Cord Blood: Thawing & Infusion – Practical Methods

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Objectives

- Present variety of product configurations
- Summarize current thawing practices
- Suggest a menu of post thaw testing, indicative of quality and potency
- Determine the method appropriate for thaw
- Recognize adverse events associated with HPC, Cord Blood thawing

- Disclaimer – not endorsing particular products or vendors
CB Product Types

- **Physical configuration**
  - Single bag
    - Baxter Cryocyte
    - Origen Cryostore
  - Compartment bag
    - Pall (Med Sep)
  - Vials
Product Composition

- Unmanipulated
- Plasma reduced
- Red cell reduced / plasma depleted
- Buffy coat enriched
Cryopreserved Products
Thawing Practices

- Wash
- Dilution or Reconstitution
- Bedside
Rubinstein Method


- **Statistics**
  - TNC recovery improved to 61% from 35%
  - Mononuclear recovery much greater than PMN (grans)
  - CFU losses were undetectable
Traditionally thawed using a 5% albumin/dextran wash protocol

Rationale that reconstituting and washing frozen products would:

- Restore osmolarity & extend cell viability
- Reduce hemoglobin load
- Diminish DMSO toxicity
- Decrease infusion volumes
- Remove potentially ABO-incompatible plasma
Pediatric Setting

- When this procedure was developed, products:
  - were not red cell or plasma reduced
  - were cryopreserved in larger volumes
  - contained greater amounts of DMSO
Equipment

- Laminar Flow Hood
- Waterbath
- Scale
- Refrigerated Centrifuge
- Plasma Extractor
Reagents & Supplies

- Sealable bag
- Syringes & Needles
- USP Albumin, 5%
- 10% Dextran
- Alcohol wipes
- Sampling Site couplers (transfer sets)
- Transfer packs
- Hemostat
Preparation

- Verify physician order
- Pull product and recipient files
- Prepare thawing solutions
  - Dextran to equal one-half total volume of product
  - Albumin to equal one-half total volume of product

- E.g.: 20 ml product + 5 ml cryoprotectant = 25 ml
- 12.5 ml Dextran, 12.5 ml Albumin
Remove from Storage

- Verify identity of product & recipient with paperwork
- Leave in vapor for 5 minutes before thawing
- Remove segments (if present)
Thawing the Product

- Place into clean bag
- Submerge into 37°C waterbath
- Ports up out of water
- Warm just until slushy
Add Wash Solutions

- Insert sampling site coupler or transfer set
- Inject one half of wash solutions
Drain into Transfer Pack
Add remaining wash solutions
to rinse the bag
Drain into transfer bag
Alternatively...

- Prepare wash solution from 250 ml Gentran + 50 ml 25% Albumin
- Prepare syringes in appropriate amounts
- Insert spike of wash bag into one of the ports of the cryobag
Reconstitute to 150 ml

- Insert port of transfer pack into second port of cryobag
- Drain successive additions of wash solutions into transfer pack
- Rinse bag with wash solution
Centrifugation

- Temperature = 10ºC
- Speed = 420 x g (1200 rpm)
- Duration = 10 minutes
- No brake
Express supernatent
Expression Tips

- Calculate amount of supernatent to remove from the centrifuged product
  - 12.5 ml Dextran
  - 12.5 ml Albumin
  - 5 ml cryoprotectant
- Safely remove 30 ml supernatent
- Perform cell count on supernatent
Aspirate concentrated product into syringe for administration
Rinse with fresh wash solutions

- Include with product for infusion
- Use for sterility testing
Mix well & remove sample for post thaw assays

- Nucleated cell count
- Hematocrit
- Viability
- CD34+ cell count
- CFU
Limited cells for testing??

