Ultra-Low Temperature (ULT) Handling and Transport

Protecting valuable biospecimen integrity with a portable, stable -75˚ to -50˚C cooled environment

White Paper

BioCision

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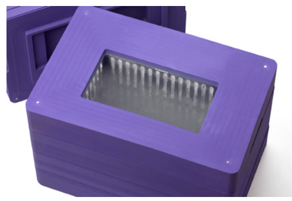
Introduction

The growth of biobanking[1], cell therapy[2], and complex biopharmaceutical therapies like cancer vaccines[3] has created a strong need for products and processes to ensure the stability of temperature-sensitive biospecimens. Proper collection, handling, characterization, packaging and shipping of these materials is critical, as poor sample handling impacts researchers working on discovery with patient samples[4], as well as clinicians treating patients with cutting edge therapies.

Figure 1: BioT™ ULT Transporter



Specifications

Hours of < -50˚C cooling:	> 8 hrs (lid off) > 24 hrs (lid on)
Amount of dry ice required:	12.8 lbs (5.4 kg)
Weight empty:	8.0 lbs (3.6 kg)
Weight with dry ice:	20.8 lbs (9.4 kg)
Working depth:	0-8 inches from floor
Capacity:	8 Standard cryostorage boxes, or 2 CoolCell® freezing containers

The BioT™ ULT Transporter with its patent-pending DIR™ cooling technology provides over 24 hours of below -50°C cooling with only one charge of dry ice.

This white paper describes in detail the BioCision BioT™ ULT Transporter (Figure 1), the first-of-its-kind dry ice-based portable cooling system that maintains a stable -75° to -50°C environment for over 24 hours on one charge of dry ice, enabling reliable handling and transfer of valuable temperature-sensitive biospecimens as part of a larger cold chain standardization process.

The Biospecimen and Biotherapeutic Cold Chain

The cold chain is a system designed to standardize the transport and storage of drugs, vaccines, patient biospecimens, and other biomaterials within the optimal temperature range. For biopharmaceuticals, the cold chain begins when the drug product is manufactured and ends in the clinic at the time of administration to the patient. For biobanking (e.g. of tissues or cord blood), the cold chain begins upon sample collection and ends with delivery to a storage facility or end user.

While particularly critical for biopharmaceuticals, the cold chain is also important, but often neglected, in a research setting. Proper handling of frozen cell and enzyme stocks during storage, sorting, and transfer can have a significant impact on the reproducibility of experimental results.

Amazingly, cold chain-related spending accounts for 80% of the cost of vaccines[5]. For antibiotics, failures in cold chain can result in sub-therapeutic doses that are associated with the development of antibiotic-resistance, a major public health concern[6]. For temperature-sensitive biospecimens, strict temperature control must be maintained to minimize potentially destructive transient warming events that can affect the viability, functionality and efficacy of the drug product or biological sample. Ideally, the cold chain should remain unbroken as any damage to the samples is cumulative and cannot be reversed. It is estimated that defects in maintaining the cold chain cost vaccine companies alone million of dollars each year.[7,8]

As part of the strategic vision to develop products that address gaps in and provide improved standardization of the biopharmaceutical and biospecimen cold chain, BioCision has studied current cold chain procedures and identified several areas of unmet need. These include (1) biopharmaceutical packaging and handling at ultra-low (< -50°C) temperatures, (2) ultra-low temperature freezing and handling of biospecimens at point of collection and during transfer to banks, and (3) biospecimen handling upon receipt to end users and when being handled outside of -80°C freezers.

