Hematopoietic Progenitor Cell Graft Processing & Testing

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Director Cell Processing Laboratories
Medical College of Wisconsin
Types of “Processing”

- Minimally Manipulated
  - Preparation for Infusion
  - Plasma Removal (Minor ABO incompatibility)
  - Red Blood Cell Removal (Major ABO incompatibility)
  - Cryopreservation and thawing
  - Cell enrichment or depletion approved devices

- Extensive manipulation
  - Cell enrichment or depletion-unapproved devices or reagents
  - Ex vivo expansion of specific subsets (e.g. CTLs)
  - Gene manipulation (e.g. “Suicide genes”)
Preparation for Infusion ± Processing

Sample Removal & Testing
- Cell counts & viability
- Stem cell content-Flow assessment of CD34
- Sterility cultures
- Archive sample storage (mostly cells to be frozen)

Labeling
- Composition (Cell count, volume, additives)
- Storage conditions and expiration
- Patient identification, Unit Identifier
- Collection and processing center identification
- Warnings and precautions

Documentation
- Records of all steps of product receipt, testing, processing, and infusion.
Labels

DIN

Collected by and when:

Product Code:

Vol & Additives:

Donor ID

Expiration

Patient ID

Processing Center ID

For Use by Intended Recipient(s) Only

Donor ID: 1234-5678-9

Expiration Date/Time

01 Feb 2014 10:47 CST

(01 Feb, 2014 16:47 UTC)

Intended Recipient
Example Patient
Recipient ID: 11-11-11-11

MCW/FH Cell Processing Lab
9200 W. Wisconsin Ave.
Milwaukee, WI 53226

Caution: New Drug--Limited by United States law to investigational use.
## Cell Counting

**Total Nucleated Cell Counts**
- Surrogate measure of graft quality
- Does not measure potency

<table>
<thead>
<tr>
<th>Aspect</th>
<th>Manual Method</th>
<th>Electronic Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red Blood Cells</td>
<td>Manual Lyse, or Distinguish</td>
<td>Lysed automatically</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Fewer events but better for marrow</td>
<td>More events but may ct marrow fat</td>
</tr>
<tr>
<td>Precision</td>
<td>Less (more manual steps)</td>
<td>More</td>
</tr>
<tr>
<td>Cost</td>
<td>Less</td>
<td>More (could share)</td>
</tr>
<tr>
<td>Subjectivity</td>
<td>More</td>
<td>Less</td>
</tr>
</tbody>
</table>
Viability Methods

- Dye Exclusion Assays - Taken up by dead cells, excluded by cells with intact membranes
- Light or phase contrast Microscope
  - **Trypan Blue** - Most common in HPC laboratories
  - Erythrosin B
- Fluorescent Microscope
  - Acridine Orange with Propidium Iodide - Detects living and dead cells with two dyes
- Flow Cytometry Based Assays
  - **7-amino-actinomycin D (7-AAD)** - Most common
  - Propidium Iodide (PI)
CD34+ Cell Analysis

CD45 Pos

TNC Viability

CD34 Pos

CD45 Pos 99.4

TNC Viability

CD34 Pos 0.66

Expected % CD34+ HPC by HPC Product

<table>
<thead>
<tr>
<th>Allo-HPC(M)</th>
<th>Allo-HPC(CB)</th>
<th>Allo-HPC(A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0%±0.33%</td>
<td>0.93%±0.44%</td>
<td>0.85%±0.46%</td>
</tr>
</tbody>
</table>
Overall viability and recovery of viable CD34+ cells was excellent over a 4 day storage period. However, there is a larger decline in colony forming cells in the same samples. N=3 experiments

While possible to store for autologous use expect a decline in engraftment potential.
Cryopreservation-Goals

• Short term or long term storage of cellular therapy products with preservation of function

• Allows for:
  - Banking of products such as HPC, Cord Blood
  - Storage while patients to undergo addition disease treatment or conditioning for transplant
  - Allogeneic donors to be collected in advance of infusion (several reasons)
  - Storage for potential or planned future use (DLI, serial infusions, etc)
  - Products to complete release testing
Cryopreservation - Basic Requirements

• Preparation of cells for freezing
• Selection and use of cryoprotectants. Mitigate freezing-induced membrane damage due to hyperosmolality, ice crystals and heat generated during the transition from liquid to solid (heat of fusion)
• A controlled slow rate of freezing to allow water to leave the cytoplasm
  - Trigger freezing and reduce heat of fusion (good but not essential)
• Storage at cold temperatures, <-80°C at minimum, Colder is better
Pre-Processing

