CRYOPRESERVATION BEST PRACTICES FOR CAR-T CELL STORAGE

Preserving Viability and Functionality in T-Cells at -80°C and -190°C

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INTRODUCTION

The quality of procedures and products used to prepare, transport and store cells at -80°C and -190°C temperatures have a direct impact on post-thaw viability and functionality. Sub-standard preparation, handling, storage, and products may subject cells to improper cryoprotectant exposure, poorly controlled shipping conditions, and variable storage temperatures.

The objective of this study is to compare two methods of preparing, transporting, and storing live T-cells at both -80°C and -190°C to demonstrate best practices to achieve the highest post-thaw viability.

MATERIALS

Samples

- The Jurkat (Clone E6-1) human acute T-cell leukemia cell line (ATCC, Manassass, VA) was cultured in RPMI 1640 (Lonza, Walkersville, MD) supplemented with 10% FBS (Atlas Biologicals, Fort Collins, CO).
- FluidX® 2mL jacketed, external thread, 2D cryovials

Cryoprotectants

- CryoStor® CS5 (BioLife Solutions, Bothell, WA)
- 95%/5% FBS/DMSO. FBS was obtained from Atlas Biologicals, Fort Collins, CO and 100% DMSO was obtained from BioLife Solutions (Bothell, WA) under the brand name BloodStor® 100.

