Next Generation Technology Procedures and Products Facilitate Biopreservation Best Practices and Increased Viability for Cellular Therapy

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ISCT Singapore 2016 ANNUAL MEETING MAY 25 – 28, 2016

Introduction:

The quality of procedures and products used for preparing, transporting and storing cells at cryogenic temperatures have a direct impact on the post-thaw viability and functionality of the cells. Sub-standard preparation, handling, storage, and products may subject cells to improper cryoprotectant exposure. Inadequate transport packaging and negligent shipping practices can cause variability in product temperatures and unknown transient warming events throughout the handling, storage and logistics chain. This can negatively impact the viability, recovery and functionality of sensitive cells and therapies.

The objective of this study is to compare two methods of preparing, transporting and storing living cells (Cold Chain) to achieve the highest post-thaw viability. One method is intended to show an optimized cold chain and improved best practice, the other is considered current common practice. The outcome of this study recommends best practices for procedures and products to ensure consistency, visibility, and documented control of the cold chain.

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.

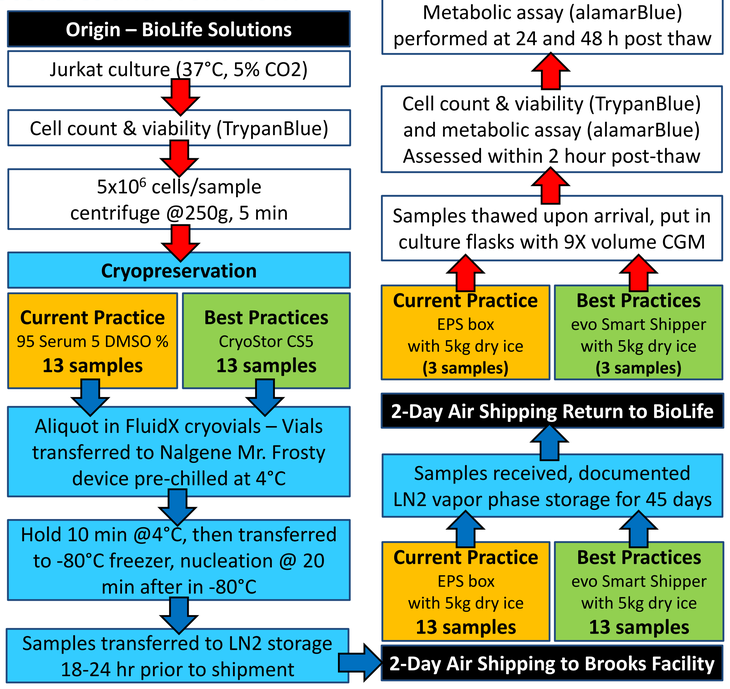
Shipping

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

Storage & Handling

- BioStore™ III Cryo -190°C Storage system
- CryoPod™ carrier
- TempAura™ temperature monitoring

Procedures and Work Flow:



Post-Thaw Testing Methods

Following return transit to BioLife, cryovials were immediately thawed in a 40°C water bath for 2.5 minutes along with reference (non-shipped) controls of both CS5 and 95% FBS/5% DMSO conditions. Post-thaw viability was determined via manual counting (trypan blue exclusion method) using a hemocytometer. Functional viability was assessed using the metabolic indicator alamarBlue (AbD Serotec, Bio-Rad, CA). Briefly, 1.25x10^6 cells were removed from the flask immediately post thaw and also at 24 and 48 hours, and pelleted at 250g for 5 min. 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 every 5 min for 1 hour using a Tecan SPECTRAFluorPlus plate reader (TECAN Austria GmbH, Austria) at 530nm/590nm excitation/emission.

Statistical Analysis

Where indicated, statistical analysis was conducted using 2-way ANOVA with Tukey's post-hoc comparisons (*p=0.0001, #p<0.0001). Data are presented as mean±S.E.M of 3 independent samples (n=5 replicates/sample).

