Cryopreserved Leukopaks Maintain Cell Viability and Functionality: A Solution for Cell Therapy Logistics

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Abstract

Human cells are critical raw materials for research and manufacturing of cell therapy products. However, accessibility to freshly procured cells can be limited, creating a crucial need for a suitable alternative to fresh cells that are viable and functional —especially when transporting materials globally. At HemaCare, we have investigated the viability and functionality of lymphocytes, both fresh and cryopreserved, from leukopaks (leukapheresis collections) procured within our FDA registered cGMP donor collection facility. Fresh leukopaks (LP) from healthy donors were evaluated for cell viability via flow cytometry over the course of seven days. Studies found that viability of LPs in autologous plasma at room temperature were greater than 80% up to 144 hours post collection. However, the cell counts decreased steadily over time, leading us to look at how cryopreservation might help to ameliorate these drawbacks in typical LP transport. The viability of T-cells, monocytes, B-cells, and natural killer (NK) cells from LP were evaluated from the same donor pre- and post-cryopreservation. Preliminary data for whole LPs shows that post-cryopreserved viability averages 97.5% (+/- 1.2 SEM). The distribution of the CD3+, CD4+, and CD8+ populations were 43.4%, 28.7%, and 12.2%, respectively, within the total LP. Distribution of B-cells, NK cells, and monocytes shows 8.38%, 12.5%, and 20.4%, respectively. T-cell functionality data was also obtained, as this cell type is sensitive to the cryopreservation process. Results of CFSE-labeled T-cells functional assays show multiple divisions over 5 days, and high expression of Ki67 and CD25 after 5 day monocyte derived DC stimulation. These preliminary results suggest that cryopreserved LPs can serve as an acceptable alternative to fresh LPs. Thus, cryopreserved LPs are a valuable and significant option for emerging autologous and allogeneic cell therapies which require apheresis shipments from collection centers to cell therapy processing facilities around the world.

Materials and Method

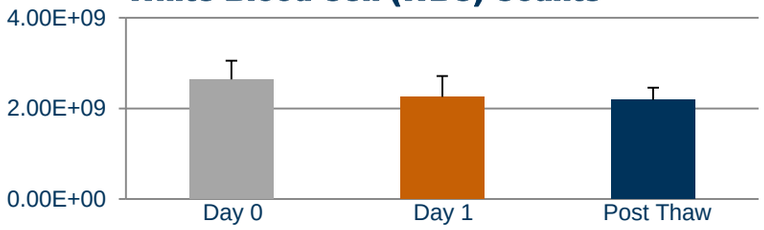
- Fresh LP were stained for cell count and cell distribution, then split into two equal halves. One half incubated at room temperature for 24 hr (Day 1 LP) to simulate shipping, the other half was cryopreserved in CryoStor® CS10 (BioLife Solutions, Bothell, WA) the same day as draw.
- Cell count and distribution were quantified on the Day 1 LPs and the post-thaw cryopreserved LPs.
- CD3 and CD14 were isolated from the Day 1 and post-thaw LPs using magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany).
- Observed and compared T-cell proliferation ability by monitoring CFSE division between Day 1 and post-thaw cryopreserved LPs.
- Observed and compared the differentiation ability of CD14 cells into dendritic cells (DC) from Day 1 LPs and post-thaw LPs.

Conclusion

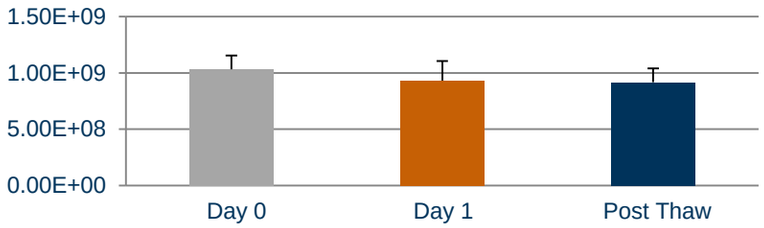
- Initial total WBC counts between fresh LPs (Day 0), Day 1 LPs, and post-thaw cryopreserved LPs were not significantly different (2.64 billion, 2.26 billion and 2.19 billion average cells per timepoint).
- The number of CD3 in fresh LPs (Day 0), Day 1 LPs, and post-thaw cryopreserved LPs were not significantly different (1.03 billion, 0.93 billion, and 0.92 billion average cells per timepoint).
- The number of CD14 cells in fresh LPs (Day 0), Day 1 LPs, and post-thaw cryopreserved LPs were not significantly different (0.40 billion, 0.38 billion, and 0.40 billion average cells per timepoint).
- Viability of the cells was greater than 99% in all cases when gated on FSC/PI.
- DC generation was similar from the CD14 cells isolated from both the Day 1 and post-thaw cryopreserved LPs.
- DC purity was greater than 99% in all cases when gated on CD1c/CD45.
- CD3 cells isolated from both the Day 1 and post-thaw cryopreserved LPs showed similar high proliferation function.

