Summary of cryotechnology equipment and consumables for cell therapy

Introduction

Somatic cell therapy medicinal products, a sub-category of advanced therapeutic medicinal products (ATMPs), consists of live cells or tissues intended to provide a therapeutic benefit to patients. Both starting material (SM), used to manufacture these ATMPs, and final product (FP) contain live cells. These live cells are often preserved by cryopreservation and stored at temperatures < -120°C to prolong their shelf life and to allow flexibility around logistics and scheduling. Cryopreservation provides increased time at some key points of the ATMP manufacturing workflow, such as for the transport of SM and/or FP between clinical and manufacturing sites, for quality release testing, etc. Cryopreservation also allows greater scheduling flexibility. This is seen both in terms of starting the manufacturing workflow of the ATMP at the manufacturing site (or putting manufacturing on hold, if needed), and in administering the therapy at the clinical site.

Cryopreservation of ATMP SM and FP requires the application of optimised cooling rates in order to maximize the recovery of live and active cells post-thaw. Passive freezing units are a less expensive option to achieve cooling rates close to the optimum, but they are limited to cryovials (not appropriate to freeze samples in cryobags). Additionally, passive freezing devices provide no control and no logging of the freezing process. On the contrary, controlled-rate freezers (CRFs) are more versatile in terms of compatibility with various types and sizes of samples; they enable a tight control of the cooling profile and provide a record of the cooling process. Historically, cryogens such as liquid nitrogen (LN_2) or methanol were used to deliver the desired cooling profile in CRFs, until LN_2-free, cryogen-free CRFs were developed in the last decade or so. These LN_2-free CRFs use electricity and Stirling engine cryocoolers to cool samples down at a controlled rate.

Although still widely used, there are indeed some concerns associated with the use of LN_2. Specifically, using LN_2 poses safety hazards (i.e., cold burns, asphyxiation, etc), can prove to be costly as there is possibly limited availability of LN_2 infrastructure at some manufacturing/clinical sites, and is nonsterile in nature.

Cryopreserved ATMPs are typically stored in large cryogenic tanks in the vapor phase of LN_2 and transported in dry shippers: vessels internally coated with a porous zeolite material in which LN_2 vaporizes. The presence of LN_2 vapor is what keeps samples in cryogenic storage tanks and dry shippers cryogenically cold, slowly venting over time. Cryogenic storage tanks are therefore constantly monitored and automatically refilled with LN_2, while dry shippers last until all the LN_2 vapor has vented. A cryogenic, LN_2-free alternative to dry shippers has also recently been developed using Stirling engine cryocoolers.

Before use of a cryopreserved ATMP SM or FP, thawing will typically be performed by immersion of the sample(s) in a prewarmed water bath. This practice can be quite cumbersome as it requires

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cleaning the water bath between each use and filling it with sterile water to limit contamination risks. Additionally, water baths are difficult to standardize and do not provide a record of the thawing process. Water-free and partly water-free thawing units are available on the market and address these limitations to achieve thawing of cryopreserved ATMP SM or FP in a more controlled and standardized way, as well as being less prone to waterborne contamination.

Below, is a nonexhaustive list of the main equipment and consumables available in the cell therapy space as of May 2022.

Equipment and consumables available

Freezing containers

Table 1. Overview of available freezing containers

Range name	Manufacturer	Hermetically sealed cryovials	Cryobags*	Cryostraws
AT-Closed Vial	Aseptic Technologies	X		
CellSeal Clear Access Closed-System Cryogenic Vial	Sexton Biotechnologies, BioLife Solutions	X		
Daikyo Crystal Zenith vials	West Pharmaceutical	X**		
Aramus single-use bags	Entegris		X	
Blood cryoconservation bag	Valmed s.r.l.		X	
CellStor freezing bags	CellBios Healthcare and Lifesciences Pvt. Ltd		X	
Cell Freeze freezing bags for cryogenic storage	Charter Biomedical		X	
Cord blood freezing Bbag	Pall Corporation		X	
CryoMACS freezing bags	Miltenyi Biotec		X	
CryoStore freezing bags	Origen Biomedical		X	
EVA bags and kits for cell freezing and storage	Macopharma		X	
KryoSure cryopreservation bags	Saint-Gobain		X	
SAFE2 lines of bags	ADVATIS		X	
CBS straws	Cryo Bio System, IMV Technologies			X

** Cryobags are typically inserted in cryo-cassettes – also called canisters – before cryopreservation to ensure they are frozen flat and improve thermal transfers. There is a wide variety of cryo-cassettes/canisters available from various manufacturers (Thermo Fisher Scientific, BioLife Solutions, Air Liquide, Aviamax, etc.)*

*** not hermetically sealed cryovials but made of cyclic olefin polymer which presents a lower risk of loss of container closure integrity at cryogenic temperatures compared to standard cryovials (made of polypropylene)*

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Freezing technology

Table 2. Overview of available freezing technology (CRF: controlled-rate freezer)

