Hematopoietic Progenitor Cell Product Characterization

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Product Testing and Characterization Goals

- Required to ensure product:
  - Safety
  - Purity
  - Potency

Questions to be addressed this talk:
- What to test
- When to test
- How to test
What Testing (1)

- Function of product type and processing
- All Product Types and Processing
  - TNC
  - Viability
  - Sterility
- HPC Products \{HPC(A), HPC(M), HPC(CB)\}
  - Hematopoietic stem cell content (CD34)
- TC Products \{TC-T, TC-CTL, etc\}
  - TC content (e.g. T cells)
What Testing (2)

- Based on Processing
  - RBC removal
    - RBC content
  - Mononuclear Cell Enrichment
    - MNC content
  - Subset enrichment or depletion
    - Enriched or depleted target cells
**When To Test**

- **At receipt**
- **TNC**
- **Viability**
- **Sterility**—Not required but may be useful
- **Post Processing known to affect content**
- **Relevant subset (s)**

Example: Density gradient separation to remove plasma and red blood cells. Also expect loss of CD34+ cells, therefore should repeat testing for CD34+ cells in addition to assessment of rbc content.
When To Test

- Prior to Infusion or/Cryopreservation
  - TNC
  - Viability
  - HPC content if relevant
  - Sterility-Required

Testing requirement for product release must be defined
How To Test

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

<table>
<thead>
<tr>
<th>Expected % CD34+ HPC by HPC Product</th>
<th>Allo-HPC(M)</th>
<th>HPC(CB)</th>
<th>Allo-HPC(A)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.0%±0.33%</td>
<td>0.93%±0.44%</td>
<td>0.85%±0.46%</td>
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</tbody>
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Manual Methods

Electronic Methods
Manual Counting Method

1. Take representative sample - Ensure product is well mixed
2. Prepare dilution - Typically 1:10 for apheresis products. Target ≤100 cells per quadrant.
3. Load Counting Chamber, both sides, Do not overfill

1. Count WBC in 4 outer Quadrants, determine average

Average cells in 4 outer quadrants
\[ \times 10^4 \times \text{dilution} = \text{cells per mL} \]
Electronic Counting Methods

- **Method**
  - Take representative sample—Ensure product is well mixed
  - Prepare dilution—Typically 1:10 for apheresis products
  - Apply sample to hematology analyzer
  - Results report from analyzer

<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)
Trypan Blue Viability

Dye excluded = Live Cells
Dye Uptake = Dead Cells

% Viability = \( \frac{\# \text{ Living}}{\# \text{ Living} + \# \text{ Dead}} \times 100 \)
Dead cells take up 7-AAD

Titration with 100% Dead Cells

Advantages compared to Trypan
• Less subjective
• Can be used in conjunction with CD34 assessment
• Most accurate with lyse/no wash methods for assessing thawed products (e.g. cord blood)
• Documentation of results

R² = 0.99651
Sterility

• To assess Aerobic and Anaerobic Bacteria and Fungus
• Culture Based Methods
  • Bactec/ BacTAlert- Most Common in US Labs
  • Requires validation by each laboratory for the products and reagents used. Should include:
    • Innoculum size (most 0.5 to 1 mL product)
    • Detection of appropriate range of bacteria and fungus for your situation
  • Guidance available in US
  • Typically product samples inoculated in processing laboratory, cultures performed in institution Microbiology Laboratory. Must have notification system
• Rapid Methods- May be needed for extensively manipulated products (e.g. cultured cells)
  • Gram stain
  • Endotoxin testing
  • Mycoplasma testing
Hematopoietic Stem Cell Assessment (Potency Assays)

- Assays to detect HSC found to be associated with engraftment
  - Flow Cytometry Based
    - CD34+ Cell content- Most common
    - CD133+ Cell content
    - Aldehydehydrogenase bright (ALDH) cells
  - Hematopoietic Colony Forming Cell Assays (CFU)- Required for Cord Blood Banks, not typically performed for other HPC products
**CD34+ Cell Assessment**

