Our Stem Cell Transplant Laboratory Program:

• Operating since Fall 2001
• Mostly Autologous HPC-A
• One laboratory technologist / coordinator
• One half time technologist
• Average 20 transplants per year (range 12-33)
• 100% engraftment
Freezer Storage – How much is enough?

Friday, September 19, 2008 at 2030 hours

• Alarm sounding on stem cell freezer!!
• Temperature -130 C
Freezer Storage – How much is enough?
Freezer Storage – How much is enough?

SANYO MDF-1155ATN Ultra-Low Temperature Freezer

• Electricity
• Battery backup
• 180L liquid nitrogen tank – set to engage at -135 C
• Internal alarm
• Remote alarm
• Temperature -145 to -150 C
• Holds about 150 component bags
Freezer Storage – How much is enough?

2100 hours

- Arrived at hospital
- Freezer temp -130 °C
- **LN2 backup not working!**
- **2nd freezer almost full!**
- LN2 tank fine
- Freezer valve not opening to allow LN2 in
- Compressor failure?
- Freezer shut down
Freezer Storage – How much is enough?

2300 hours

• Moved most HPCs and all cryovials to -80 C freezer
  – Freezer locked but in public corridor
  – No remote alarm
  – Monitored every 4 hours

September 23, 2008

• New -85 C freezer set up in laboratory
  – HPCs and cryovials moved again
  – New freezer alarmed and monitored
Freezer Storage – How much is enough?

QUESTIONS???

• Has there been ice crystal formation causing cell damage or death?
• Should the cells be returned to colder storage?
Freezer Storage – How much is enough?

October 3 – 7, 2008
• Attended AABB Conference in Montreal
• Tried to get answers…..no one knew…

October 22, 2008
• Renowned expert answers…

"you are in unchartered waters..."

• 3 cryovials
• 2 bags: 1 stored at -150C and one moved to -80C
<table>
<thead>
<tr>
<th>Sample</th>
<th>Storage</th>
<th>Pre-freeze CD34/uL</th>
<th>Post-thaw CD34/uL</th>
<th>Pre-freeze CD34 % viability</th>
<th>Post-thaw CD34 % viability</th>
<th>% recovery CD34+ cells</th>
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</thead>
<tbody>
<tr>
<td>Cryovial</td>
<td>-80C</td>
<td>7048.4</td>
<td>2451.0</td>
<td>N/A</td>
<td>42.8</td>
<td>35%</td>
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<tr>
<td>Cryovial</td>
<td>-80C</td>
<td>4508.5</td>
<td>1836.1</td>
<td>N/A</td>
<td>43.4</td>
<td>41%</td>
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<tr>
<td>Cryovial</td>
<td>-80C</td>
<td>610.8</td>
<td>196.46</td>
<td>98.7</td>
<td>20.7</td>
<td>32%</td>
</tr>
<tr>
<td>Bag</td>
<td>-80C</td>
<td>610.8</td>
<td>523.34</td>
<td>98.7</td>
<td>68.5</td>
<td>86%</td>
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<tr>
<td>Bag</td>
<td>-150C</td>
<td>6589.8</td>
<td>6037.0</td>
<td>N/A</td>
<td>90.4</td>
<td>92%</td>
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</tbody>
</table>
Freezer Storage – How much is enough?

Results were encouraging for bags whether stored at -150 C OR moved from -150 C to -80 C

- Bag at -150 C – 92% recovery CD34+ cells
- Bag moved to -80 C – 86% recovery CD34+ cells