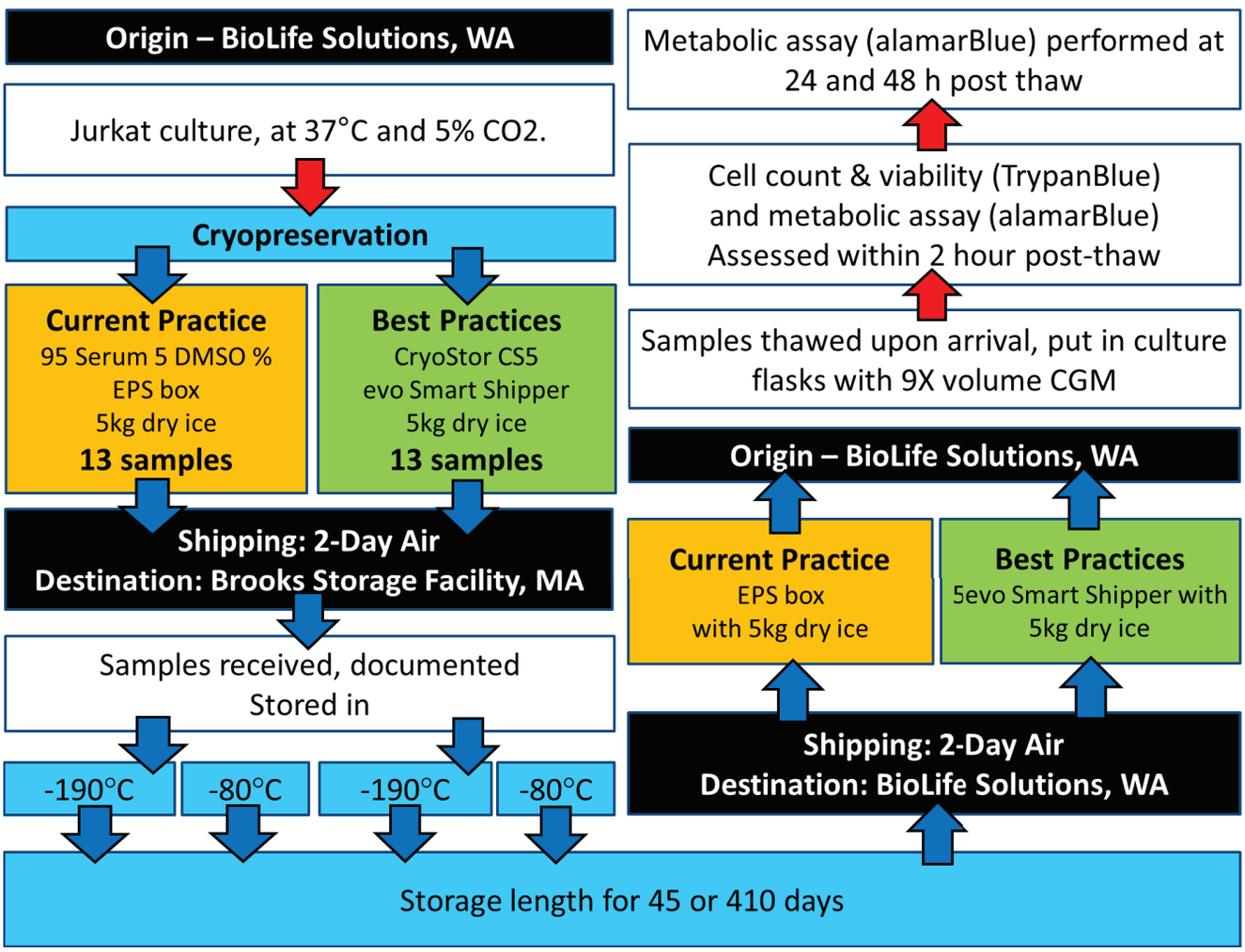
Shipping

- CRYO evoTM -80°C Smart Shipper with integrated data collection and monitoring, and 5kg of dry ice.
- EPS box with 5kg of dry ice

Storage and Handling

- BioStore™ III Cryo -190°C Storage system (Brooks, Chelmsford, MA)
- CryoPod™ carrier (Brooks, Chelmsford, MA)
- TempAura™ temperature monitoring (Brooks, Irlam, UK)
- Biocision BioT™ ULT1.9 -80°C Freezer

WORKFLOWS AND PROCEDURES



Post-Thaw Testing Methods and Analysis

Following return transit to BioLife after 410 days of storage, cryovials were immediately thawed in a 40°C water bath until full-sample thaw was observed. Reference (non-shipped) controls of both CS5 and 95/5 cryomedia were previously thawed under similar conditions for a previous study. Post-thaw viability was determined via trypan blue exclusion on hemocytometer. Functional viability was assessed using the metabolic indicator alamarBlue (AbD Serotec, Bio-Rad, CA). Briefly, 1.25x106 cells were re-suspended in 600µL of alamarBlue at a 1:20 dilution in Hanks Balanced Salt Solution without phenol red. 100µl of cells/alamarBlue were added to 5 wells of a 96-well microplate and alamarBlue fluorescence evaluated after 1 hour using a Tecan SPECTRAFluor Plus plate reader (TECAN Austria GmbH, Austria) at 530nm/590nm excitation/emission. Where indicated, statistical analysis was conducted using 2-way ANOVA with Tukey's post-hoc comparisons. Data are presented as mean+S.D. of 3 independent samples.

RESULTS

Comparing Best Practice (BP) and Current Practice (CP) at -190°C and -80°C Storage Temperatures.