• HPC, Marrow or HPC, Cord Blood
  - RBC reduced using:
    • Buffy coat preparation
    • HES sedimentation or Density gradient separation
  - Cell concentration- 1-2 x $10^8$ TNC/mL marrow

• HPC, Apheresis
  - Cell concentration- commonly 4.0 x $10^8$ TNC/mL, up to 5.6 x $10^8$ shown to have acceptable recovery
  - Granulocyte content- Aim for a MNC content ≥ 70% at collection

• In general low concentrations better, less clumping and lower viscosity
Computer Controlled Freezing

Let product = Chamber
Before start

Need to determine during validation

End temp may vary from -60 to -100

Region I
1°/min

Latent Heat of fusion

Region II
Supercooling
Then resume
1°/min (some 2-3°)

Region III
Increase Rate to
5-10°/min

Product Temperature

Chamber Temperature

Temperature

Time
Storage, Monitoring, Shipping
Storage of Products

- **Liquid/Vapor nitrogen tanks**
  - -196 °C Best for long term storage
  - Less susceptible to power interruptions

- **Mechanical Freezer**
  - -80 °C to -150 °C
  - Need back up power supply

- Both methods need back up plan with alternate storage location
Storage of Products

- Temperature monitoring of storage location
  - Continuous recording or regular frequency
- Alarm system to notify of abnormal temperature
- Inventory system to track/locate products
Long Distance Transportation (frozen product)

- Required for cord blood from bank
  - May also ship for transfer pt
- Shipped in “dry shipper”
  - Liquid nitrogen in absorbent material
- Holds temperature for several days
  - Usually shipped without courier
- Monitor temperature during shipment
Thawing for Infusion

Direct Thawing (At bedside)
Less Time
Fewer Manipulations
Higher Infusion Reaction Rate

Dextran/Albumin Wash (In Laboratory)
Controlled Thawing Environment (safer!)
Better Cell Recovery
Easier Transport
Requires Good Communication with Infusion Team
# ABO Compatibility

<table>
<thead>
<tr>
<th>RBC ABO Type</th>
<th>Plasma Antibodies</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>Anti-B</td>
</tr>
<tr>
<td>B</td>
<td>Anti-A</td>
</tr>
<tr>
<td>AB</td>
<td>None</td>
</tr>
<tr>
<td>O</td>
<td>Anti-A &amp; Anti-B</td>
</tr>
</tbody>
</table>

May need to remove plasma, RBC or both. RBC limit 0.3 mL/kg.
Red Blood Cell Removal

PURPOSE:
To reduce the content of donor red blood cells reactive with recipient antibody. MCW limit <20mL total or 0.3 mL/kg.

Volume reduction

METHOD:
Gel Sedimentation (Plasmagel, HES). Hct 1-2%
Mononuclear Cell Preparation
Density Gradient Method. Hct <0.5%
Centrifugation Method. Hct 1-5%

Do not work for PBPC
Subset Depletion or Enrichment

**PURPOSE:**

- To remove undesired WBC subsets leaving behind everything else. Most often:
  - CD3+ T cells to prevent GVHD (@ transplant)
  - CD8+ T cells to prevent GVHD (DLI products)
  - CD19+ B cells to reduce chances of PTLD

- To enrich the desired population, discarding everything else. Most often:
  - CD34+ or CD133+ hematopoietic stem cells
  - Subsets for immunotherapy, e.g. CD4+CD25+ (Treg), CD56+ (NK cells)

- Performed using cell selection devices such as the Miltenyi CliniMACS
# Target Infusion Cell Dose

## Non Manipulated HPC Products

<table>
<thead>
<tr>
<th>Source</th>
<th>Nuc Cells/kg</th>
<th>CD34/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allo Marrow</td>
<td>$2-4 \times 10^8$</td>
<td>$2-4 \times 10^6$</td>
</tr>
<tr>
<td>Auto Marrow</td>
<td>$1-2 \times 10^8$</td>
<td>$1-2 \times 10^6$</td>
</tr>
<tr>
<td>PBSC *</td>
<td>$2-10 \times 10^8$</td>
<td>$2-5 \times 10^6$</td>
</tr>
<tr>
<td>Cord Blood**</td>
<td>$&gt;4 \times 10^7$</td>
<td>$&gt;0.5 \times 10^5$</td>
</tr>
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</table>

*Cell dose varies widely depending upon mobilization

**Required doses likely attainable only for Pediatric recipients
CD34 Dose

Day of engraftment versus CD34 dose per kg. Allogeneic patients.