All cryovials were moved to -80 C
- results 32 – 41% recovery CD34+ cells

Nov 3 – 4, 2008

Tested 10 pairs (20) cryovials
- 10 still at -80 C
- 10 moved back to -150 C for 4 days
<table>
<thead>
<tr>
<th>#</th>
<th>Pre-fryze CD34/uL</th>
<th>Post-thaw CD34/uL</th>
<th>Pre-freeze CD34 % viability</th>
<th>Post-thaw CD34 % viability</th>
<th>Storage</th>
<th>% recovery CD34+ cells</th>
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<tbody>
<tr>
<td>1</td>
<td>3664.7</td>
<td>1041.4</td>
<td>N/A</td>
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<td>-85C</td>
<td>28.4</td>
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<td></td>
<td>1057.5</td>
<td></td>
<td>33.9</td>
<td>-150C</td>
<td>28.8</td>
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<tr>
<td>2</td>
<td>1126.3</td>
<td>357.0</td>
<td>N/A</td>
<td>42.4</td>
<td>-85C</td>
<td>31.7</td>
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<td></td>
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<td>303.3</td>
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<td>42.0</td>
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<td>26.9</td>
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<td>3</td>
<td>594.3</td>
<td>194.1</td>
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<td>32.7</td>
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<td></td>
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<td>188.3</td>
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<td>-150C</td>
<td>31.7</td>
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<td>4</td>
<td>8041.5</td>
<td>1427.1</td>
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<td>-85C</td>
<td>17.7</td>
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<td>1467.8</td>
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<td>-150C</td>
<td>18.3</td>
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<td>40.6</td>
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<td>6</td>
<td>227.4</td>
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<td>5.5</td>
<td>-85C</td>
<td>23.9</td>
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<tr>
<td></td>
<td></td>
<td>100.4</td>
<td></td>
<td>40.7</td>
<td>-150C</td>
<td>44.2</td>
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<td>7</td>
<td>1532.0</td>
<td>671.8</td>
<td>96.6</td>
<td>17.6</td>
<td>-85C</td>
<td>43.8</td>
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<td></td>
<td></td>
<td>658.9</td>
<td></td>
<td>40.9</td>
<td>-150C</td>
<td>43.0</td>
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<td>8</td>
<td>1250.2</td>
<td>539.2</td>
<td>98.5</td>
<td>10.8</td>
<td>-85C</td>
<td>43.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>438.2</td>
<td></td>
<td>35.2</td>
<td>-150C</td>
<td>35.0</td>
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<tr>
<td>9</td>
<td>24631.1</td>
<td>11578.6</td>
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<td>47.0</td>
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<td></td>
<td></td>
<td>11713.3</td>
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<td>47.1</td>
<td>-150C</td>
<td>47.6</td>
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<td>10</td>
<td>4226.9</td>
<td>2118.4</td>
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<td>50.1</td>
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<tr>
<td></td>
<td></td>
<td>2183.3</td>
<td></td>
<td>38.3</td>
<td>-150C</td>
<td>51.7</td>
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</tbody>
</table>
**Freezer Storage – How much is enough?**

Cryovial results:

Cryovials left at -80 C: 17.7 – 50.1% recovery CD34+ cells  
Moved back to -150 C: 18.3 – 51.7 % recovery CD34+ cells

Cryovials left at -80 C: 3.1 – 44.6 % viability  
Moved back to -150 C: 13.8 – 47.1 % viability

**Good news:**
No further cell loss moving back to colder temperature

**Bad news:**
Cryovials cannot be used to determine CD34+ recovery or viability
Freezer Storage – How much is enough?

Next steps:
• CD34+ count and viability again
• 10 matching pairs of cryovials, half moved back to 150 C

Results November 15, 2008:

Cryovials left at -80 C: 14.0 – 55.3% recovery CD34+ cells
Moved back to -150 C: 17.6 – 59.7 % recovery CD34+ cells

Cryovials left at -80 C: 28.5 – 67.4 % viability
Moved back to -150 C: 28.1 – 72.2 % viability

Again - No further cell loss moving back to colder temperature
Freezer Storage – How much is enough?

Oct 27, 2008
• Decided to purchase LN2 vapor phase freezers instead of mechanical, less parts to break
• As long as we have LN2 the cells should be safe

Nov 18, 2008
• First MVE 1500 Series - 150 LN2 freezer set up

Dec 13, 2008
• Second MVE 1500 Series - 190 LN2 freezer set up

Freezer capacity - approximately 600 small component bags or 800 large component bags
Freezer Storage – How much is enough?

With LN2 freezers in lab, insufficient room to continue processing

Dec 2 – 4, 2008
• Paul Cartlidge, Quality Specialist, Stem Cell and Bone Marrow Transplant Program, Hamilton Health Sciences arrived to review space and make recommendations to set up a new processing lab

November 2008 – January 2009
• Validated MVE freezers using validation protocol developed by Paul Cartlidge
We felt optimistic that the cells in the bags had probably survived the warmer temperature better than the cryovials.

<table>
<thead>
<tr>
<th>Bag</th>
<th>Temperature</th>
<th>Viability</th>
<th>Recovery CD34+ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bag 1</td>
<td>-150C</td>
<td>90.4%</td>
<td>92%</td>
</tr>
<tr>
<td>Bag 2</td>
<td>-80C</td>
<td>68.5%</td>
<td>86%</td>
</tr>
</tbody>
</table>

Transplant team decided we did not want to thaw anymore bags until we could have CFU assays and CD34+ counts.

