

Human-Derived Raw Materials: Controlled, Consistent Collections Enable Successful Manufacturing of Cell-Based Regenerative Medicine Products

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Abstract

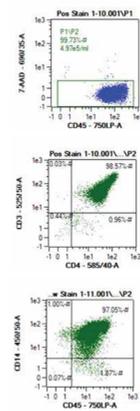
Human cells are critical raw materials for manufacturing cell therapy products, but often introduce significant variability. Rigorous operational controls and quality systems, however, enable optimal collection of high-quality, consistent cellular material. HemaCare, a long-standing supplier of human-derived blood components, controls apheresis procedures and collection sites under a formal quality system, with GMP-compliant, validated procedures and equipment, and GTP-compliant donor screening and tracking. HemaCare performed 86,799 cellular apheresis collections in the last seven years (year ending July 31, 2013), including patient and normal-donor peripheral blood mononuclear cells (PBMCs), and plateletpheresis products, for research, clinical trials, and commercial products. The apheresis collections were completed using the COBE® Spectra Apheresis System with validated IQ, OQ, and PQ specifications. HemaCare's unmodified apheresis products showed consistently high mononuclear cell (MNC) purity, with 93.8% of products containing $\geq 75\%$ MNC, and an average of $85.66\% \text{ MNC} \pm 7.1\%$ (mean $\pm 1 \text{ SD}$). Red blood cell contamination was low, with hematocrit averaging $1.78\% \pm 0.7\%$. Approximately 85% of HemaCare donors have donated apheresis products 5 or more times, and this repeat-donor pool also contributes to product consistency, as MNC content of individual donor apheresis products had an average coefficient of variation of 3.5%, compared to a CV of 7.7% for all apheresis products. HemaCare's BioResearch Products laboratory is equipped with Miltenyi Biotec technology for isolation of cellular raw material into purified cellular subpopulations. The consistency and viability of the purified end products are measured with flow cytometry; products lower than 90% in either parameter are discarded. Using BioLife Solutions' serum-free and protein-free fully-defined cGMP CryoStor™ cryopreservation media with purified cells, post-thaw recovery rates of CD4, CD8, CD19, NK and PBMC fractions have been above 95%, based on 7AAD staining. Dendritic cell and macrophage have demonstrated post-thaw recovery rates of $\geq 90\%$. CryoStor™ cryopreservation medium, in combination with freezing in the BioCision CoolCell™ freezing container, has enabled HemaCare to standardize the cryopreservation process, reducing variability while optimizing post-thaw viable cell recovery of its research products.

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About HemaCare

HemaCare is a leading provider of apheresis products, human blood cells, apheresis collection services, and therapeutic apheresis services

- Apheresis collections and blood-derived products for preclinical research, clinical studies from Phase I to Phase IV, and commercial applications
- Supports applications in immunotherapy, cell therapy, assay development, and medical devices
 - Apheresis PBMC
 - G-CSF-mobilized PBSC
 - Bone marrow
 - Cord blood
 - Peripheral blood
 - Plasma, serum
 - Cell subpopulations
 - CD34⁺
 - CD3⁺, CD4⁺, CD8⁺
 - CD19⁺, CD56⁺, others
 - Healthy-donor and disease-state products
 - Fresh and cryopreserved products



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The HemaCare Advantage

HemaCare is committed to providing our customers with experienced, personalized, responsive, cost effective, and value added services.

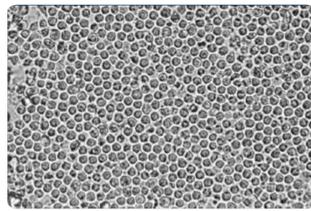
Research Products and Cellular Therapy Services

- Donor pool is already pedigreed and will continue to be expanded
- Extensive donor registry with ability to request repeat donor collections
- Predictable, reliable, and validated collection procedures
- Optimized Standard Operating Procedures (SOPs) leading to high degree of standardization and control
- Ability to collect based on specific, customizable protocols
- High-yield, consistent cell collections
- Validated, automated cell counts and five-part WBC differentials
- Established distribution redundancies leading to the ability to ship via FedEx, UPS, World Courier, and various local couriers
- Access to our scientific/technical support 24/7/365

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Human Cells: Standardizing Living Biological Raw Material Through Quality Processes

- The quality and consistency of human blood-derived cells are critical to enable research for cell therapy, tissue-engineered products, and ex vivo gene therapy products.
- Controlling cell collections minimizes variability and provides an optimal product for use in research and/or manufacturing.
- Training is rigorous and all employees are signed off on critical SOPs for collection.
- Experienced staff lay the foundation of the collections and are critical for success.
- Quality Systems standardize and guide operations aimed to yield optimal, consistent products.

