Cryopreservation and transport of Jurkat T cells using Current and Optimized Practices: The impact of temperature and length of storage

Alireza Abazari[1], Brian J. Hawkins[1], John Fink[2], Trevor McCormick[3], Kevin O'Donnell[3], Matthew Albert[2], Aby J. Mathew[1]

1 BioLife Solutions, Inc., Bothell, WA United States, 2 Brooks Life Sciences Systems, Chelmsford, MA, United States, 3 SAVSU Technologies LLC, Santa Fe, NM, United States

BioLife Solutions® BIOPRESERVATION TOOLS FOR CELLS, TISSUES, AND ORGANS

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Introduction:

The quality of procedures and products used for preparing, transporting and storage of cells at cryogenic temperatures have a direct impact on the post-thaw viability and functionality of the cells. Two of the most important parameters affecting the cell stability are:

1. The storage temperature
2. The length of storage at a given temperature

These parameters are frequently faced with in the context of cell therapy process development. In specific, storage and shipping at -80°C may sound attractive as it is more affordable and eases the operations in cell manufacturing facilities and clinics. However, the results on specific cell types and temperatures are scarce. The purpose of this study is to clarify whether storage and shipping at -80°C proves advantageous for process optimization in cellular therapies. In this study, we look into the stability of the Jurkat cells, an immortalized human T lymphocyte, across different freezing and shipping scenarios.

Materials and Methods:

Samples

- The Jurkat (Clone E6-1) human acute T-cell leukemia cell line (ATCC, Manassas, 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 (Brook Life Sciences, MA)

Cryoprotective agents

- 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.

Cryopreservation Protocol

- 5x10^6 cells/sample centrifuge @250g, 5 min, Aliquot in FluidX cryovials – Vials transferred to a typical isopropyl alcohol freezing device pre-chilled at 4°C, Hold 10 min @4°C, then transferred to -80°C freezer, nucleation @ 20 min after in -80°C, Samples transferred to LN2 storage 18-24 prior to shipment

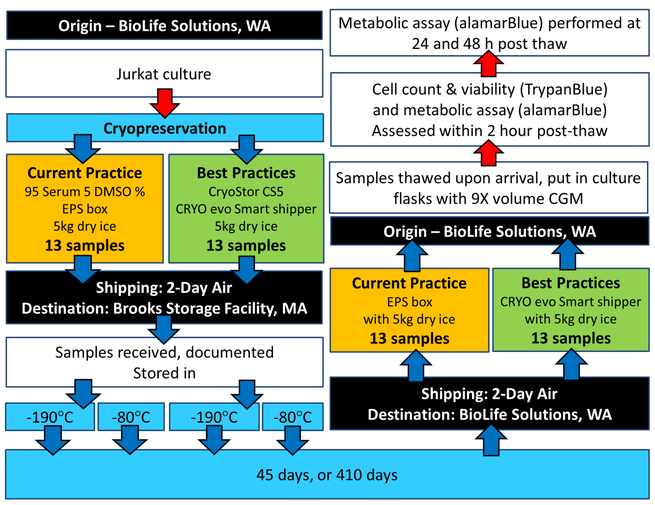
Shipping

- Best Practice: CRYO evo™ -80°C Smart shipper with integrated data collection and monitoring, and 5kg of dry ice pellets
- Current Practice: EPS box with 5kg of dry ice pellets

Storage & Handling

- BioStore™ III Cryo -190°C Storage system (Brooks Life Sciences, MA)
- -80°C mechanical freezer (Brooks Life Sciences, MA)
- CryoPod™ carrier (Brooks Life Sciences, MA)

Work Flow Diagram:



Post-Thaw Testing Methods and Analysis

Following return transit to BioLife after 45 or 410 days, 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 along with 45 day samples. 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, 1.25x10^6 cells were resuspended 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.

