Novel Container for Optimal Cryostorage

Sean R Werner, Aby J Mathew, Adam R Shields, Steven Thompson

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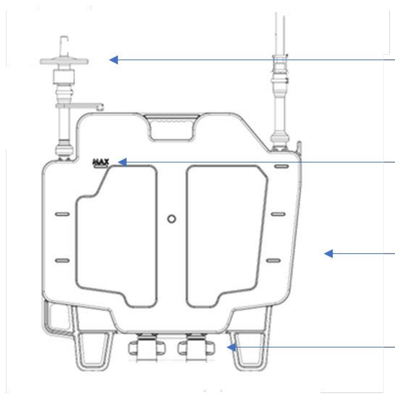
INTRODUCTION

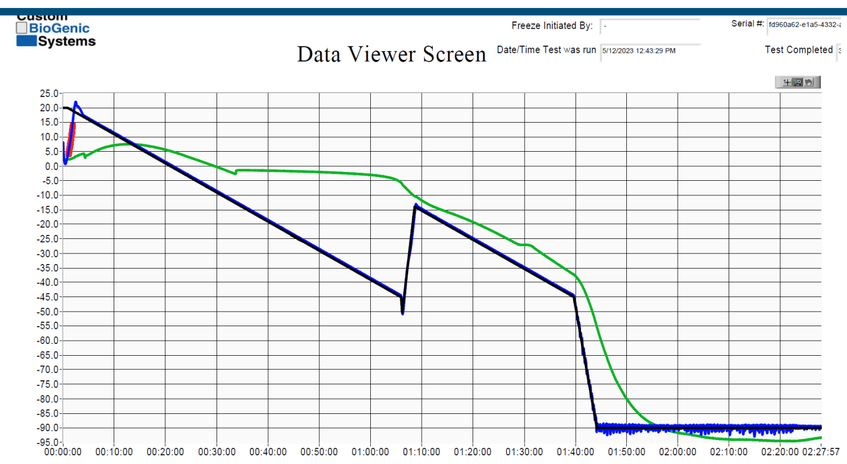
Background & Aim: Soft plastic bags are the most common primary container for larger volume cell and cell-based gene therapies. When filled with small volumes relative to the total capacity, this packaging configuration provides a wide range of storage volumes with a reproducible freezing profile for cryopreservation. Increasing volume within a stoppered vial configuration requires a change in the surface area-to-volume ratio of the product. While bags are accepted as the best available option for storage of larger volumes, they carry significant, difficult to manage risks. Known risks include fractures, difficult preparation steps such as air removal, difficulty in maintaining uniformity, bulky and expensive secondary packaging, multi-step filling, and particulates. In addition, bags are not well-suited for manufacturing at scale, unable to be used in automated filling lines. Overall, the risks and handling challenges of bags leave an opportunity for packaging optimization of larger volume cellular products.

