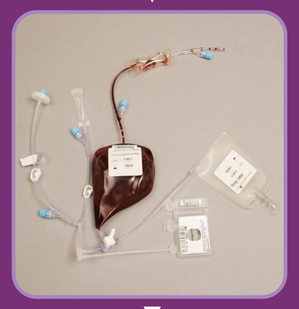
FIGURE 3: The mass balance for MNC and TNC were 101 ± 5% and 100 ± 5%, respectively. The percentage of mass balance was calculated as total found cell number in the freezing bag, RBC bag and processing/plasma bag was divided by total input cell number. This data demonstrates the ability to account for all of the cells initially present in the cord blood unit.

CONCLUSIONS: The AXP AutoXpress Platform is compatible with the use of HES for a cord blood volume reduction processing. The system consistently yields cord blood MNC and TNC cell recoveries of greater than 90%. More significantly, there is essentially complete CD34+ stem cell and CFU recovery. No effect of the AXP process could be detected on cell viability. The system reproducibly achieved the intended final volume in the freezing bag. The results from this study, together with historical data, demonstrate that the AXP AutoXpress Platform can achieve high efficiency cell recovery during automated volume reduction of cord blood with or without HES addition in a functionally closed system.

For additional information, contact John Chapman at jchapman@thermogenesis.com.

PROCESSING CORD BLOOD USING THE AXP AUTOXPRESS PLATFORM

Collect placental blood into CPD

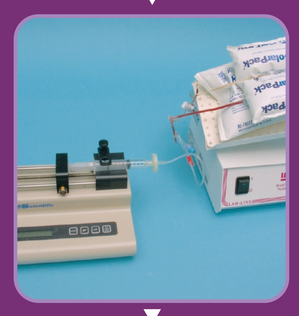


Spike or sterile dock collection bag to AXP processing set and transfer blood to processing/ plasma bag

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 10 min-utes to express the RBC to the RBC bag and WBC to freezing bag

Remove bag set from AXP device



After separating freezing bag and cryopro-tectant line from bag set, introduce 5 mL DMSO/Dextran 40 into freezing bag

Tube seal and separate freezing bag from cryopro-tectant line