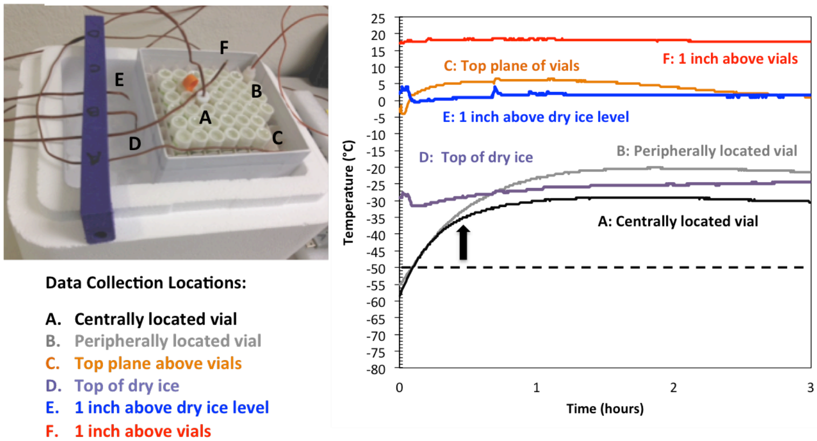
BioT™ ULT Transporter provides a solution for handling biomaterials at ultra-low temperatures (with a target of maintaining less than -50°C) that, as described below, is far superior to current practices for sample handling.

Current Practices Do Not Maintain Samples at Stable Temperatures During Handling

Valuable biospecimens such as patient tissues and serum are carefully stored at -80°C in freezers with complex monitoring and backup systems to provide reliable, consistent low temperatures that reduce the risk of sample damage. However, during sample collection and transfer to freezers or when samples are received and sorted by end users, the common practice is to put the samples in Styrofoam containers or other insulated pans partially or fully filled with dry ice as a means to keep the samples cold.

In order to understand the temperature fluctuations the samples are subjected to during this process, BioCision has examined the temperature profile of samples placed on dry ice in two commonly used non-standardized configurations.

Figure 2: Current Method - Handling Samples Placed on Top of Dry Ice

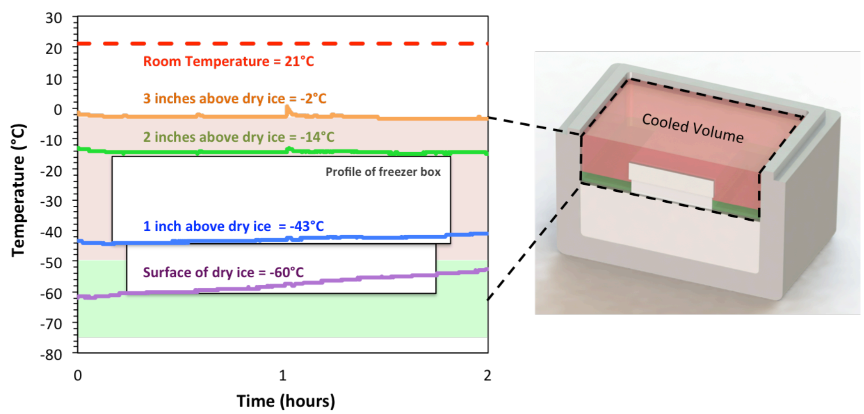


Temperature profile of samples in a cryostorage box removed from a -80°C freezer and placed directly on top of 6 inches of dry ice in a 7-inch deep Styrofoam container. The temperature was measured using thermocouples at the locations shown in the picture. Notice that the box of vials warms to -60°C in the time needed to transfer from the freezer to the Styrofoam box. In addition, in less than thirty minutes (arrow) the vials have reached approximately -35°C, which is a total of *45°C warming* since being removed from the freezer.

Handling Samples Sitting on Top of Dry Ice. A standard cryostorage box of samples removed from a -80°C freezer and placed on top of a large volume of dry ice (Figure 2) is commonly used as a means to confirm sample receipt or cherry pick samples for testing. Unfortunately, thermocouple measurements of temperature in and around test vials shows that this procedure leads to very rapid sample warming: within 30 minutes, the vials have warmed from -80°C to over -40°C while the top of the vials are above 0°C. Even the temperature measured at the surface of the dry ice is only -30°C due to mixing with room temperature air. Clearly, under these conditions, samples are detrimentally exposed to rapid warming that is practically akin to a freeze/thaw cycle.

