Peripheral Blood Mononuclear Cell Isolation

Application Note

CORNING

Content originally prepared for ThermoGenesis by Christy Kim, Jon Ellis, Stephen Truong, John Perea, Zelenia Contreras, Jillian Miller, and Philip Coelho

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.

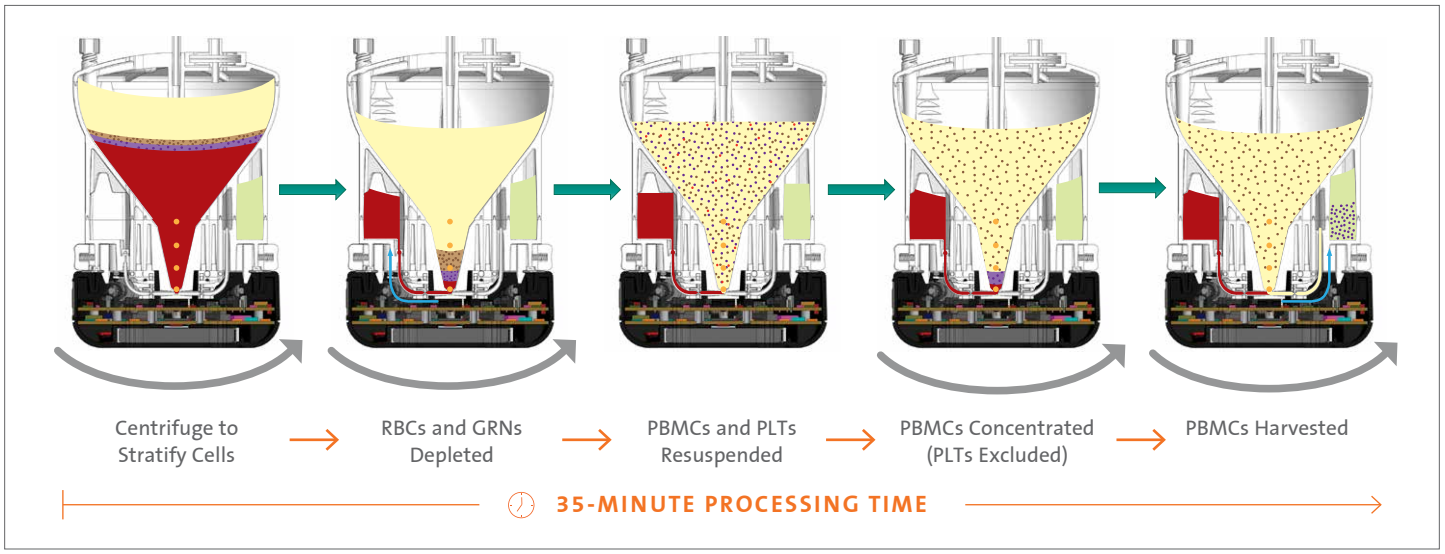
Methods and Materials

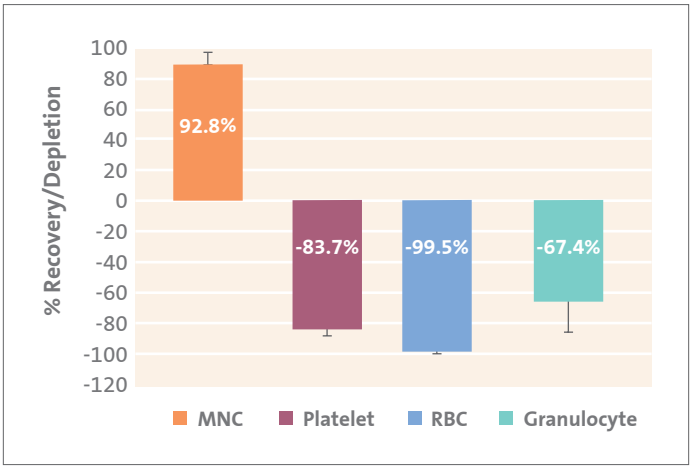
To evaluate the performance of the PBMC Protocol, 23 X-LAB Cartridges were loaded with peripheral blood (mean volume 148.8 ± 2.0 mL) less than 24 hour post-collection. Cartridges were then mated with their pre-programmed X-LAB Control Modules and placed in a 750 mL swinging bucket centrifuge.