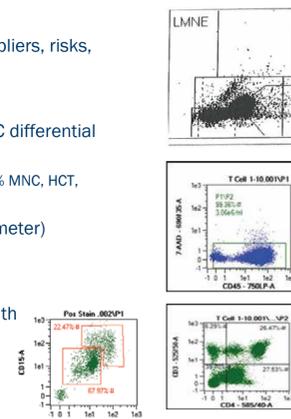


Human PBMCs after a Ficoll centrifugation

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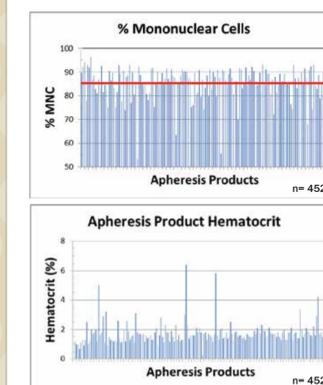
Quality Indicators

- Tracking and trending
 - Donor reactions, deviations, exceptions, suppliers, risks, equipment performance, etc...
- Product QC analysis
 - Automated cell counter/analyzer, 5-part WBC differential (Horiba Pentra analyzer)
 - Nucleated cell (WBC) content and subpopulations, % MNC, HCT, product volume, etc...
 - Immunophenotyping (MACS Quant flow cytometer)
 - Functional Assays
- Donor Testing
 - Screening, infectious disease testing, CBC with 5-part WBC differential
- Internal and external audits



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Selected Apheresis Product Quality Indicators Collected from May 2011 to July 2013



MNC Purity
Mean $85.66\% \pm 7.1\%$ (mean $\pm 1 \text{ SD}$)
93.8% of products $\geq 75\%$ MNC

MNC Content
 $11.5 \pm 4.0 \times 10^9$ MNC (mean $\pm 1 \text{ SD}$)

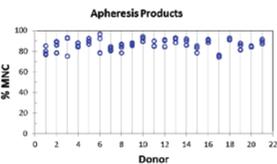
Volume
 360.62 ± 82.71 mL (mean $\pm 1 \text{ SD}$)

RBC Contamination
Hematocrit $1.78\% \pm 0.7\%$ (mean $\pm 1 \text{ SD}$)
91.3% of products $\leq 2.5\%$ hematocrit

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Donors – The Critical Source

- All donors are qualified per regulations and protocol requirements, with IRB-approved informed consent
- Pedigreed, well-characterized apheresis donor population
 - 85% of HemaCare donors have donated ≥ 5 times/year
 - Facilitates recruitment of donors with specific characteristics required by investigator
 - Medical history, HLA type, other laboratory test results, age, gender, ethnicity, etc...
 - Repeat donors further minimize variability



% MNC of a donor sample pool

- Mean 3.5% CV for products per donor
- Mean 7.7% CV for all products
- n= 21 donors, 3-5 products/donor

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Best In Class Partners

A major strategy to maintain HemaCare's quality of cells has been to partner with companies which have high quality standards of their own.

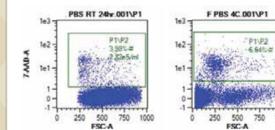
- Cold (4°C) shipment in HypoThermosol® (BioLife Solutions) has been shown to increase stability of a variety of cell types, and could extend shelf-life of apheresis PBMCs and other cell types.
- Cell processing procedures are performed with isolation technology from Miltenyi Biotec. Large scale isolations are done on the Prodigy® and CliniMACS® for an output of billions of cells. Smaller focused isolations are done on the AutoMACS® and manual columns. Both systems were optimized to achieve over 90% purities and over 90% viabilities.
- Cryopreservation was implemented with the use of the CoolCell® manual freezing container (BioCision). The containers contain no liquid component and ensure a consistent cooling rate of $1^\circ\text{C}/\text{min}$ each use. This in combination with CryoStor™ cryopreservation medium (BioLife Solutions) allows for highly efficient procedures during cryopreservation.