Handling Samples Placed Deeper in a Box of Dry Ice. A more standardized method of handling samples at ultra-

Figure 3: Current Method – Placing Samples Deeper In Styrofoam Box on Dry Ice

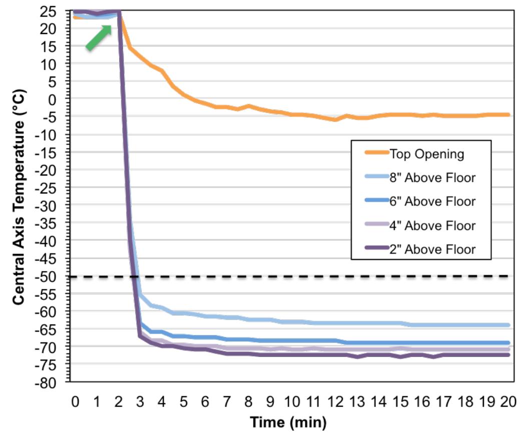


Temperature profile in the "Cooled Volume" of a 7 inch deep Styrofoam container filled with 3.5 inches of dry ice. After equilibrating for 20 minutes, the temperature was measured using thermocouples at the dry ice surface, 1, 2, and 3 inches above the dry ice over 2 hours. The temperature falls out of desired -75 to -50°C region (green shading) less than 1 inch above the dry ice. The profile of a 2 inch tall freezer box is shown for comparison.

low temperatures involves filling a Styrofoam box approximately half full with dry ice and placing the samples on top of the dry ice inside the box. The remaining "empty" volume of the box is cooled by the dry ice vapor and potentially provides better temperature stability during sample handling. As shown in Figure 3 at steady state, the temperature profile in the cooled volume of the box based on thermocouple measurements is lower compared to the box full of dry ice in Figure 2, but surprisingly, the target -75° to -50°C temperature region only exists for 0.6 inches (1.5 cm) above the dry ice. At 3 inches (7.6 cm) above the dry ice, the temperature is almost 0°C. The top of a hypothetical freezer box placed inside would be at -14°C, again subjecting samples to large temperature variation during handling or transfer.

Overall, these results demonstrate the use of Styrofoam boxes with dry ice does not maintain samples at a stable ultra-low temperature near that of dry ice (-78.5°C) and, furthermore, exposes the samples to significant warming that could alter their properties in unknown ways. As shown below, the solution is to use the BioT™ ULT Transporter, which provides a deep, stable, ultra-low temperature environment for sample handling, transport and storage that lasts over 24 hours.

Figure 4: Rapid Cooling of BioT™ ULT Transporter After Addition of Dry Ice

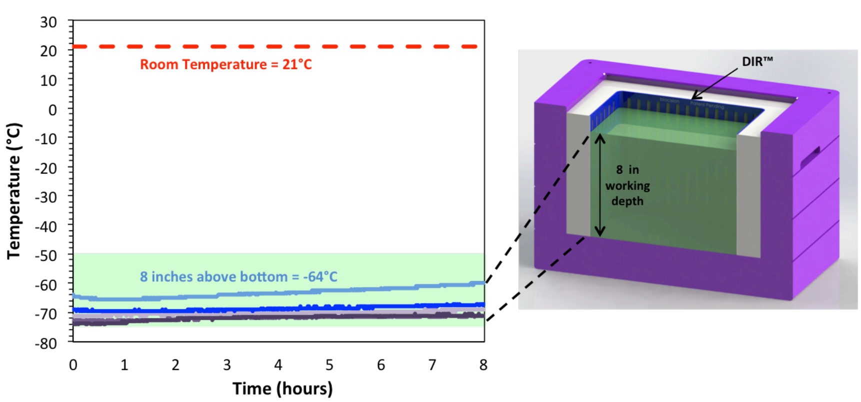


The central axis temperature profile in BioT™ ULT Transporter measured using thermocouples after addition of dry ice (without lid). Temperature readings were taken every 30 seconds at 2 (dark purple), 4 (light purple), 6 (dark blue), 8 (light blue) inches, and at the top opening (orange). Stable working temperature is reached in < 5 min. Dashed line shows -50°C. Dry ice is added at 2 min (green arrow).

BioT™ ULT Transporter

The BioT™ ULT Transporter (Figure 1) is a light-weight, insulated transporter that uses dry ice to create a deep and stable -75° to -50°C temperature environment due to the patent-pending DIR™ cooling technology developed by BioCision scientists. In addition to efficient cooling, the insulated housing and DIR™ insert minimize the amount of dry ice required for ultra-low temperature cooling, while also ensuring operator safety and comfort by positioning the dry ice against the rear and side walls of the BioT™ ULT Transporter. The exterior of the system will not be cold to the touch even when the system is loaded with dry ice and frozen materials. This configuration also leaves the central cavity open and accessible (as shown in cut-away view in Figure 5).

