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

Thomas V. Ramos, MS, MBA, Wenshi Wang, PhD, Jacob Okhovat, Gaytha McPherson • HemaCare BioResearch Products • (877) 397-3087 • taramos@hemacare.com
Scott R. Burger, MD • Advanced Cell & Gene Therapy, LLC • (919) 969-1103 • celltherapy@ac-gt.com

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 equipment sites under a total quality system, with GMP-compliant, validated procedures and equipment, and GDP-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 non-patient/endor-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 unmobilized apheresis products showed consistently high mononuclear cell (MNC) purity, with 93.8% of products ≥ 75% MNC, and an average of 80.66% MNC ± 7.1% (mean ± 1 SD). Red blood cell contamination was low, with hematocrit averaging 1.78 ± 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 cytometric products less than 90% in either parameter are described. Using Biologic Solutions’ serum-free and protein-free fully-defined uGM Cryosert™ cryopreservation media with purified cells, post-thaw recovery rates of CD4, CD8, CD19, NK and PBMC fractions have been above 95%, based on 7AAD 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 cytometric products less than 90% in either parameter are described. Using Biologic Solutions’ serum-free and protein-free fully-defined uGM Cryosert™ 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 100%. Cryosert™ cryopreservation medium, in combination with freezing in the BioClean CoolCell™ freezing container, has enabled HemaCare to standardize the cryopreservation process, reducing variability while optimizing post-thaw viable cell recovery of its research products.

Human Cells: Standardizing Living Biological Raw Material Through Quality Processes

- The quality and consistency of human blood-derived cells is 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.

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 PMC
- G-CSF-mobilized PBSC
- Bone marrow
- Cord blood
- Peripheral blood
- Plasma, serum

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
  - 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

The HemaCare Advantage

HemaCare is committed to providing our customers with experienced, personal support, 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

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

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.
- Use of cGMP, serum-free, protein-free biopreservation media such as HypoThermosol® allows for highly efficient procedures during cryopreservation.

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

- MNC Purity
  - Mean 85.66% ± 7.1% (mean ± 1 SD)
  - 93.8% of products ≥ 75% MNC
- MNC Content
  - 11.5 ± 4.0x10^9 MNC (mean ± 1 SD)
- Volume
  - 360.62 ± 82.71 mL (mean ± 1 SD)
- RBC Contamination
  - Hemacrit 1.78% ± 0.7% (mean ± 1 SD)
  - 91.3% of products ≤ 2.5% hematocrit

Non-Cryopreserved PBMC Storage Pilot Data

Cell Viability in PBS
- Ambient Temperature and 4°C x 24 hrs
- 360.62 ± 82.71 mL (mean ± 1 SD)

Cell Viability in HypoThermosol®
- 4°C x 24 and 48 hrs

Post Thaw QC Data

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

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 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 potential to enable worldwide shipment of fresh or frozen cellular products isolated from apheresis collection, extending shelf-life of cellular 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.

Functional Assay

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

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