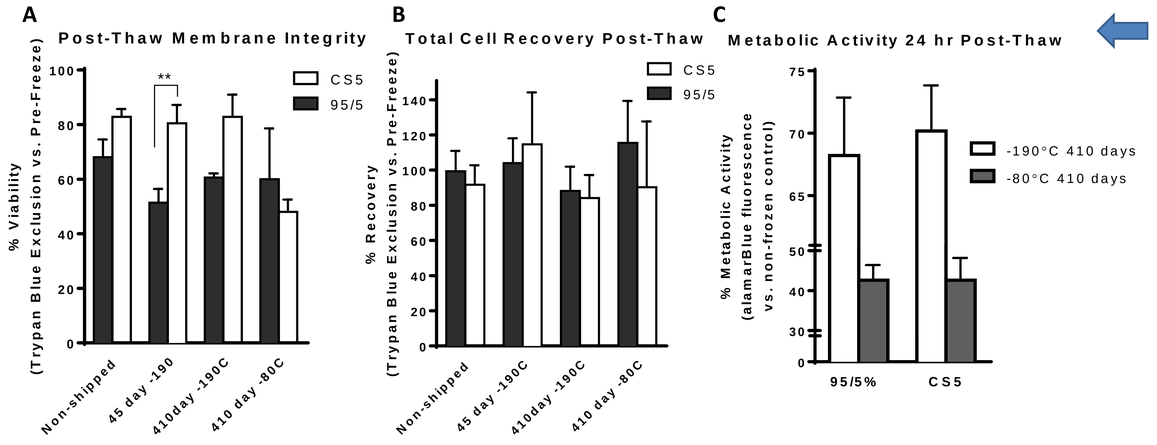


Figure 1: Cell viability following storage at -190°C and -80°C for 45 or 410 days.
(A) The viability of non-shipped Jurkats, estimated by Trypan Blue Exclusion, decreased after freezing in 95/5 compared to CS5 group. After storage for 45 and 410 days, a trend was observed suggesting higher post-thaw viability of the cells frozen in CryoStor CS5. More replicates will determine statistical significance. The protective effect of CryoStor CS5 disappeared when samples were stored in -80°C. At -80°C, post-thaw viability of Jurkats stored in CS5 or 95/5 were not statistically significant. **(B)** Total cell recovery post-thaw was identical in all groups and no statistically significant difference was observed. **(C)** Metabolic activity of Jurkat cells, normalized to non-frozen control were identical in both 95/5 and CS5 groups that were stored at -190°C for 410 days. The samples that were stored at -80°C during the same period experienced identical losses in metabolic activity 24 hr post-thaw.

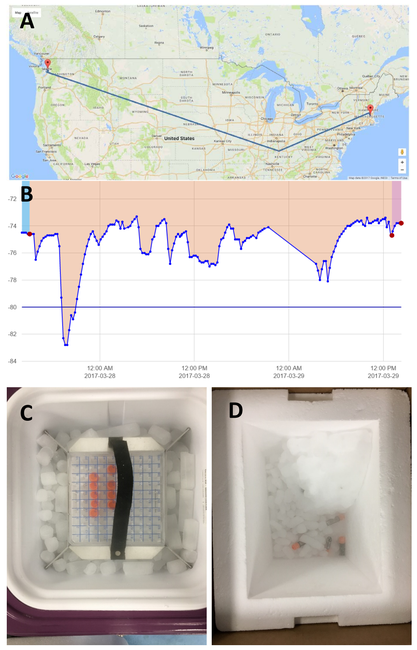


Figure 2: Routing **(A)** and internal payload temperature data **(B)** of CRYO evo shipment from Brooks Life Science Systems to BioLife Solutions. **(C)** The design of CRYO evo maintained a consistent payload temperature throughout the 48 hr cross-country transit despite dramatic fluctuations in ambient temperature **(D)** Current practice for dry ice shipments results in the partial exposure of the product payload to internal air due to the unavoidable physics of dry ice sublimation. This is often exacerbated by frequent and improper package orientation during transport and inadequate containment of the product payload within the container.