CBS Stem Cell Lab, Edmonton agreed to perform testing:

*Jan 12, 2009* – sent 10 component bags with matching cryovials in LN2 cryoshipper (one bag broke during thawing)

*Feb 2, 2009* – moved all cells from -85C to MVE freezer
# CRYOVIAL RESULTS - EDMONTON

<table>
<thead>
<tr>
<th>#</th>
<th>Pre-freeze CD34+ cells (x10⁶/ml)</th>
<th>Post-thaw CD34+ cells (x10⁶/ml)</th>
<th>% Recovery Post-thaw CD34+ cells</th>
<th>Post-thaw CFU-GM/plate</th>
<th>Post-thaw BFU-E/plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.59</td>
<td>1.57</td>
<td>24%</td>
<td>0</td>
<td>2</td>
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<tr>
<td>2</td>
<td>9.7</td>
<td>1.13</td>
<td>12%</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>7.53</td>
<td>2.12</td>
<td>28%</td>
<td>1</td>
<td>2</td>
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<td>4</td>
<td>7.1</td>
<td>3.97</td>
<td>56%</td>
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<td>5</td>
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<td>48%</td>
<td>14</td>
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<td>6</td>
<td>2.92</td>
<td>0.96</td>
<td>33%</td>
<td>0</td>
<td>5</td>
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<tr>
<td>7</td>
<td>0.82</td>
<td>0.87</td>
<td>106%</td>
<td>1</td>
<td>8</td>
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<tr>
<td>8</td>
<td>1.3</td>
<td>0.66</td>
<td>51%</td>
<td>15</td>
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<td>9</td>
<td>7.9</td>
<td>4.26</td>
<td>54%</td>
<td>5</td>
<td>25</td>
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<td>#</td>
<td>Storage Temp</td>
<td>Pre-freeze CD34+ cells (x106/ml)</td>
<td>Post-thaw CD34+ cells (x106/ml)</td>
<td>% Recovery Post-thaw CD34+ cells</td>
<td>Post-thaw CFU-GM (x105/ml)</td>
</tr>
<tr>
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<td>----------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>1</td>
<td>-150°C</td>
<td>6.59</td>
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<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bag broke during thawing</td>
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<tr>
<td>2</td>
<td>-150°C</td>
<td>9.7</td>
<td>7.31</td>
<td>75%</td>
<td>5.68</td>
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<tr>
<td>3</td>
<td>-150°C</td>
<td>7.53</td>
<td>6.22</td>
<td>83%</td>
<td>5.83</td>
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<tr>
<td>4</td>
<td>-85°C</td>
<td>7.1</td>
<td>6.3</td>
<td>89%</td>
<td>6.74</td>
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<td>6.13</td>
<td>4.83</td>
<td>79%</td>
<td>3.82</td>
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<tr>
<td>6</td>
<td>-85°C</td>
<td>2.92</td>
<td>2.39</td>
<td>82%</td>
<td>1.53</td>
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<td>7.9</td>
<td>5.71</td>
<td>85%</td>
<td>1.8</td>
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</tbody>
</table>
Freezer Storage – How much is enough?

Acceptable limits: CBS Stem Cell Lab, Edmonton:
• Percent recovery CD34+ cells post-thaw: >60%
• Colony growth for CFU-GM: >15 colonies per plate

Our component bags:
• Percent recovery CD34+ cells post-thaw: 75 – 102%
• Colony growth for CFU-GM: 23 – 96 colonies per plate

Amazingly enough, there was very little harm to the cells after the move!
Process Improvements

February 2009 – began post-thaw testing of cryovials before reinfusion required

Percent recovery CD34+ cells post-thaw from February 2009 to February 2010:
- 25 collections
- Mean 83% recovery post-thaw CD34+ cells
- Range: 69 – 100%
Freezer Storage – How much is enough?

At least enough empty storage space for the contents of one full freezer

Lessons learned:

- Be sure to have a current freezer failure plan in place
- Back-up systems don’t always work
- Alarms are very necessary
- Test remote alarms and response regularly
- Expect the best but be prepared for the worst
- Document everything
Freezer Storage – How much is enough?

Many thanks to:

Brenda Letcher – Tech III, CBS Stem Cell Lab, Edmonton
Dr. Locksley McGann – Stem Cell Lab Director, CBS Edmonton
Paul Cartlidge – Quality Specialist, Stem Cell Transplant Program, Hamilton Health Sciences
Mike Halpenney – Charge Technologist, Stem Cell Processing Laboratory, CBS - Ottawa

And…of course to:
Dr. Kirsty Tompkins – Clinical Director, Bone Marrow Transplant Program
Dr. Jason Tay – Hematologist / Transplant Physician
Dr. Lucinda Whitman – Divisional Chief, Laboratory Medicine (Hematology)
Yvonne Gulliver – Bone Marrow Transplant Coordinator