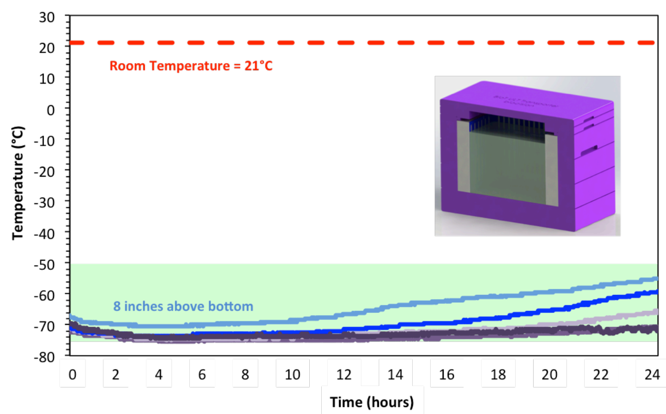
Figure 5: Temperature Profile in BioT™ ULT Transporter (No Lid)



Steady state temperature profile measured with thermocouples in the central axis through the 8 inch working depth of the BioT™ ULT Transporter. Temperature readings were taken for 8 hours at 1 (dark purple), 2 (plum), 4 (light purple), 6 (dark blue), and 8 (light blue) inches above the bottom. Desired -75° to -50°C region shown with green shading.

Mechanism of DIR™ Insert-Mediated Cooling. The DIR™ cooling insert fuels the BioT™ ULT Transporter cavity by a very efficient process known as cold convection. Cold CO_2 gas generated by sublimation of the dry ice is denser than the warmer air inside the working cavity of the BioT™ ULT Transporter. As a result, the cold CO_2 gas will settle toward

Figure 6: Temperature Profile in BioT™ ULT Transporter (With Lid)



Steady state temperature profile measured using thermocouples at the central axis through the 8 in working depth of the BioT™ ULT Transporter with the lid on. Temperature readings were taken for 24 hours at 1 (dark purple), 2 (plum), 4 (light purple), 6 (dark blue), and 8 (light blue) inches above the bottom. Desired -75 to -50°C region shown with green shading.

the cavity floor. This movement creates a downward convective draft through the dry ice in the DIR™ cooling insert to the cavity floor, then inward toward the central portion of the cavity. Because the DIR™ cooling insert holds dry ice at the rear and side walls of the cavity, the gas flow converges and upwells near the center front of the cavity. This convective cooling process allows the BioT™ ULT Transporter to reach working temperature in less than 5 minutes even when the lid is off (Figure 4) and stay at ultra-low temperature for hours.

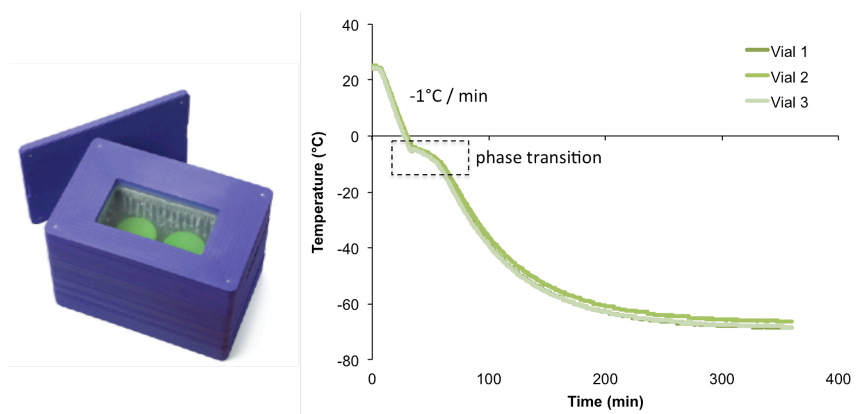
At steady state, the entire 8-inch working depth is close to the temperature of the dry ice (-78.5°C, Figure 5), and as a result, the convection rate will slow. However, the convective flow will persist in the same pattern as long as there is environmental heat influx into the BioT™ ULT Transporter cavity (i.e. when being used without a lid). Thus, ultra-low temperatures can remain stable for hours.