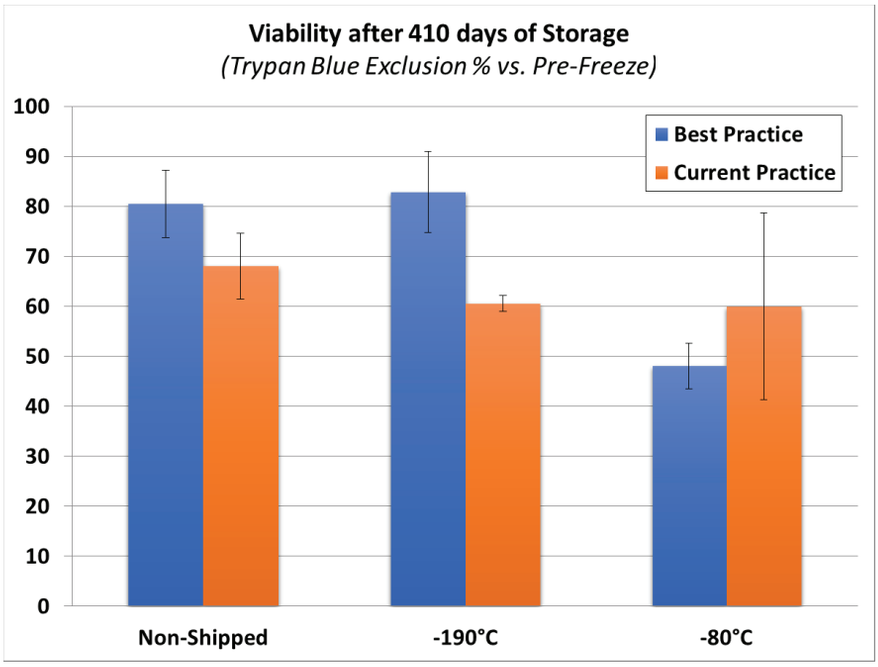


Figure 1.
Jurkat T-cells stored at -190°C with BP products and processes had the highest viability post thaw – nearly identical to non-shipped. Whereas, the CP batch had reduced viability vs BP, but showed little difference between storage temperatures.

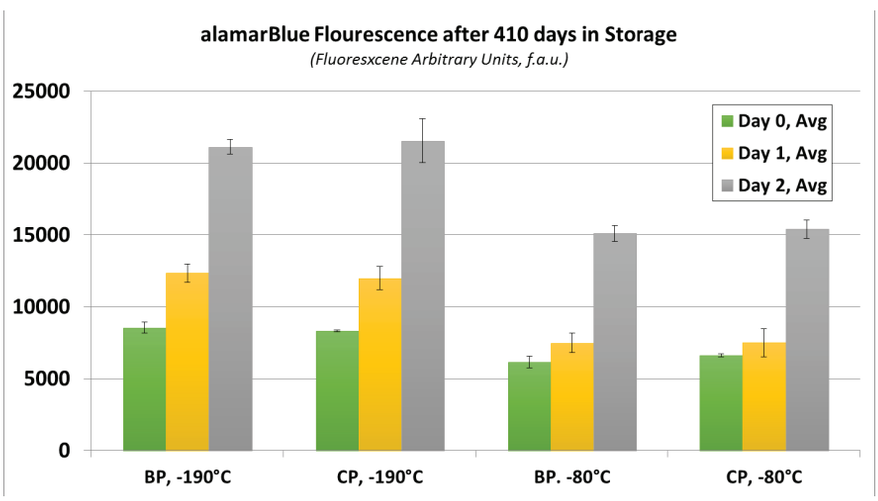


Figure 2.
Metabolic activity was largely influenced by storage temperature as seen by the significantly higher values of both -190°C batches. Best vs. Current Practice had little to no effect on the post-thaw metabolic activity.

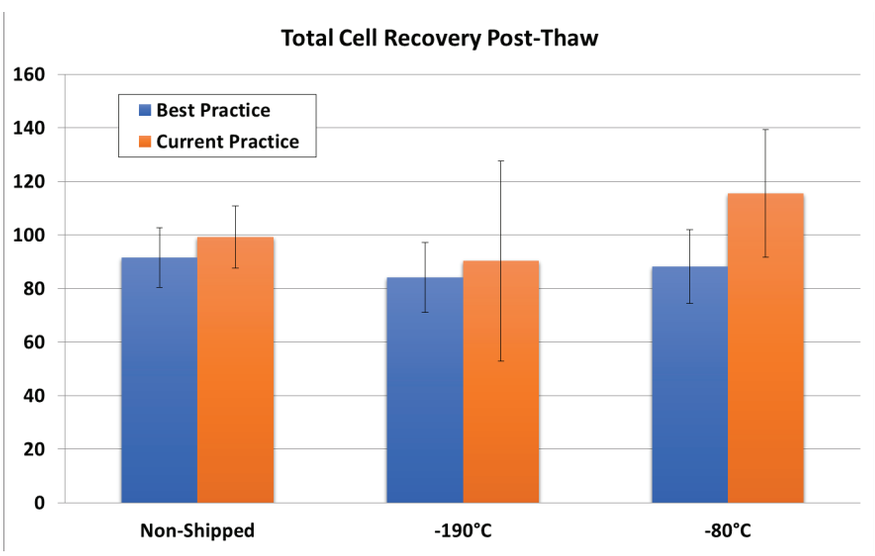


Figure 3.
Cell recovery percentage was nearly the same in all groups when considering the standard deviation ranges. More replicates are required to determine accurate trends.

