Transient Warming Events ("TWEs") are brief exposures of a frozen blood component to ambient air during post-freeze processing, storage, shipping and transfer between cryogenic containers. The viability of cryopreserved hematopoietic stem and progenitor cells from PCB units may be reduced as a result of the TWEs that affect them after initial freezing. We question whether the loss of viability is sufficient to threaten the clinical effectiveness of PCB transplants. In these experiments we assessed temperature range and multiple exposures.

**EXPERIMENTS**

A. Cell viability after multiple exposure of frozen (-196°C) to different temperatures.
- PCB leukocytes were controlled-rate-frozen in 10% DMSO to -196°C in 1 ml aliquots.
- Vials were transferred to alcohol baths maintaining -25°C, -40°C or -80°C, for 4 minutes and then returned to LN2.
- After 5 minutes in LN2, the exposed aliquots were returned to the same alcohol baths. Each aliquot underwent a total of five identical cycles from LN2 to either -25°C, -40°C or -80°C and back into LN2.
- Control aliquots were maintained at -196°C.
- All exposed and control aliquots were thawed and DMSO removed (1).
- Recoveries of total and viable leukocytes were assessed by acridine-orange and ethidium bromide staining.
- Recoveries of viable progenitor cells were ascertained by double staining of CD 34+ cells and formation of hematopoietic cell colonies, (CFU-G).
- The results are shown in Table 1.

**Summary Conclusion:** Definite reduction of viability with increased temperature and higher number of exposures was encountered in the three tests.

B. Timed exposure to room temperature and humidity.
- Placental blood collections, matched for ABO group, were combined into two separate pools (Pool 1 and Pool 2), each containing >10^9 total viable nucleated cells.
- Hydroxyethyl starch was added to 1% v/v (standard technique, ref 1).
- After 6 minutes centrifugation at 50 x G the leukocyte-rich plasma from each was collected. The supernatant was then transferred into five processing bags and concentrated to a volume of 20 ml per bag by centrifugation (400 x G, 13 minutes).
- Five ml of 50% DMSO / 5% Dextran 40 was added and each mixture transferred to a freezing bag (Pall Medical (Model 791-05) Cryo Bag 25 ml with Overwrap Bag in S. Steel Canister) for at least 48 hours.
- After 5 minutes in LN2, the exposed aliquots were returned to the same alcohol baths. Each aliquot underwent a total of five identical cycles from LN2 to either -25°C, -40°C or -80°C and back into LN2.
- Control aliquots were maintained at –196°C.
- PCB leukocytes were controlled-rate-frozen in 10% DMSO to –196°C in 1 ml aliquots.
- Timed exposure to room temperature and humidity.
- Probe Placement
- Table 2: Timed Exposure to Ambient Air

**CONCLUSIONS**

1. Viability loss was demonstrated in several of the TWE modes.
2. Viability losses upon repeated exposure were additive.
3. These losses, UNDER CONDITIONS THAT MAY OCCUR IN ROUTINE PRACTICE, suggest that units that may be used for transplantation should be monitored for TWE and all incidents recorded.
4. Documentation of TWE should include minimally the time and the temperature of each exposure.

### Table 1: Loss of Cell Viability from TWEs

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<tr>
<th>Temperature (°C)</th>
<th>Leukocytes (% Loss) Median</th>
<th>Range</th>
<th>CFU-C Median</th>
<th>Range</th>
<th>CD34+ cells (% Loss) Median</th>
<th>Range</th>
<th>Recovery Pool 2 650,000</th>
<th>% TNC Viability Pool 3</th>
<th>% TNC Viability Pool 1</th>
<th>% CFU Pool 1</th>
<th>% CFU Control</th>
<th>% CD34+ Viability Pool 3</th>
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<td>13</td>
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**REFERENCES:**
3. New York Blood Center & ThermoGenesis Corp.