Effective Hours-Long Cooling. Due to the effectiveness of the DIR™ cooling technology, thermocouple temperature measurements at the central axis of the cooled cavity show that the entire 8-inch (20.3 cm) working depth of BioT™ ULT Transporter is maintained within the target -75° to -50°C temperature range for up to eight hours even with the lid off (Figure 5). With the lid on, and exposure to ambient temperatures minimized, BioT™ ULT Transporter maintains temperatures below -50°C for up to 24 hours (Figure 6).

Applications

Because BioT™ ULT Transporter rapidly reaches ultra-low working temperature and can maintain those temperatures for over 24 hours, it is ideally suited for several applications related to improving cold chain handling of critical temperature-sensitive biospecimens. These include (1) sorting or other short-term handling of biospecimens outside a -80°C freezer; (2) controlled temperature transfer of frozen biospecimens between

Figure 7: Controlled-Rate Freezing of Samples Using a BioT™ ULT Transporter



Freezing rate profile of samples in a CoolCell® placed into the BioT™ ULT Transporter. Three vials filled with cell freezing medium (10% DMSO, 20% FBS, 70% DMEM) were equipped with thermocouples (as in Figure 1) to measure their temperature and put in a CoolCell® LX freezing container separated by 3 filler vials so that all 12 positions were filled. Temperature was monitored over 6 hrs. The temperature decrease is virtually identical to that of a CoolCell® placed inside a -80°C freezer and shows the usual -1°C / min drop until approximately -40°C with the typical phase transition plateau highlighted.

laboratories or collection and processing sites; (3) transfer of temperature-sensitive biologics and other biological samples to clinical sites; and (4) freezing samples (e.g. locally at a collection site and/or on the bench-top) in conjunction with CoolCell® controlled-rate cell freezing containers and CoolRack® snap freezing modules.

Example: Bench-Top or Portable Passive Cell Freezing Using the BioT™ ULT Transporter and CoolCell® Freezing Containers. Due to the BioT™ ULT Transporter ability to maintain approximately -75°C for many hours, it provides an ideal portable environment for freezing valuable biospecimens prior to transfer to more permanent LN_2 vapor phase storage. The freezing profile of cell samples within a CoolCell® container placed in the BioT™ ULT Transporter (Figure 7) shows nearly identical performance to a CoolCell® container placed inside a -80°C freezer[9]. Importantly, the freezing rate of the vials using this configuration shows the desired -1°C/min rate that has been found to maximize cell viability post-thaw. Thus, the BioT™ ULT Transporter coupled with CoolCell® controlled-rate freezing containers can replace expensive and hard-to-maintain programmable freezers (i.e. controlled rate freezers) as a bench-top, or even portable, solution for highly reproducible passive cell freezing.

Summary

BioT™ ULT Transporter is the first-of-its-kind portable ultra-low temperature transporter that maintains a -75° to -50°C working environment for hours with a minimal amount of dry ice. With BioT™ ULT Transporter, burying boxes or individual samples in dry ice is no longer necessary. BioT™ ULT Transporter allows researchers and health care professionals to easily sort, transfer, freeze, or otherwise manipulate valuable biospecimens in an ultra-low temperature environment that minimizes thawing, inadvertent freeze/thaw cycles and potential loss of integrity, while also allowing easy access and handling of samples.

About BioCision

BioCision is a life science research and development company that develops products and solutions for process standardization throughout the healthcare industry through the application of advanced thermal regulation principles and technologies. The intuitive design and interconnectivity of BioCision products enables researchers, clinicians and manufacturers to protect the integrity of temperature-sensitive therapeutics, biological samples, and biospecimens. By comprehensively addressing temperature stability, BioCision strives to improve the success of therapeutic discovery and development, and enable effective care delivery. Scientists worldwide in the pharmaceutical, biotechnology, and biobanking industries use BioCision products to improve their workflow efficiency and the quality of their products.

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