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Non-Cryopreserved PBMC Storage Pilot Data

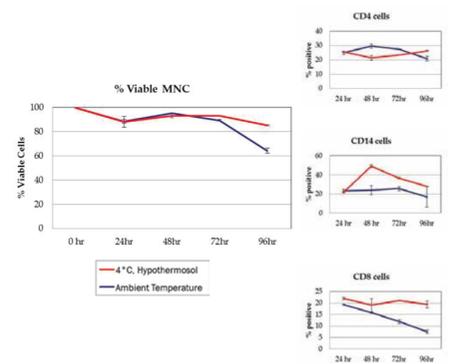
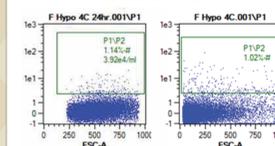
Cell Viability in PBS

Ambient Temperature and 4°C x 24 hrs



Cell Viability in HypoThermosol

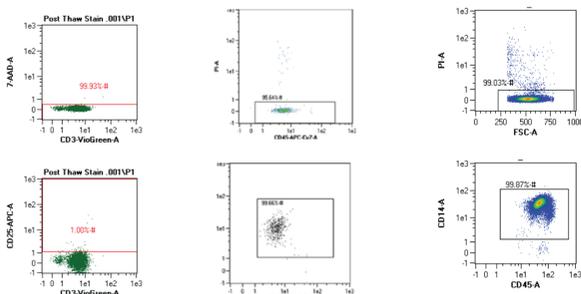
4°C x 24 and 48 hrs



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Post Thaw QC Data

Upon thawing cryopreserved cells, HemaCare's recovery has been consistently stable with respect to purity and viability.



CD3 T cells post thaw showing low death nor any activation

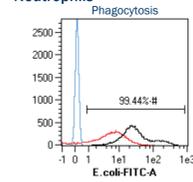
CD34 BM cells post thaw showing low death and high purity

CD14 cells post thaw showing low death and high purity

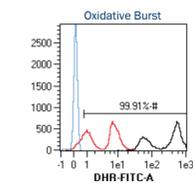
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Functional Assay

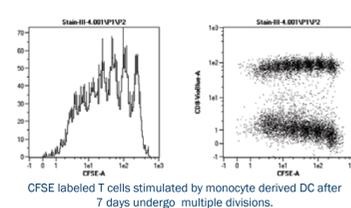
Neutrophils



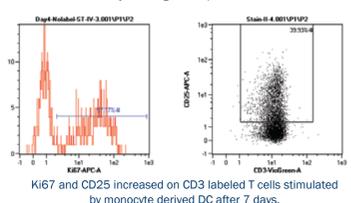
The light blue line represents the condition: neutrophil only;
The red line represents the condition: neutrophil with non-opsonized E. coli-FITC particles;
The black line represents the condition: neutrophil with opsonized E. coli-FITC particles.



The light blue line represents the condition: neutrophil only;
The red line represents the condition: neutrophil with DHR;
The black line represents the condition: PMA activated neutrophil with DHR.



CFSE labeled T cells stimulated by monocyte derived DC after 7 days undergo multiple divisions.



Ki67 and CD25 increased on CD3 labeled T cells stimulated by monocyte derived DC after 7 days.

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Summary

- Collecting blood-based cellular products in a manner that minimizes variability brings a higher degree of reproducibility to the research project or manufacturing effort.
- Quality-based controls such as standardized SOPs, staff training and competency assessments, equipment management, and monitoring of quality indicators reduce this variability.
- Availability of repeat donors from a pedigreed donor base enhances the quality and value of this critical, living biological material.
- Relationships with other best in class organizations allows for consistent product development and collaborations focused in improving the cell manufacturing process.
- Use of cGMP, serum-free, protein-free biopreservation media such as HypoThermosol and CryoStor shows great promise to enable worldwide shipment of fresh or frozen cellular products isolated from apheresis collection, extending shelf-life of cell therapy products.
- Development of functional assays are established to monitor the health and functionality of a variety of cells after processing. These assays are applied to both fresh and cryopreserved cells.

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