METHODS

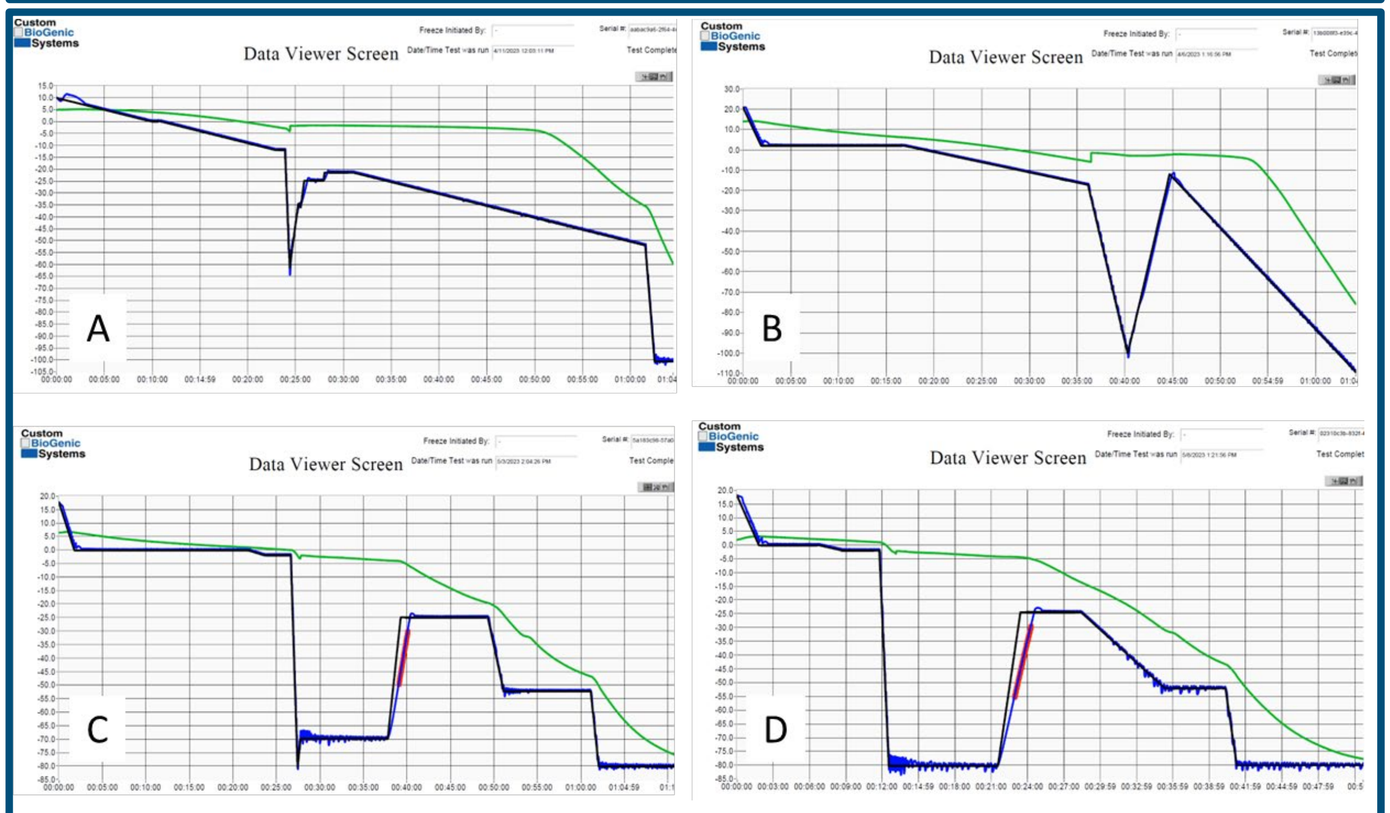
To investigate the potential of alternative form factors to address these challenges, the freeze profiles of a novel, integrated (storage vessel and protective cassette) rigid body cryocontainer (LVC) were evaluated. The LVC container was designed to mimic the surface area-to-volume ratio and use profile of the CellSeal cryovial, a liquid and vapor phase nitrogen compatible vial already in use for commercial cell therapy products (CellSeal®). The LVC cryocontainer can be filled with volumes ranging from 20mL to 70mL. In this study the LVC was evaluated for freeze profiles, post-thaw cell viability, and sample recovery. Results were compared to those obtained from the reference 2mL and 5mL CellSeal vials as well as a commercially available cryobag used to cryopreserve up to 70mL volumes.



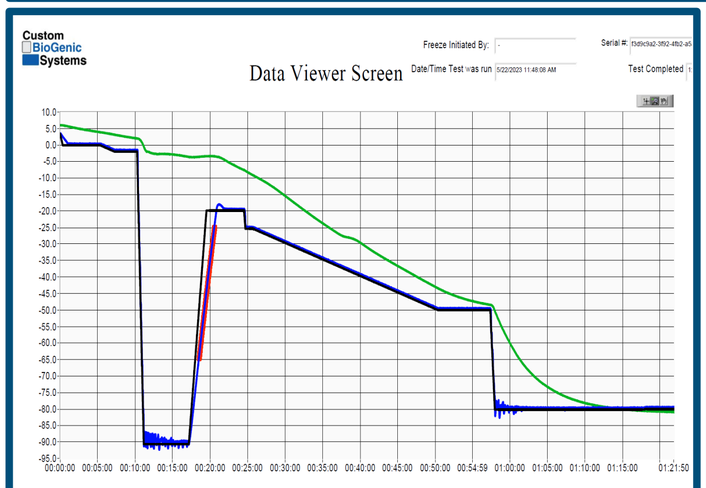
RESULTS



Preprogrammed bag freezing curve from the Intellirate 67i freezer (CBS). Sample is 70 ml DPBS. Under the conditions of the study, ice nucleation occurred at approximately -2.5 ℃ with the evolution of heat during the phase transition taking approximately 30 minutes.



To evaluate the ability to tune freezing profiles in the LVC and the impact on ice nucleation and phase transition, protocol parameters were adjusted. A) A protocol validated for 5mL CellSeal vials was used to freeze 50mL of DPBS. Phase transition lasted approximately 25 min. B) Using a cycle with a deeper plunge cycle (-100 ℃) with immediate return to -10 ℃. Phase transition lasted approximately 18 minutes. C) A low temperature hold was added during phase transition. Phase transition lasted about 12-13 min. D) The low temperature hold was lowered to -80 ℃ and held longer with a slower temperature reduction after return to higher temperature. Phase transition lasted approximately 11 minutes.



Protocol optimized for LVC freezing. A) Freeze curve for DPBS. Low temperature hold after plunge results in an approximately 10 minute phase transition. Controlled temperature rise and subsequent reduction results in temperature change of -1.5 ℃.

