

# Cryogenic Storage of Human Hematopoietic Progenitor Cells

## Comparability of Cell-Freeze® to Cryocyte™ Freezing Bags

Successful cryopreservation of hematopoietic progenitor cells (HPC) depends on a number of factors: biopreservation media, freezing rates, and storage using sterile, cryogenically durable storage bags. Cell-Freeze® cryogenic storage containers are 510(k) cleared for storage, preservation, and transfer of hematopoietic progenitor stem cells (HPCs). These bags are made from a unique polyolefin film that offers excellent durability while remaining flexible when stored at ultralow temperatures (–196 °C). Their proprietary membrane port design offers thinner walls for increased flexibility, and an industry-standard label pocket design lends ease of use and traceability for product labeling. Available interface tubing and connectors are compatible with sterile connection and smart-seal technologies for closed-system applications.

We investigated bioequivalence of a Cell-Freeze® (Charter Medical, Ltd. Winston-Salem, NC) freezing bag to a Cryocyte™ (Baxter Healthcare, Deerfield, IL) freezing bag. The Cell-Freeze® bags were designed to directly replace those now-discontinued Cryocyte™ bags. Data shown here demonstrate that for post-thaw recovery of human HPCs, these bags perform comparably.

### DATA SUMMARY

Post-thaw recovery values following storage in liquid nitrogen (LN<sub>2</sub>) — based on total viable mononuclear cells (MNC), CD34+, and CFU — were observed to be bioequivalent for Cell Freeze® and previously standard Cryocyte™ storage bags (Figure 1). We investigated additional sets of bags for physical integrity. Following six weeks of storage in LN<sub>2</sub>, frozen bags were removed, exposed to ambient temperature for one minute, and then returned to LN<sub>2</sub> for five minutes. Thermal cycling exposure was repeated five times before thawing to simulate a worst-case scenario. All bags passed the physical integrity tests that included both microbial-challenge and dye-immersion tests, indicating no fracturing or breakage (Table 1).

### METHODS

For cell preservation assessment, we compared 25 Cell-Freeze® bags from three separate manufactured lots with 25 Cryocyte™ bags from one lot. Bags were filled to the validated maximum freezing fill volume with diluted human HPC product and 10% DMSO at an average  $4.64 \times 10^6$  cells/mL, frozen in a controlled-rate freezer, and subsequently transferred to LN<sub>2</sub> storage for 42–43 days. After thawing, all units were tested for post-thaw viable MNC and CD34+ (as determined by flow cytometry) and CFUs of hematopoietic lineage (by culturing in MethoCult™ medium). For physical integrity, 135 Cell-Freeze® bags (three lots) were investigated. Tryptic soy broth (TSB) containing 10% DMSO was added, and the units were frozen and stored as

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Figure 1: Poststorage recovery comparison

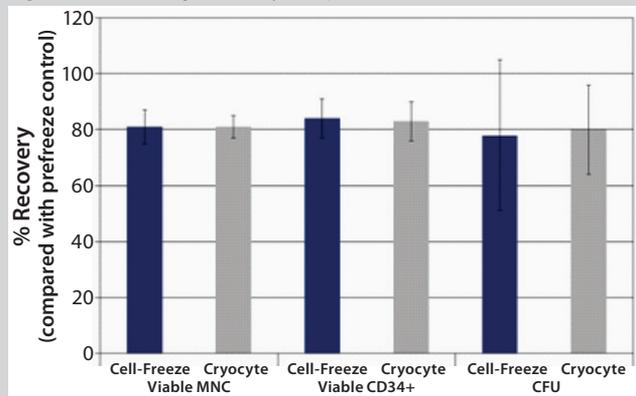


Table 1: Physical integrity testing

Freezing Bag	Pressure/Leak Inspection	<i>B. Diminuta</i> Challenge	Methylene Blue Immersion
Cell Freeze®	No leaks observed	No growth observed	No leaks observed

described above. After cryopreservation, bags were thawed and tested for leakage by pressure and visual inspection before microbial challenge and dye-immersion tests were performed. For microbial challenge, thawed bags were immersed in a bath of *B. diminuta* followed by 14 days of storage at 30–35 °C. For additional leak testing, bags were immersed overnight in a methylene blue dye bath.

### RESULTS AND CONCLUSION

Human HPC post-storage recovery data demonstrate that Cell-Freeze® cryogenic storage bags are bioequivalent to the discontinued Cryocyte™ bags. Average post-thaw recoveries of viable MNC, CD34+, and CFU were comparable. All bags remained intact (no breakage or fracturing) after extensive storage in LN<sub>2</sub> and thermal cycling before thawing. So the Cell-Freeze® cryogenic storage containers from Charter Medical, Ltd. offer comparable cell recoveries to Cryocyte™ bags. The unique polyolefin film offers the added benefit of flexibility at cryogenic (–196 °C) temperatures. 🌐

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