WHITE PAPER

Peripheral Blood Mononuclear Cell Isolation Protocol

ThermoGenesis®
A CESCA Company

Purification Through Automated Depletion of Red Blood Cells, Granulocytes, and Platelets

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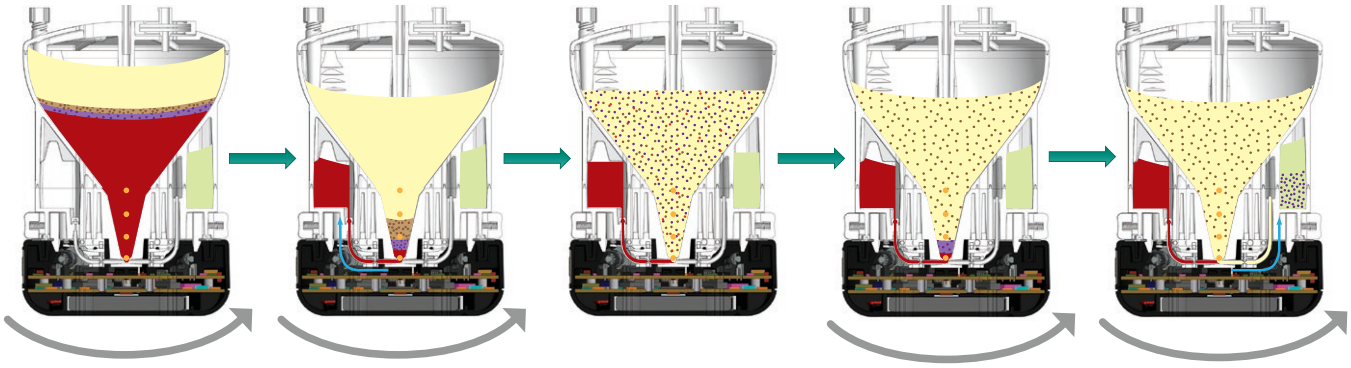
INTRODUCTION

Peripheral blood mononuclear cells (PBMC) are valuable for both clinical and research applications. Isolating pure populations of PBMC from whole blood traditionally requires sample dilution and use of a density gradient medium to deplete red blood cells (RBC), granulocytes (GRN) and platelets (PLT).[1] This open, manual process involves a high risk of contamination. In addition, selective loss of specific populations of lymphocytes[2,3] and phenotypic discrepancies have been associated with the use of density gradient media.[4-6] Further, this method involves multiple tedious steps that are dependent upon highly skilled laboratory personnel, making the process cost-ineffective and standardization very difficult.[7] To be compliant with current good manufacturing practices (cGMP), manufacturers of cellular therapies must find alternative methods of PBMC isolation that are user independent, reproducible, and closed to ensure sterility.

X-LAB® SYSTEM

PBMC PROTOCOL USING THE X-LAB SYSTEM

The X-LAB System is a functionally closed, sedimentation-based system that reliably and reproducibly isolates PBMCs without the need for density gradient media or manual transfer steps. The X-LAB System features fully customizable protocols that can process 40 to 240 mL of source material and isolate MNCs in a user-defined harvest volume between 2 and 45mL in just 35 minutes. The PBMC Protocol using the X-LAB System automates MNC isolation by compartmentalizing RBC/GRN, MNC, and plasma/PLT fractions using highly sensitive infrared sensors to ensure reproducibility of the manufacturing process.



X-LAB® SYSTEM

METHODS

To evaluate the performance of the PBMC Protocol, 23 X-LAB Cartridges were loaded with peripheral blood (mean volume 148.8 ±2.0 mL) less than 24 h post-collection. Cartridges were then mated with their pre-programmed Control Modules and placed in a 750mL swinging bucket centrifuge.

The automated centrifugation protocol involved:

1. Centrifugation at 2000 x g for 20 min to sediment the bulk RBC/GRN fraction
2. Centrifugation at 50 x g for 5 min for depletion of the bulk RBC/GRN fraction
3. Centrifugation at 1000 x g for 5 min to sediment residual RBC/GRNs
4. Centrifugation at 50 x g for 1min for further depletion of residual RBC/GRNs*
5. Centrifugation at 1000 x g for 1 min to sediment the MNCs, leaving the platelets suspended
6. Centrifugation at 50 x g for 2 min to harvest the purified MNC fraction

*[Cartridges were then removed from the centrifuge, briefly agitated to re-suspend MNCs and PLTs in the main chamber, and returned to the centrifuge]

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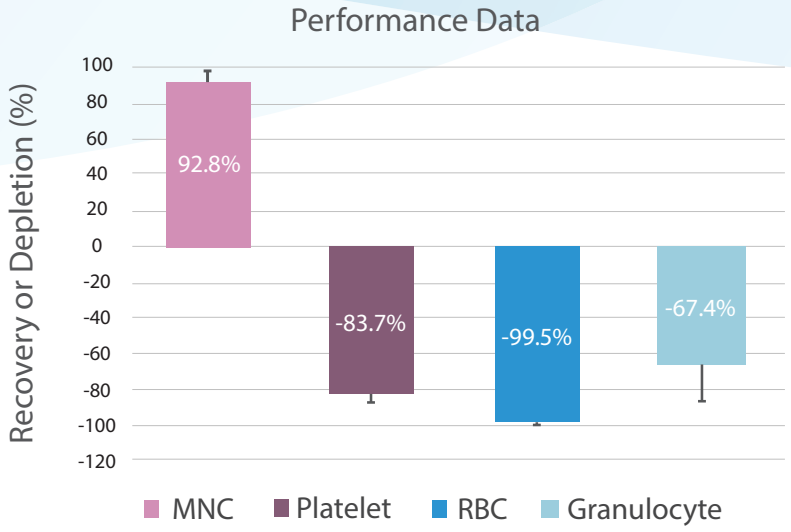
RESULTS

The X-LAB PBMC protocol generated MNC recoveries of 92.8±4.8% while efficiently depleting PLTs (83.7±3.3%), GRNs (67.4±19.6%), and RBCs (99.5±0.1%). Average post-processing CD45⁺ cell viabilities were 96.7% with a 15.6-fold hematocrit reduction.



	PRE-PROCESSING		POST-PROCESSING	
	Hematocrit	CD45⁺ Viability	Hematocrit	CD45⁺ Viability
Average	39.0%	97.8%	2.5%	96.7%
SD	2.9%	1.0%	0.4%	1.3%

CONCLUSION

The PBMC Protocol using the X-LAB System overcomes the limitations of traditional density gradient separation by providing an automated, closed system that isolates MNCs with high recoveries, viability and purity, and that is compliant with cGMP.

Efficient depletion of unwanted cellular fractions is essential for downstream assays and applications. For instance, in positive magnetic activated cell selection (MACS) of CD34⁺ cells, high RBC, GRN and PLT contamination have been shown to significantly reduce the purity and yield of CD34⁺ cells due to nonspecific binding and sequestering of cells of interest in clumps and clots.[8-10] Further, if the isolated MNCs are to be cryopreserved, RBC contamination impairs MNC function following thawing, as RBC are prone to lysis.[11,12]

The adoption of the X-LAB PBMC Protocol reduces process variability while optimizing the recovery of physiologically relevant cell populations, providing performance and consistency suitable for clinical scale applications.

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X-LAB® SYSTEM

ADVANTAGES

- Sediment-based (density gradient media free)
- Functionally-closed and sterile
- High precision sensor detection of PBMCs
- High recovery and purity

X-Counterweights

X-Balance Rings

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