

Peripheral Blood Mononuclear Cell Isolation

Application Note



CORNING

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Introduction

Peripheral blood mononuclear cells (PBMCs) are valuable for both clinical and research applications. Isolating pure populations of PBMCs 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.

PBMC Protocol using the Corning® X-LAB® System

The Corning 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 mononuclear cells (MNC) in a user-defined harvest volume between 3 to 40 mL 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.

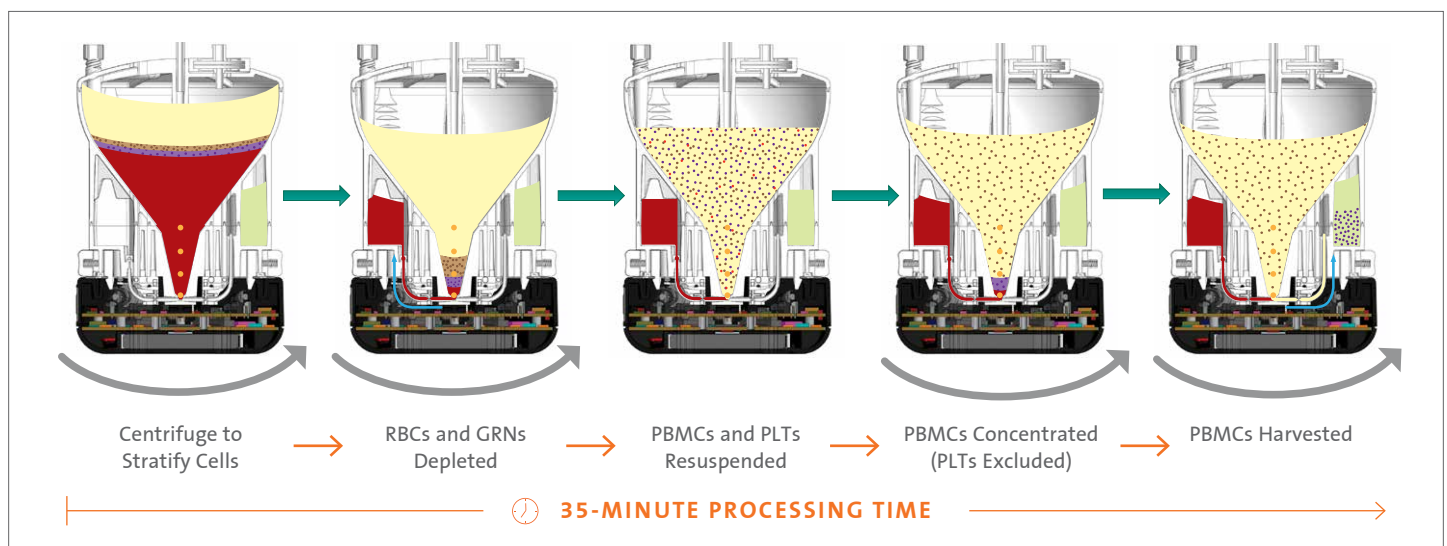
Methods and Materials

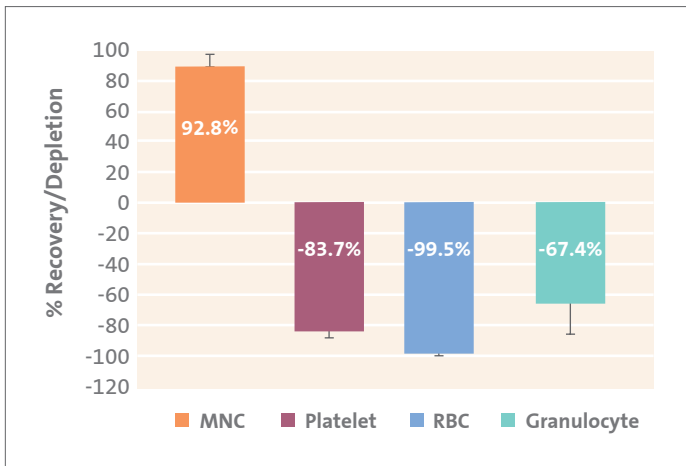
To evaluate the performance of the PBMC Protocol, 23 X-LAB Cartridges were loaded with peripheral blood (mean volume 148.8 ± 2.0 mL) less than 24 hour post-collection. Cartridges were then mated with their pre-programmed X-LAB Control Modules and placed in a 750 mL swinging bucket centrifuge.

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 1 min. 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 resuspend MNCs and PLTs in the main chamber, and returned to the centrifuge.





	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%

Results and Discussion

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

Conclusions

The PBMC Protocol using the Corning 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 designed to meet user cGMP needs.

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 RBCs are prone to lysis.^{11,12}

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

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