Results:

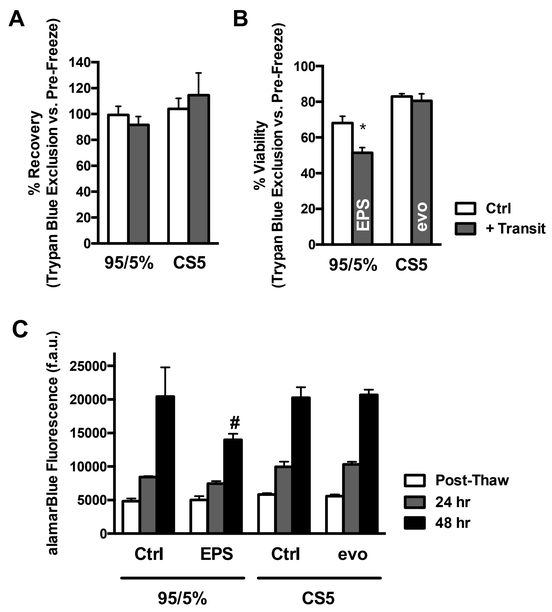


Figure 1. Optimized Cold Chain Management increases cell viability.

Despite similar cell recovery in all samples **(Fig. 1A)**, Jurkat cells in the **DMSO/EPS** group experienced a significant decline in viability immediately post-thaw **(Fig. 1B)** as determined by membrane integrity (Trypan Blue Exclusion). Jurkat cells in the **CryoStor/evo** group did not experience a loss in viability during transport and were statistically similar to non-shipped controls in identical cryomedia (Ctrl). Similarly, Jurkat cells in the **DMSO/EPS** group experienced a significant delay in functional recovery at 48 hr **(Fig. 1C)** as measured by the metabolic indicator alamarBlue. Jurkat cells frozen in the **CryoStor/evo** group experienced a more rapid rise in metabolic function that was identical to non-shipped controls in identical cryomedia (Ctrl) at all time points.

Best Practices:

Cryopreservation

The selection of a cryomedia is an essential component of proper cold-chain management for biologic-based therapies. 'Home-brew' medias composed of various mixtures of cryoprotectants (such as DMSO), sugars, salts, and animal animal/human proteins (including fetal bovine serum and human albumin) have historically been employed for the successful cryopreservation of primary human cells and research cell lines. However, 'home-brew' cryomedia containing animal/human protein introduce the potential for disease transmission to patients and may not be amenable to the Good Manufacturing Practices (GMP) required of biologic-based therapies. CryoStor media is an intracellular-like solution that is specifically designed to provide enhanced protection to cells during cryopreservation. CryoStor media is chemically-defined, devoid of animal/human proteins, and contains a mix of cell permeable and impermeable cryoprotectants (including DMSO) that provide improved cellular performance post-cryopreservation and have a regulatory framework that facilitates inclusion into the manufacturing of cellular therapy/regenerative medicine products.

Shipment Monitoring & Tracking

It is important that samples arrive on time and in known condition. The shipment in the CRYO evo Smart Shipper was tracked with known internal temperature of the payload (cryovials), along with time, GPS location and a record of any unanticipated open events. All this information was automatically sent during shipment so that the entire sample transportation history and environmental conditions were recorded and could be viewed at any time throughout the transport (**Figs. 2A & 2B**). The whereabouts 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 or chain of custody or any indication when or where the package was opened or altered.

The CRYO evo Smart Shipper design and high thermal conductivity components represents a new level of packaging sophistication and understanding of thermodynamics by eliminating temperature stratification, a condition common in dry ice shipping configurations when the product payload does not remain completely surrounded by dry ice on all sides. Continuous contact between the dry ice and product payload retainer ensures uniform distribution of temperature throughout the payload to within ±1°C, regardless of package orientation or quantity of dry ice remaining.