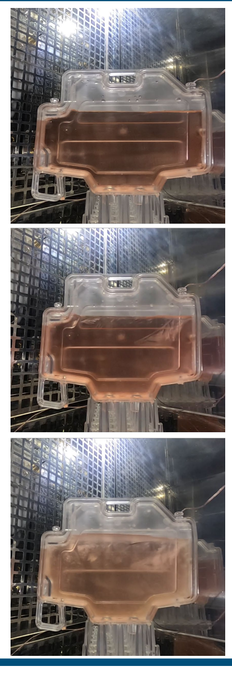
Images of LVC at various stages of freezing.

LVC with DBPS plus red dye at approximately 0 ℃

Initiation of ice formation at –3.5 ± 1.0 ℃

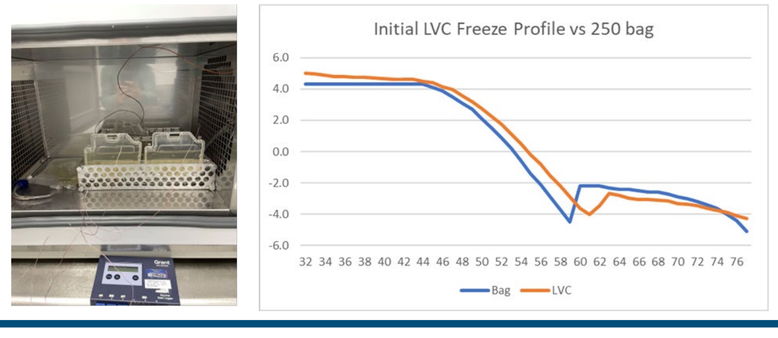
End of ice formation

Time-lapse video of the LVC during phase transition in the CRF is available at the following URL:
https://youtu.be/6qkeVhETnaU



Optimization of the freezing protocol results in overlap of the freezing curve of the LVC container with both the 2mL and 5mL CellSeal vials and the cryobag. Cell viability was equivalent across all container formats when optimized. In addition, the new container exhibited significantly reduced fill processing time and fracture resistance when frozen. These results demonstrate that this novel design provides significant risk reduction while maintaining the optimal freezing profiles developed for cellular products.

Using a protocol optimized for use with a cryobag, the freezing profile of the new container demonstrated a delay in evolution of heat and subsequent return to reduction in temperature. Initial testing was performed in a CryoMed CRF with temperature probes placed within the sample of either a cryobag (CM250) or in the LVC; in both formats the sample was 50mL of fluid (DPBS, 5% DMSO, 15% hPL). The freeze profile in the LVC demonstrated a 7% longer time to reach ice nucleation. The duration of heat evolution was similarly extended.

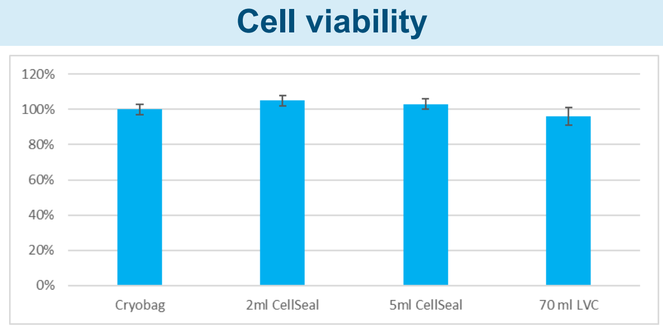


Ice Nucleation

Average (n=13)	**-3.50 ℃**
Low	**-4.78 ℃**
High	**-2.50 ℃**
StDev	**1.04 ℃**

Ice Nucleation within the LVC occurs within a narrow range of temperatures

Cell viability



Cells were cryopreserved using a freeze protocol optimized for the appropriate storage container type. Frozen samples consisted of adipose-derived MSCs at $1x10^7$ cells/mL. For this study, viability was only assessed using live/dead cells (trypan blue) for simplicity.

CONCLUSIONS

Studies were performed with prototypes of a novel rigid cryocontainer (LVC) from Sexton Biotechnologies intended for up to 70mL storage volumes The cassette is manufactured from cyclic olefin co-polymer (COC) and ethyl-vinyl acetate (EVA). The same materials are used in the construction of the CellSeal® cryogenic vials. Strength of the LVC was confirmed by drop test onto epoxy coated concrete from two meters after each freeze cycle. The LVC is designed with features to improve visibility, particulate levels, and result in a more consistent freeze profile.

Controlled rate freezing was performed in either a CryoMed CRF (Thermo Fisher) or the Intellirate 67i CRF (CBS). The freezing profiles observed for the LVC demonstrated that targeted temperature changes can be achieved by modifying the CRF settings. Even though the cross-sectional area of the sample is larger than that of the same volume sample in a cryobag, adjustments to settings results in similar freeze profiles and viability of frozen materials.