Range name	Manufacturer	Passive freezing containers	LN_2 CRF	Other cryogen based CRF	LN_2-free, cryogen-free, CRF
Thermo Scientific Mr. Frosty Freezing Container	Fisher Scientific	X			
Corning CoolCell cell freezing vial containers	Fisher Scientific	X			
Digitcool freezers	IMV Technologies		X		
High-capacity rate freezers	BioLife Solutions		X		
Kryo controlled rate freezers	Planer PLC		X		
Thermo Scientific CryoMed controlled- rate freezers	Thermo Fisher		X		
BioCool controlled rate freezers	SP Scientific Products			X (methanol)	
CRF-1 liquid nitrogen and cryogen free controlled freezer	Grant Instruments				X
CYTO Sensei controlled-rate freezer	Strex				X
VIA Freeze™ controlled-rate freezers	Cytiva				X

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Cryogenic storage technology and storage services

Table 3. Overview of available cryogenic storage technology and storage services

Range name	Manufacturer	Monitoring system	Automated sample management
Bio Series and Biosystem Series	Statebourne		
MVE HEco series liquid nitrogen freezers, MVE Fusion Freezer and MVE Variō series	Chart Industries		
S Series liquid nitrogen freezers and isothermal freezers with patented LN_2 jacket technology	BioLife Solutions		
CryoExtra high-efficiency cryogenic storage systems	Thermo Scientific	X	
GT, Arpege, Espace, and RCB cryo-preservation vessels (small, mid-sized, large, and extra-large, respectively) for long-term biological samples storage	Cryopal, Air Liquide [MD]	X (CryoMemo)	
BioStore -190°C LN_2-based fully automated storage systems	Azenta Life Sciences*	X	X

** formerly Brooks Life Sciences; 'MD' stands for medical device.*

Cryogenic shipping technology

Table 4. Overview of available cryogenic shipping technology

Range name	Manufacturer	Dry vapor shipper	Liquid nitrogen-free shipper	Smart/cloud-connected shipper
Arctic Express transport systems	Thermo Scientific	X		
Biotrek Series	Statebourne	X		
DS-3 LN_2 dry shipping container	Biolife Solutions	X		
MVE cryoshipper series and the Doble series	Chart Industries	X		
Voyageur dry shipper containers for sample storage and transport	Cryopal, Air Liquide	X [MD]		
YDH liquid nitrogen dry shippers	Haier BioMedical	X		
CryoPod Carrier*	Azenta Life Sciences**	X		
evo DV10 LN_2 Shipper + evo.IS software	Savsu Technologies, BioLife Solutions	X		X
VIA Capsule™ system and Chronicle™ software	Cytiva		X	X

** LN_2 vapor-based portable hand carrying solution ** formerly Brooks Life Sciences; 'MD' stands for medical device*

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Thawing units

Table 5. Overview of available thawing technology

Range name	Manufacturer	Water bath	Partly water-free	Fully water-free	For cryo-bags	For cryo-vials	Medical device
CytoTherm plasma thawers	CytoTherm LP	X			X		
QuickThaw plasma thawing systems	Helmer Scientific	X			X		
Barkey plasmatherm	Barkey		X		X		X
CytoTherm dry plasma thawers	CytoTherm LP		X		X		
Plasma thawers	KW Apparecchi Scientific		X		X		X
SmartThaw systems	CPSI Biotech		X		X	X	
Sahara warming/thawing systems	Sarstedt			X	X		X
VIA Thaw™ dry automated thawers	Cytiva			X	X		X
CellSeal automated thawing system	Sexton Biotechnologies, BioLife Solutions			X		X	
ThawSTAR thawing systems	BioLife Solutions			X	X	X	

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Concluding remarks

Over the recent years, LN_2-free technology has emerged in the cryopreservation space. Whether this emergence was for safety reasons — as LN_2 is associated with risks of cold burns, asphyxiation, and explosion; for environmental and cost-saving purposes — the carbon footprint and running costs of using the electrically-powered VIA Freeze™ Quad system versus an LN_2-based system are minimal[1]; or other reasons, LN_2-free controlled rate freezers and most recently an LN_2-free cryogenic shipper (The VIA Capsule™ shipper from Cytiva) were developed.

Though cryogenic storage technology hasn't drastically evolved since the development of the first LN_2 tanks, the monitoring systems associated with this technology for the control and refill of liquid nitrogen has been refined.

Cryobags are the typical cryogenic containers for cell therapies SM and FP; however, hermetically sealed cryovials have emerged more recently. These hermetically sealed cryovials present the advantage of being more robust to fracture than cryobags and can contain volumes ranging from a milliliter or less to several tens of milliliters.

When it comes to thawing, automated, water-free, or partly water-free devices are now widely available and used in the cell therapy space. Moving away from operator-dependent, prone to contamination water baths indeed helps reduce risks to the therapeutic product, and ultimately to the patient.

All of these new systems also tend to be digitally connected to allow remote control and monitoring of collected data in real time. For instance, the Chronicle™ software from Cytiva — a GMP manufacturing automation software — was developed for this purpose. The benefits of this software are manyfold. Specifically, one benefit is tracking and traceability: helping to ensure unbroken chains of identity (COI) and custody (COC) throughout the manufacturing operations and supply chain logistics of therapeutic products.

[1] In the Cytiva white paper "Going liquid nitrogen-free for low-impact cryopreservation," we demonstrate that switching to an LN_2-free VIA Freeze™ Quad system could reduce carbon emissions by 87%, as well as reduce the average running cost by 97% compared to an LN_2-based system over a 10-year period: https://www.cytivalifesciences.com/solutions/cell-therapy/knowledge-center/resources/cryopreservation-co2-impact

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