Controlled-rate cell freezing and thawing are crucial to optimizing cell survival and functionality, and a reproducible, standardized process is necessary when operating in a controlled or regulated environment. It is incumbent upon operators in cGMP and other regulated environments to identify and adopt tools and procedures that minimize variability and subjectivity at every possible point in the process.

**Integration of CoolCell® and ThawSTAR™ Technologies into a Manufacturing Process - UCSF**

BioCision’s CoolCell® cryopreservation systems and ThawSTAR™ automated cell thawing systems are engineered to significantly reduce variability and subjectivity at many points in the cell processing workflow. The economical and intuitive solutions can be integrated into regulated workflows with relative ease (Figure 1).

**Fig. 1 Cell Cryopreservation (snowflake icon) and thawing (melting ice cube icon) steps in the manufacturing of regulatory T cells (Tregs) for use in transplant rejection. CoolCell® controlled-rate cell freezing containers and ThawSTAR™ transport and thawing system were utilized for freeze-thaw steps in this manufacturing process.**
Controlled-Rate Cell Freezing

The current recommendation for freezing stem cells, primary cells, PBMCs and cell lines is that they be cooled gradually to approximately -80°C at a controlled rate of -1°C per minute. This can be achieved using a CoolCell® alcohol-free controlled-rate cell freezing container in conjunction with a -80°C freezer.

CoolCell containers accommodate cryogenic vials and injectable ampules.

Comparable to a Controlled-Rate Freezer (CRF)

UCSF Division of Transplant Surgery adopts CoolCell® containers over controlled-rate freezers. UCSF Division of Transplant Surgery integrated CoolCell controlled-rate cell freezing modules into their allo-antigen reactive (ar) regulatory T-cell (Tregs) manufacturing process and found the cell recovery and viability comparable to a controlled-rate freezer (Figure 2).

CoolCell® containers
- are less expensive
- do not contain liquid
- can be acclimated quickly for fast turn-around
- are transportable

Proven GMP Compatibility

TxCell integrates CoolCell® controlled-rate containers into their Treg clinical trial. In a feasibility study for their Ovasave® clinical trials, France-based TxCell, SA determined CoolCell cell freezing containers performed equivalent to a controlled-rate freezer (CRF) for the development of their cell therapy products (Figure 3). CoolCell containers are adaptable to a GMP environment (Figure 4), considerably easier to deploy to clinical sites, more cost-effective and require no maintenance, and were adopted over a CRF for the Ovasave phase 2b trial.

Fig 2. CoolCell performance compared to a controlled-rate freezer. Multiple leukapheresis-like products were processed into PBMCs and frozen using either a CoolCell or controlled-rate freezer (CRF). In both cases, cells were frozen at approximately 108 cells per mL in CryoStor® CS10. Vials were stored in liquid nitrogen for approximately 2-3 weeks and thawed the same day using a water bath. CoolCell and CRF methods were comparable in both recovery and viability.

Fig 3. Effects of freezing on antigen-specific Treg (Ag-Treg) cell therapy products. Ag-Tregs (n = 6) were frozen at a concentration of 1 to 10 x 10^6 cells/mL and a freeze rate of -1°C/min using the CoolCell system or programmable freezer; viability and absolute viable cell count of thawed Ag-Treg cell therapy products were evaluated by flow cytometry. The CoolCell freezing method was comparable to the CRF method.

Fig 4. Particle release performance data
Particle release performance was tested three times on three different CoolCell freezing containers (nine measurements). Upper line on each graph indicates maximum acceptable particle count for each particle size measured. CoolCell cell-freezing containers are suitable for use in an active GMP cleanroom.

-Data generated by UCSF Division of Transplant Surgery
-Data generated by TxCell, SA
Thawing cells in a regulated environment presents many challenges and the need to minimize variability through standardization is crucial. Ice recrystallization during thawing is a commonly observed phenomenon, making a rapid and controlled rate of thawing just as important as a controlled rate of freezing in retaining optimal cell survival and function. ThawSTAR™ cell transport and automated thawing system ensures the thawing process is standardized from the point of vial retrieval through hood-based thawing.

