PALL Medical

Application Note

PN33536

Umbilical Cord Blood CD34+ Cell Selection and Expansion Using Mononuclear Cells Enriched by the Purecell™ Select System

Enriched mononuclear cells (MNC) from fresh umbilical cord blood (UCB) are used in a variety of research applications. Routinely, researchers utilize either a centrifugation-based method using density gradient reagents or a sedimentation technique employing hydroxyethyl starch to enrich MNC from UCB. Reproducibility of results is highly user-dependent with these labor-intensive and time-consuming methods.

Pall has developed the Purecell Select System utilizing Pall's specialty materials, including a device and protocol for enrichment of MNC from whole blood (WB) samples. This system eliminates open-tube processing and is designed for high recovery of MNC in only 8-15 minutes with highly reproducible results. The easy-to-use, needleless system includes a set with sterile fluid path and an optimized Harvest Solution. Briefly, the sample is first transferred to the sample bag via a sterile Luer-Lok◆ port. The sample then flows through the system under gravity, and MNC are captured within the filter. After flow has stopped, the user back-flushes the filter with the Harvest Solution to recover the enriched MNC population. Internal studies with 50 mL of WB have consistently achieved recoveries of > 70% of MNC [see Application Note *Performance Characterization of the Purecell Select System for Enrichment of Mononuclear Cells from Human Whole Blood (PN33505)*]. These studies include side-by-side comparison to density gradient reagent (Ficoll-Paque◆, GE Healthcare); results showed that the Purecell Select System consistently recovers 20-50% more MNC.

Based on this experience, the Purecell Select System was evaluated for enrichment of MNC from UCB in a comparison study with density gradient reagent. UCB-enriched MNC from either method were further processed to enrich CD34+ cells followed by cell culture and expansion using a proprietary culture method. The main objective of the study was to compare the purity and viability of the cells after CD34+ enrichment using the MNC enriched cells from the Pall system and the Ficoll method. A total of 8 direct comparison procedures were performed and the methods and results are summarized below.

Materials and Methods

Umbilical Cord Blood Samples

- Collected UCB units that were rejected for clinical use were used for this study. Maternal consents were obtained for use of the cells for research purposes.
- Cells were collected in blood collection bags (Charter Medical, Winston-Salem, NC) containing 15 mL of CPD (Sigma, St. Louis, MO) and were maintained at ambient temperature and processed within 24 hours after collection.
- The pre-processing product volume range was 90-165 mL (mean: 126 +/- 23 mL) which consisted of either a single UCB unit (n=5) or two pooled units (n=3).
- Total of eight comparison runs were performed. Products were divided into two equal fractions prior to the MNC enrichment step to compare directly the Purecell Select System with a density gradient method.

Mononuclear Cell Enrichment by Purecell Select System

- UCB sample was transferred to the input bag with a syringe via the luer port on the Purecell Select System.
- Cells were allowed to flow through the filter by gravity flow. To reduce red cell numbers, a rinse step was performed by stopping the flow when cells reached the bottom of the sample input bag. 6 mL of Dulbecco's Phosphate Buffered Saline (DPBS: Cellgro/Mediatech, Herndon VA or Gibco/Invitrogen, Carlsbad, CA) supplemented with 0.5% Bovine Serum Albumin (BSA: Sigma) was added to the system. Flow was resumed until the rinse solution flowed through the filter.

- The filter was back-flushed with 24 mL of the Harvest Solution (buffered saline with Dextran), and eluted cells were collected into a syringe.
- Harvested cells were concentrated by centrifugation at 400 x g for 15 minutes, without brake. The supernatant was decanted, and concentrated cells were resuspended in DPBS/EDTA (Miltenyi Biotec, Auburn, CA) supplemented with 0.5% BSA (DPBS/EDTA/BSA).

Mononuclear Cell Enrichment by Density Gradient Method

- UCB sample was transferred into 50 mL conical tubes with 15 mL of cord blood in each tube and was diluted with 20 mL DPBS/EDTA.
- Samples were under-layered with 15 mL of Ficoll-Paque PLUS. Tubes were centrifuged at 400 x g for 35 minutes at 18 ºC without brake.
- Buffy coat interface layers were collected and diluted in DPBS/EDTA and washed three times by centrifugation at 18 ºC with high brake. Centrifuge speed was 400 x g for the first two wash steps and 300 x g for the last wash step. Cells were centrifuged for 15 minutes for the first wash step and 10 minutes for the subsequent two washes.
- Washed cells in each tube were resuspended in 1 mL of DPBS/EDTA/BSA and pooled into one tube.

CD34+ Cell Enrichment

- MNC enriched cells were incubated with a CD34+ antibody (Miltenyi Biotec) for 30 minutes at 6 ºC as per manufacturer's protocol.
- Unbound antibody was removed by washing the cells with PBS/EDTA/BSA.
- Washed cells were processed on autoMACS® (Miltenyi Biotec) following manufacturer's instructions.