- **Assay Requirements**
  - Class III CD34 antibody conjugated to bright fluorochrome (PE or APC recommended)
  - CD45 in every tube
  - Viability dye in every tube - 7-AAD recommended
  - Use multiple parameters to ensure accurate detection to include:
    - Moderate to bright expression of CD34
    - Moderate to dim expression of CD45
    - Low forward and low side scatter
  - Acquire sufficient cells to collect minimally 100 events in final CD34+ gate (if possible)
ISHAGE Gating Method - CD34

- Viable
- CD45 Pos
- CD34 Pos

- CD45 DiM
- Lo FSS Low SS
Allogeneic
ANC 500

Allogeneic
Platelets 20K

Day of engraftment versus CD34 dose per kg. Allogeneic patients.
Mononuclear Cell (MNC) Differentials

- Defined as lymphocytes and monocytes within the Cellular Therapy Product
  - Useful as a surrogate for CD34+ cells, since CD34+ cells have the characteristics of mononuclear cells
  - MNC correlate best with recovery post thawing for HPC(A) products
- Methods of detection
  - Manual Differential- Most common?
  - Flow Cytometry Differential- Accurate and can be performed with CD34-assessment
  - Hematology Analyzer Differential- Poorly correlates with manual method for BM, moderate correlation with HPC(A) products
MNC Content Vs Thaw Recovery

\[ R^2 = 0.3763 \]
\[ P < 0.0001 \]
MNC Assessment - Flow Differential

HPC(A)  HPC(BM)

Monocytes
Immature Myeloid
Lymphocytes
Red Blood Cells in HPC Products

- Bone Marrow
  - High RBC content, High Volume
  - Typically give fresh
- Cord Blood
  - High RBC content, Low Volume
  - RBCs lyse at thaw
- HPC, Apheresis Products
  - Low RBC content, typically ≤5%
  - Standard Methods for RBC removal ineffective
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

• Methods For Removal:
  • 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
RBC Test Methods

- Hematocrit-Ratio of RBC to the total volume
  - Manual Centrifugation Methods
    - Requires specialized centrifuge
    - Can be difficult to distinguish packed RBC layer from WBCs for highly cellular products
  - Hematology Analyzer- Most common method
- RBC Count
  - Manual Method- Uses counting chamber as described for TNC
  - Hematology Analyzer
Rare Cell Detection-Subset Depletion/Enrichment

- Most Common Procedures
  - CD34+ Cell Enrichment
    - Typically performed for CD3-removal
    - Requires rare event detect of CD3+ cells
  - CD3+ cell Reduction, ± CD19+ cell reduction
    - Detection of CD34+ cells
    - Rare event detection of CD3+ and CD19+ cells
  - CD56+ NK Cell-Enrichment ± CD3- Reduction
    - Rare event CD3+CD56- cell detection
Rare Cell Detection Methods

- Multi-parameter Flow Cytometry - Only reliable method currently in use
  - Use enriched population to help set gates for reduced population
  - Ensure that antibody used for detection not blocked by antibody used for cell separation
  - Collect sufficient events - Target 100 cells in final analysis gate
  - Use bright fluorochrome for rare events
CliniMACS CD3-Reduced

Pre Treat

CD3 Reduced
CliniMACS CD34-Enriched

Pre Treat

CD34 Enriched
NK Cell Enrichment

Pre-Enrichment

Post Enrichment
**CD34 & CD3 Detection Limits**

**CD34 Observed vs Expected**
- $R^2 = 0.99976$

**CD34 Low Frequency**
- $R^2 = 0.98947$

**CD3 Observed vs Expected**
- $R^2 = 0.9971$

**CD3 Low Frequency**
- $R^2 = 0.99716$
Other Assessments for Release

- Labeling
  - Composition (Cell count, volume, additives)
  - Storage conditions and expiration
  - Patient identification, Unit Identifier
  - Harvest and Processing Center identification
- Warnings and Precautions
- Product Appearance
  - Appears as expected, no aggregates, clumps, unexpected color or turbidity
  - Container is intact, no evidence of damage or leakage