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±4.8% while efficiently depleting PLTs (83.7±3.3%), RBCs (99.5±0.1%), and GRNs (67.4±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.

References

1. Boyum A. (1968). Isolation of mononuclear cells and granulocytes from human blood. Isolation of monuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. Scand J Clin Lab Invest Supple 97:77-89.
2. Hokland P and Heron I. (1980). The Isopaque-Ficoll method re-evaluated: selective loss of autologous rosette-forming lymphocytes during isolation of mononuclear cells from human peripheral blood. Scand J Immunol. 11(3):353-356.
3. Hokland P and Heron I. (1980). Analysis of the lymphocyte distribution during Isopaque-Ficoll isolation of mononuclear cells from human peripheral blood. J Immunol Methods 32(1):31-39.
4. Alexander EL, et al. (1978). Quantification of Fc receptors and surface immunoglobin is affected by cell isolation procedures using plasmagel and ficoll-hypaque. J Immunol Methods 22(3-4):263-272.
5. Lin SJ, et al. (2002). Expression of adhesion molecules on T lymphocytes in young children and infants – A comparative study using whole blood lysis or density gradient separation. Clin Lab Haematol 24(6):353-359.
6. Romeu MA, et al. (1992). Lymphotcyte immunophenotyping by flow cytometry in normal adults. Comparison of fresh whole blood lysis technique, Ficoll-Paque separation and cryopreservation. J Immunol Methods 154(1):7-10.
7. Nilson C, el al. (2008). Optimal blood mononuclear cell isolation procedures for gamma interferon enzyme-linked immunospot testing of healthy Swedish and Tanzanian subjects. Clin Vaccine Immunol 15(4):585-589.
8. Hildebrandt M, et al. (2000). Immunomagnetic section of CD34+ cells: factors influencing component purity and yield. Transfusion 40(5):507-512.
9. Reiser M, et al. (2000). High platelet contamination in progenitor cell concentrates results in significantly lower CD34+ yield after immunoselection. Transfusion 40(2):178-181.
10. Bruno A, et al. (2002). Positive selection of CD34+ cells by immunoadsorption: factors affecting the final yield and hematopoietic recovery in patients with hematological malignancies and solid tumors. Transfus Apher Sci 26(2):103-110.
11. Zhurova M, el al. (2012). Quality of red blood cells isolated from umbilical cord blood stored at room temperature. J Blood Transfus 2012:102809.
12. Hauxk-Dlimi B, et al. (2014). The effects of cell concentration from different cell populations on the viability of umbilical blood stem cells. Clin Lab 60(10):1635-1640.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

For additional product or technical information, visit **www.corning.com/lifesciences** or call 800.492.1110. Outside the United States, call +1.978.442.2200 or contact your local Corning sales office.

CORNING

Corning Incorporated
Life Sciences
836 North St.
Building 300, Suite 3401
Tewksbury, MA 01876
t 800.492.1110
t 978.442.2200
f 978.442.2476
www.corning.com/lifesciences

ASIA/PACIFIC

Australia/New Zealand
t 61 427286832

Chinese Mainland
t 86 21 3338 4338
f 86 21 3338 4300

India
t 91 124 4604000
f 91 124 4604099

Japan
t 81 3-3586 1996
f 81 3-3586 1291

Korea
t 82 2-796-9500
f 82 2-796-9300

Singapore
t 65 6572-9740
f 65 6735-2913

Taiwan
t 886 2-2716-0338
f 886 2-2516-7500

EUROPE
CSEurope@corning.com

France
t 0800 916 882
f 0800 918 636

Germany
t 0800 101 1153
f 0800 101 2427

The Netherlands
t 020 655 79 28
f 020 659 76 73

United Kingdom
t 0800 376 8660
f 0800 279 1117

All Other European Countries
t +31 (0) 206 59 60 51
f +31 (0) 206 59 76 73

LATIN AMERICA
grupoLA@corning.com

Brazil
t 55 (11) 3089-7400

Mexico
t (52-81) 8158-8400