BEST PRACTICES

Cryopreservation

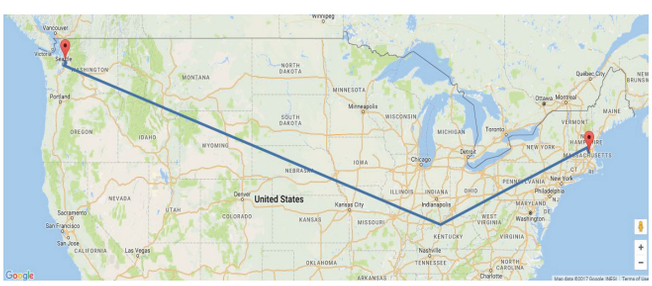
The results of this study demonstrate that using biopreservation Best Practices (BP), including utilization of an intracellular-like composition for cryomedia, provides important advantages over traditional/current practice (CP), as evidenced by the trend in post-thaw viabilities. Although our analysis did not identify significant differences at this point, we anticipate that more replicates will increase power and reveal trends.

The storage temperature proved to be essential for the stability of the cells. Storage at -80°C proved detrimental for the stability of the samples, whereas the LN2 samples (-190°C), regardless of the composition of the cryomedia, performed well.

Shipment Monitoring and Tracking

Biopreservation Best Practices include ensuring sample traceability and temperature stability during shipping. The shipment in the Best Practices group (BP) was able to be tracked via on board GPS with known temperature, time and notifications of any unanticipated events.

In contrast, the CP batch had no record of temperature, time or events and location was only available from UPS hub location scans.



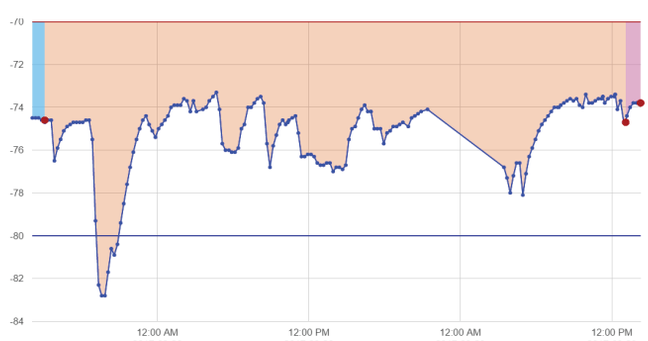


Figure 4. Report of GPS location, temperature, time and events of BP shipment.

Storage and Monitoring

The viability and functionality of cells is maximized when stored in LN2 vapor freezers, below the glass transition temperature of water (Tg), approximately -135°C, as the findings of this study show. Equally important is that during daily interactions with the freezer the thousands of samples that are removed, but not accessed ("innocent" samples), do not transiently warm past Tg.

To allow the greatest temperature protection, high efficiency -190°C vapor freezers should be used. Access should be controlled, monitored and innocent exposures should be recorded and compared to known warming rates in order to ensure innocent samples have not crossed Tg during their storage lifetime.

This can be done with SOPs and monitoring equipment or with an automated system with these features built in. In addition to a freezer's temperature monitoring, a validated third party device should be used with cloud connectivity and alarms.

Figure 5. BioStore III Cryo software demonstrating sample ID tracking (left), inventory locations (middle), and comprehensive audit trail of every freezer and sample interaction (right). BioStore III Cryo storage system (right).

CONCLUSION

Trends were observed that suggest improving viability and metabolic activity post-thaw when employing Biopreservation Best Practices.

- Samples stored at -190°C exhibited higher viability and metabolic activity post-thaw vs. the same cells stored at -80°C. -190°C samples proved to be stable, regardless of BP/CP group.
- Storing cells long-term at warmer than glass transition temperatures (i.e. -80°C) is not recommended.

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