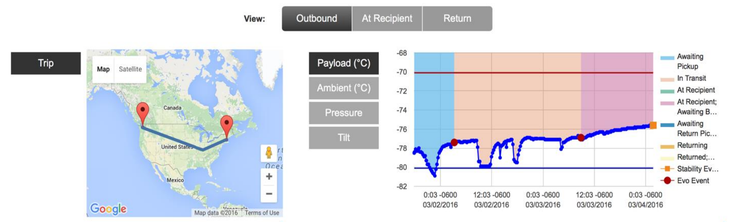


Figure 2A. Routing and internal payload temperature data of evo CRYO shipment from BioLife Solutions to Brooks Automation.

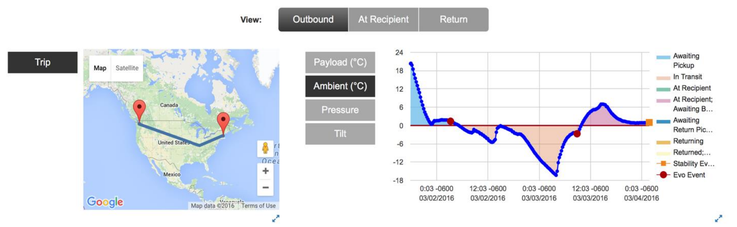


Figure 2B. Routing and ambient temperature data of evo CRYO shipment from BioLife Solutions to Brooks Automation.

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. Such conditions risk repeatedly exposing temperature-sensitive products to fluctuating temperature. In traditional EPS containers, head space increases volumetrically from on-going dry ice sublimation, causing the surrounding internal air space to potentially reach temperatures as high as -48°C. As evidenced in Fig 2, the optimally designed CRYO evo maintained a constant payload temperature throughout the 48hr cross-country transit despite dramatic fluctuations in ambient temperature. It is extremely beneficial to know the condition, status and audit trail of your samples prior to receiving them to ensure their transportation was within all the required specifications, and to catalogue any unwanted events, deviations or unforeseen challenges in the logistics.

Storage & Monitoring

The viability/functionality of cells is maximized when cryopreserved at temperatures below the glass transition temperature of water (Tg) (approximately -135°C). Also important is that any additional cells stored in the same vessel/freezer are not exposed to transient warming events above -135°C at any point during their storage lifetime (protection of innocence). To achieve temperature stability, cells should be stored in high efficiency LN2 vapour freezers at -190°C or colder with controlled access. Sample retrieval and innocent exposures should be monitored, tracked and recorded. Furthermore, should an innocent sample ever be unintentionally warmed above Tg, an indication of the event with regards to who, when and for how long the event occurred should be recorded and the sample owner/researcher notified. Additionally, all storage freezers should have a separate temperature monitoring device installed with email/cloud reporting to verify correct freezer temperature and to facilitate instant alarms of any warming.

Conclusions:

- Jurkat T-cells frozen in CryoStor CS5 were similar to, but exhibited less baseline variability, than reference 'home-brew' cryomedia.
- Jurkat T-cells frozen in traditional 95/5% cryomedia and shipped in an EPS container experienced a significant decline in viability immediately post thaw and a delayed return to function 48 hr post-thaw.
- The combination of CryoStor CS5 and the CRYO evo smart shipper afforded superior protection from cryopreservation and transportation stress with no measurable decline in structural and functional viability as a result of freezing, thawing and two cross-country transit events.
- The CRYO evo smart shipper and biologistex™ cloud-based shipment application allow real-time status, tracking and event alarms throughout the entire shipping process, permitting enhanced tracking and knowledge of any environmental excursions as they happen.
- The design of the CRYO evo smart shipper prevented warming from dry ice sublimation and maintained the Jurkat T-cells within the desired temperature range throughout transit.
- The BioStore III Cryo storage system safely stored the Jurkat T-cells below -190°C, prevented unauthorized access and monitored all activities to ensure no samples ever crossed Tg (-135°C). With LIMS connectivity, reports and alarms, storage conditions and inventory was available at all times.
- The CryoPod carrier extended the cold chain between shipping and storage by keeping the samples below -180°C during cryovial identification and internal transport.
- TempAura enabled remote temperature monitoring and reporting of the freezer's temperature and condition. It functioned as an external device to ensure the freezer's built-in temperature monitoring was accurate.