### Problem:
Water Baths and Other Manual Methods
- User dependent
- Contamination risk
- Variable end point
- Difficult to use in a GMP environment
- Not standardized

### Solution:
ThawSTAR™ Transport and Automated Thawing System
- Standardizes from vial retrieval through thawing
- Equivalent or better cell viability and recovery compared to water bath
- More consistent and reproducible results
- One-push operation can be performed in a hood

### ThawSTAR™ System Outperforms Water Bath Method

The ThawSTAR cell transport and automated thawing system has been shown to increase post-thaw cell recovery and viability and improve consistency, as the data below indicate. (Figure 5, Figure 6).

![Figure 5 Ex-vivo stimulated B cells](image)

![Figure 6 Mean viability (left figure) and mean recovery (right figure)](image)

- Data generated by UCSF Diabetes Center and Transplant Surgery

### Highly Reproducible Automated Thawing

The ThawSTAR automated cell thawing system takes the guesswork and subjectivity out of cell thawing. Engineered to mirror a “perfect” water bath thaw, the ThawSTAR system delivers a vial thermal profile comparable to that achieved in a water bath (Figure 7). The system also delivers consistent and highly reproducible thawing endpoints (Figure 8).

![Figure 7 ThawSTAR system delivers comparable vial thermal profile](image)

![Figure 8 Highly reproducible automated thawing](image)

- Data generated by Blood Systems Research Institute

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**Controlled-Rate Cell Thawing**

- 37°C Water Bath
- ThawSTAR™

- Same ThawSTAR unit tested on 5 days
- 3 ThawSTAR units tested on same day

3 ThawSTAR units tested on same day
99% conf. interval of water bath thaw time

- Fresh
- Cryopreserved
- Fresh
- ThawSTAR

Same ThawSTAR unit tested on 5 days

- 5 days
- 2-way ANOVA with post hoc Sidak test

- Thaw STAR
- 99% conf. interval of water bath thaw time

Thawing is a process that involves...
ThawSTAR™ System IQ/OQ/PQ Validation

To comply with government agencies such as the US FDA, a complete Installation Qualification (IQ) and Operational Qualification (OQ) is available for the ThawSTAR™ CFT Transporter and ThawSTAR automated cell thawing instrument. A general guideline for Performance Qualification (PQ) is also included. Please contact us at thawstar@biocision.com and let us know how we can assist you with integrating the ThawSTAR cell thawing system into your regulated workflow.

Cold Chain Management

CryoPod™ Carrier is a liquid nitrogen (LN2)-based solution that provides safe, reliable and portable < -150°C cryogenic transport for biospecimens for over 4 hours. The instrument displays and logs temperature, date and time, and features audible and visual alarms. It also integrates into an optional automated filling station, allowing hands-free handling and replenishing the LN2 charge in less than 15 minutes.

Ensures operator safety
- Designed to allow safe and quick transportation of cryogenic samples
- Hands-free auto-fill option

Maintains sample cold chain integrity
- Temperature display with audible and visual alarms
- Temperature logging and retrieval

Delivers reliable performance
- Over 4 hours < -150°C
- No direct sample contact with LN2

Portable
- Compact footprint; only ~18 lbs
- Built-in handle and bottom finger grips

BioCision offers handling and transport solutions for cold chain management at different temperatures:

<table>
<thead>
<tr>
<th>Product</th>
<th>Temperature Range</th>
<th>Duration</th>
<th>Temperature Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>CryPod™ Carrier</td>
<td>-180°C to -150°C</td>
<td>&gt; 4 hours</td>
<td>LN₂</td>
</tr>
<tr>
<td>BioT™ ULT Transporter</td>
<td>&lt; 70°C to -50°C</td>
<td>8 hours (lid open), 24 hours (lid closed)</td>
<td>Dry Ice</td>
</tr>
<tr>
<td>BioT™ Carrier</td>
<td>2°C to 8°C</td>
<td>8 hours</td>
<td>Rechargeable battery</td>
</tr>
</tbody>
</table>

Standardize your workflow.

BioCision products standardize points of variability throughout many temperature-sensitive workflows. Our economical and easy-to-implement solutions will instantly standardize your sample prep, freezing, transporting, storing and thawing workflows. Learn more at www.biocision.com.

The products noted above are for laboratory research use only. Any intended use for diagnostic purposes, direct transfusion, or in the production of therapeutic product(s) or vaccines(s) may require advance regulatory clearance which is the sole responsibility of the user, as this is not a medical device that has undergone medical device registration, clearance, or approval by the U.S. Food and Drug Administration (FDA), European Union, Health Canada, or the Australian Therapeutic Goods Administration. Research Only Device: Limited by Federal Law (United States) to Research Use Only.