- Reserve the attached segment prior to thaw
- Pellet the supernatent
- Rinse of CBU bag
- Salvage cellular material into cryovials
- Perform ABO/Rh testing & crossmatch in allogeneic settings
- Spot onto Schleicher & Schuell paper
Dilution Method

  - Cytotherapy, Volume 7
  - 18th Annual National Marrow Donor Program Council Meeting, November 4–6, 2005
  - Biology of Blood and Marrow Transplantation, Volume 12, Issue 11, Page 1224 (November 2006)
Dilution Method

- Stabilize the product coming out of thaw
- Eliminate safety risks and potential bag breakage during centrifugation
- Reduce tech time
- Increase confidence that cells are not lost in expression step
- Provide material for characterization studies without adversely affecting cell dose
- Lab is a controlled environment
- Improve clinical outcomes from TCs with limited experience in manipulating frozen cord blood products

- Product is diluted and stabilized in patient circulation
- Bedside event
- Product testing
Conventional thaw + wash (CW)
thaw only (direct thaw or DT)
thaw + albumin reconstitution (AR)

CFU, TNC, CD34, 7AAD  
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remove from LN2
Endpoints

- The results of TNC recovery, CD34 expression, CFU growth & viability were compared:
  - immediately
  - at two hour intervals for the first 8 hours
  - at 24, 32, and 48 hours post thaw
### TNC Recovery

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<th>Reconstitute</th>
<th>Reconstitute and Wash</th>
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</tr>
<tr>
<td>48</td>
<td>90.3</td>
<td>86.2</td>
<td>74</td>
</tr>
</tbody>
</table>

**Percent Recovery**

- **Thaw Only**: 90.9, 90.2, 88.1, 88.7, 88.9, 88.9, 88.4, 90.3
- **Reconstitute**: 86.3, 84.0, 84.0, 84.4, 84.3, 87.4, 87.7, 86.2
- **Reconstitute and Wash**: 79.8, 78.3, 77.7, 78.8, 78.7, 79.4, 75.8, 74
Post Thaw Testing & QC

- Essential to determining product integrity & quality of the manufacturing process
- FDA IND - Safety & efficacy
- Outcome analysis
  - Evaluating variables affecting engraftment
QC Testing

- Regulatory Requirements - post thaw not clearly defined by AABB, FACT or FDA
- Establishing Reference Ranges
- Sample size issues
- Standardization is crucial
QC Testing Recommendations

- Nucleated Cell Count
  - Dose infused
  - Recovery
- Viability
- CFU
- CD34 – total & viable
- ABO/Rh
  - Labeling / Identity
  - Patient post infusion support
- Hematocrit
  - Red Cell Volume / Dose
- Sterility Cultures

- Optional: HLA cases where segment typing not done
CB Thaw Averages - SLCBB

- 848 products thawed at 218 TCs
- NC recovery = 81%
- TB viability = 79-90%
What’s right for you?

- Laboratory procedures for different HPC-C products
  - Do laboratories follow CBB recommendations
  - Practice validated in-house procedures
- How are infrequent in house procedures validated
- NMDP practice units
To wash or not wash...

- ... what are criteria for this decision
- Physician preference
- Patient size – 10 kg pediatric or 100 kg adult
- DMSO dose
- RBC dose
- Experience and confidence
Adverse Events

- Advance prep will circumvent issues
- Proper thaw supplies
- Transport
- Patient delay
Product Issues

- Brittle bag at low temps
- Rapid expansion when removed from LN2
- Ports
- Segment exposure & Undetected cracks
  - Contain product
  - Avoid introduction
- Bag break in centrifuge
Clinical Considerations

- Filtering – not standard practice
- Rinse product container
- Push vs hang
Engraftment/Outcome Data

- Essential for evaluating quality of unit
- Each institution establishes own outcome criteria
- Providing data is crucial
- Stem Cell Transplant Outcomes Database (SCTOD)
Key Points

- Communication and advance preparation are essential to contributing to a quality process.
- TC need to develop and validate their own thaw protocol(s) and utilize those systems.
- QC testing is critical and can be done without compromising the infusion.