Cell Culture and Expansion

- Enriched CD34+ cells were centrifuged at 400 x g for 10 minutes. Concentrated cells were resuspended in 1 mL of media consisting of equal volume of DPBS/EDTA/BSA and proprietary culture media.
- Enriched cells were cultured for seven days using a proprietary method with media and growth factors specific for expansion of hematopoietic progenitor cells.

Nucleated Cell Count, Viability and CD34+ Cell Analysis

- Samples were obtained after the MNC and CD34+ cell enrichment steps and again after cell expansion.
- Data collection was performed on a Guava EasyCyte® Cytometer (Guava Technologies, Hayward, CA).
- Guava ViaCount® assay was used to determine the nucleated cell count and viability. The system consists of two fluorescent nucleic acid dyes to exclude debris and distinguish live from dead and apoptotic cells, reporting nucleated cell concentration and percentage of viable cells.
- CD34+ cell number was determined by setting the cytometer gates on nucleated cells (side and forward scatter, as per manufacturer's specifications). Percentage of nucleated cells positive for CD34+ was calculated after subtracting isotype controls.

The following formulas were used for calculations:

Nucleated cell concentration x volume = Total nucleated cells (TNC)

$$\frac{\left(\text{Expanded cell number on Day 7} - \text{cell number put into culture on Day 0}\right)}{\text{Cell number put into culture on Day 0}} = \text{Fold expansion}$$

Results

MNC Enrichment Step

The Purecell Select System resulted in a significantly higher number of TNC when compared to the Ficoll method. The MNC-enriched cell population with the Purecell Select System had a higher number of granulocytes compared to the Ficoll method.

Table 1

TNC Count from Purecell Select System Versus Ficoll Gradient Separation

N=8	Purecell Select System	Ficoll Gradient
Mean	6.3×10^8	3.7×10^8
+/- SD	2.1	1.3
p value t-test	0.0104	

MNC Enrichment Processing Time

The processing time with the Purecell Select System was significantly less than the Ficoll method. For all eight procedures, the MNC enrichment step with the Purecell Select System was completed in less than 15 minutes compared to 2 hours and 15 minutes for the Ficoll procedure (excluding the 10-minute final concentration step for both methods).

CD34+ Enrichment Step

There was no significant difference between the two methods for the total number of cells recovered and CD34+ purity after CD34+ enrichment.

Table 2

Post-CD34+ Selection TNC and CD34+ Cell Purity Using MNC Enriched with Purecell Select System Versus Ficoll Gradient Separation

	Total Nucleated Cells (10^6)		CD34+ Purity (%)	
N=8	Purecell Select System	Ficoll Gradient	Purecell Select System	Ficoll Gradient
Mean	1.6	1.8	97.8	98
+/- SD	1.0	0.9	0.7	0.8
p value t-test	0.7582		0.5057	

Viability After MNC and CD34+ Enrichment Steps

Viability data showed a statistically significant difference in favor of the Ficoll method after the MNC enrichment step, but the magnitude of the difference was only 1.5%. In contrast, the Purecell Select System, after the CD34+ enrichment step, yielded a statistically significant improvement over Ficoll by 7.8%.

Table 3

Cell Viability from Enrichment Processes Using Purecell Select System Versus Ficoll Gradient Separation

	MNC Viability (%)		CD34+ Viability (%)	
N=8	Purecell Select System	Ficoll Gradient	Purecell Select System	Ficoll Gradient
Mean	97	98.5	92.3	85.6
+/- SD	1.2	0.5	3.4	3.7
p value t-test	0.0059		0.0022	

Fold Expansion

There was no significant difference in fold expansion results when comparing the two methods.

Table 4

Fold Expansion of Cultured Cells Using MNC Enriched with Purecell Select System Versus Ficoll Gradient Separation

N=8	Purecell Select System	Ficoll Gradient
Mean	51	52.5
+/- SD	9.5	13.8
p value t-test	0.8045	

Conclusions

MNC enrichment is a common upstream processing procedure for many research applications. A fast and easy-to-use system that is capable of providing an MNC enriched cell population suitable for further downstream processing is highly desirable. Previously, data had shown that Pall's Purecell Select System can achieve high recovery of MNC and viability with whole blood samples [see Application Note *Performance Characterization of the Purecell Select System for Enrichment of Mononuclear Cells from Human Whole Blood (PN33505)*]. The data herein shows that the system is also capable of enriching MNC from UCB units for research applications in less than 15 minutes. MNC from the Purecell Select System used in downstream CD34+ enrichment resulted in high CD34+ purity (> 97%) and high viability (> 92%). Furthermore, data shows that the enriched cells from this system can be expanded in culture resulting in equivalent fold expansion when compared to Ficoll MNC used in similar downstream processing methods. Pall's Purecell Select System can be of substantial value to users by providing MNC enriched cells in a significantly shorter amount of time than Ficoll procedures while eliminating open-tube and user-dependent manipulation steps.

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