Best Practices:

Cryopreservation

The results of this study demonstrated that incorporation of Biopreservation Best Practices, including utilization of an intracellular-like composition for cryomedia, provides important advantages over traditional practice, as evidenced by the observed trend in post-thaw viabilities. Although our statistical analysis did not identify significant differences at this point, we anticipate that more replicates will increase the statistical power of the analysis. CryoStor cryopreservation media is an intracellular-like solution that is specifically designed to provide enhanced protection to cells during cryopreservation. CryoStor is chemically-defined, is devoid of animal/human proteins, and contains a mix of cell permeable and impermeable cryoprotective agents (including DMSO) that synergistically support improved cellular performance post-cryopreservation. Furthermore, CryoStor has a Quality and Regulatory footprint that facilitates inclusion into the manufacturing of cellular therapy and Regenerative Medicine products.

The temperature of storage can significantly impact the stability of samples. Storage at -80°C proved to be detrimental for the stability of the samples that were frozen to liquid nitrogen temperature (-190°C), regardless of the composition of the cryomedia. It has been reported by Kilbride et al. (BioResearch Open Access, 2016, 5 (1) 146-155) that freezing to liquid nitrogen induces ice nuclei formation within the cells. Such nuclei grow and fuse into large crystals when stored above the glass transition temperature of the medium (generally assumed to be around -130°C to -110°C). Our results are in agreement with previous findings. Further studies are in progress to determine the time course of storage at -80°C with and without LN2 plunge.

Shipment Monitoring & Tracking

Biopreservation Best Practices include ensuring sample traceability and temperature stability during shipping. The shipment in the CRYO evo Smart shipper could be tracked with known internal temperature of the payload along with time, GPS location and a record of any unanticipated open events. This information was recorded during shipment so that the sample transportation history and environmental conditions were traceable and could be viewed throughout transportation (**Fig. 2A, 2B**). In contrast, the location of the samples shipped in the EPS shipper could only be obtained from the on-line UPS tracking history (hub locations). There was no record of temperature, location, path, mode of transport, chain of custody, or any indication when or where the package was opened or tampered with. In addition, the CRYO evo Smart shipper design eliminates temperature stratification within the box (**Fig. 2C, 2D**) by ensuring continuous contact between the dry ice and product payload retainer (Fig. 2C). On the other hand, a common condition in dry ice shipping configurations is when the payload does not remain completely surrounded by dry ice on all sides, which can repeatedly expose temperature-sensitive products to temperature fluctuations, potentially reaching temperatures as high as -48°C, when the target temperature is dry ice temperature (-78.5°C).

Storage & 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. Equally important is that during daily interactions with the freezer the thousands of samples that are removed, but not accessed ("innocent" samples) do not warm past Tg during these transient exposures. 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 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 the freezer's temperature monitoring, a validated third party device should also be used with cloud connectivity and alarms.

Conclusions:

- A trend was observed in improving cell viability and metabolic function post-thaw using Biopreservation Best Practices. Further experiments with more replicates will determine statistical significance.
- Long-term (410 days) storage of cells at -190°C (below glass transition temperature) proved to be stable, regardless of the type of cryomedia.
- Storage at above glass transition temperature is detrimental to the fate of the cells post-thaw. Compared to non-shipped controls, long-term storage of cells at -80°C (above glass transition temperature) proved to be unstable, resulting in a drop in viability. More replicates are required to increase the statistical power of analysis.
- The CRYO evo Smart shipper and biologistex™ cloud-based shipment application provide a permanent record of real-time status, tracking and event alarms throughout the entire shipping process, permitting enhanced tracking and knowledge of any excursions as they happen.
- The BioStore III Cryo storage system safely stored the Jurkat T-cells below -190°C prevented unauthorized, unnecessary access, and monitored all activities to ensure no samples ever crossed Tg (-135°C). With full LIMS connectivity, reports and alarms, storage conditions and inventory was available at all times.

Figure 3: ***Left*** Shown in this figure are screen shots from the BioStore III Cryo software demonstrating sample IDs (top), inventory locations (middle), and audit trail of every freezer interaction (Bottom). ***Right*** BioStore III Cryo storage system