Total Counts

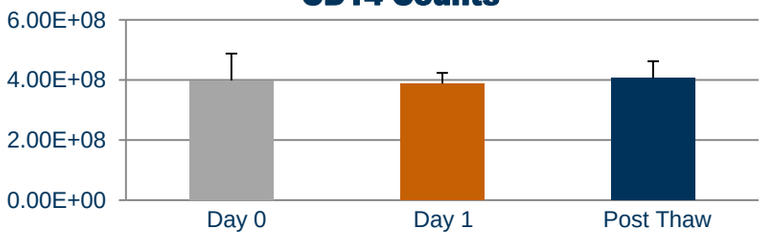
White Blood Cell (WBC) Counts



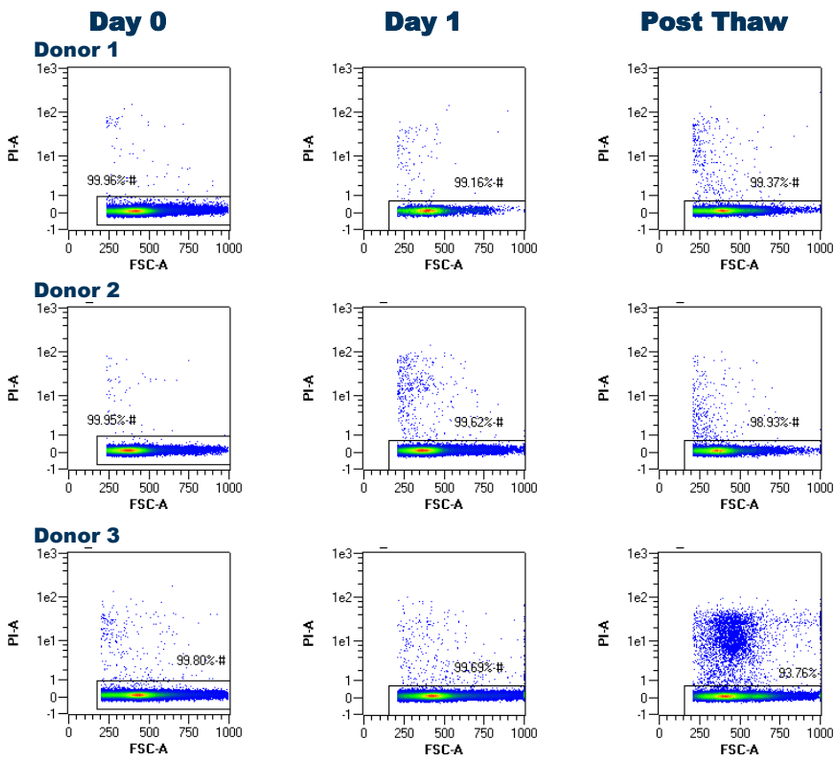
CD3 Counts



CD14 Counts

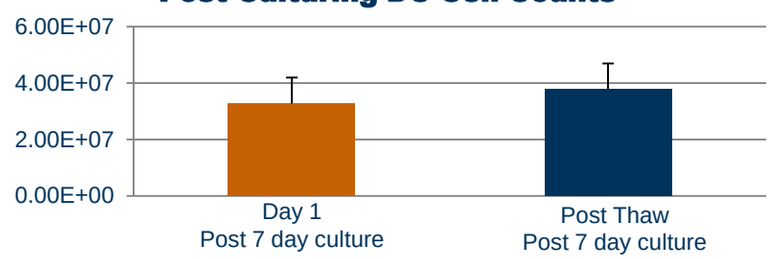


Viability

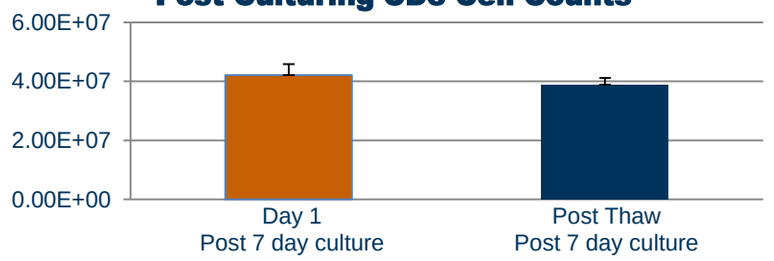


Post-Culturing Cell Counts

Post Culturing DC Cell Counts

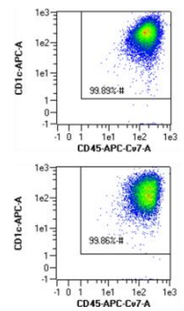


Post Culturing CD3 Cell Counts

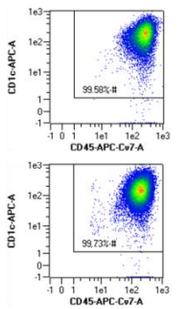


Purity of Dendritic Cells

CD14 Cells Isolated from Day 1 Fresh Leukopak

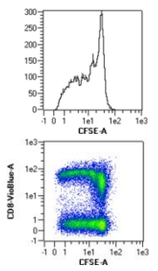


CD14 Cells Isolated from Cryopreserved Leukopak

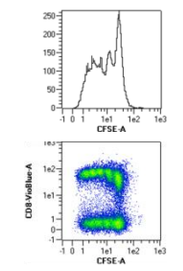


Proliferation of CFSE Labeled CD3 T Cells

CD3 T Cells Isolated from 24 hr Fresh Leukopak



CD3 T Cells Isolated from Cryopreserved Leukopak



Summary

In conclusion, leukopaks from healthy donors, cryopreserved per the HemaCare collection and processing model maintain viability and functionality of target cells. This presents a scalable option for emerging autologous and allogeneic cell therapies that require apheresis shipments from collection centers to cell therapy processing facilities around the world.