

# **BIOREPOSITORIES: INDUSTRY SOLUTIONS TO ESTABLISHING BETTER BIOBANKS**

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# Cryopreservation: Introduction

## Important points to consider

- What is to be stored?
- How many samples are to be stored?
- What is the expected duration of storage?
- What properties are the retrieved samples required to possess?
- Are there packaging requirements in addition to those dictated by the cryopreservation process?

**'Current Frontiers in Cryobiology'**  
**Precision in Cryopreservation**  
- **Equipment and Control**  
Stephen Butler, David Pegg



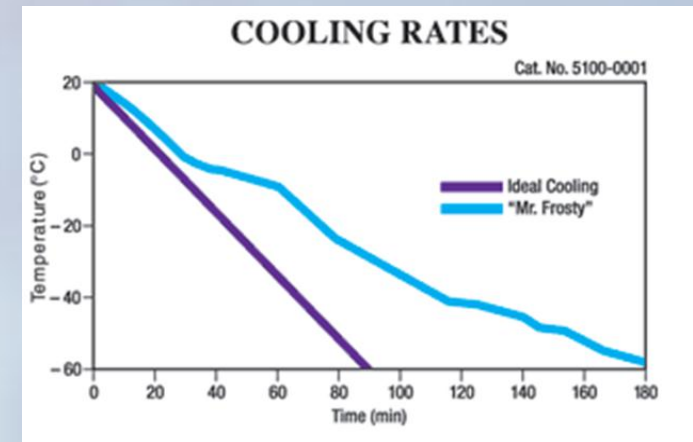


## Freezing of samples



### Freezing in mechanical freezers

- Used for most robust cells
- Low concentration of cryoprotectant
- Cells are dessicated slowly during the cooling process.
- Variations of temperature within refrigerator
- Ease of access



**"Mr Frosty"** Uses 100% isopropyl alcohol and mechanical freezer  
Able to achieve  $-1^{\circ}\text{C}$  /minute cooling rate

# ARCTIKO

The art of simplicity<sup>®</sup>



## Freezing of samples

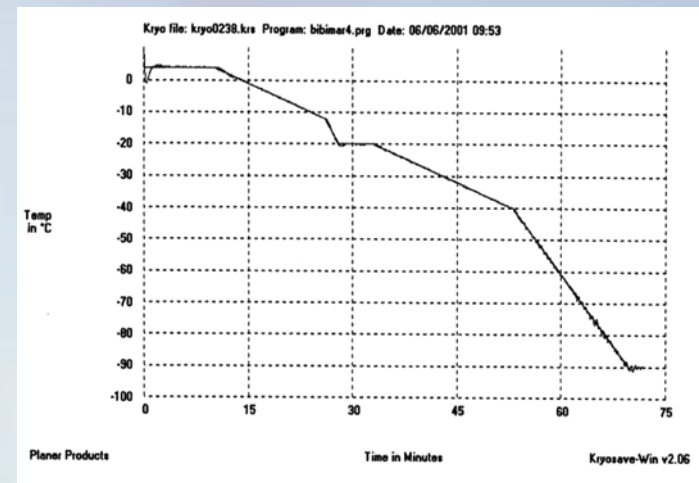


### Controlled rate freezing

- Used routinely for sensitive cells
- Very accurate cooling profile of temperature and time
- Reproducible and audit trail
- Uses liquid nitrogen and laminar airflow
- Small and large volume freezing



PLANER





## Freezing of samples



### Vitrification

- Used for robust cells
- Generally used in IVF (sperm and embryo)
- Highest concentration of cryoprotectant
- Uncontrolled cooling

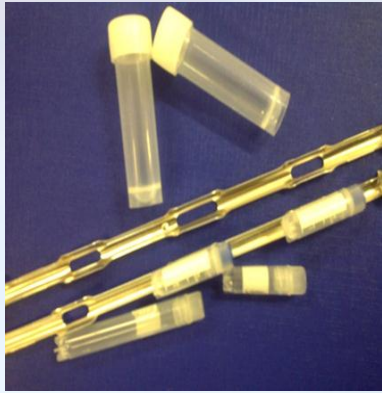




# Freezing of samples



## Freezing of samples in final storage container



### Cryo tubes/ vials

1ml -15mls

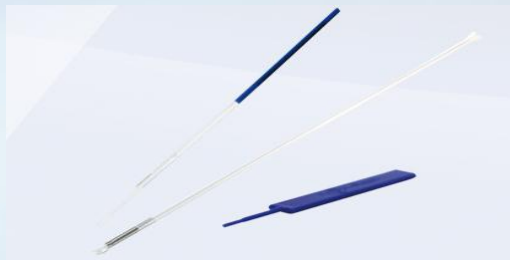
### Freezing bags

5ml -750mls



### Straws

0.3ml – 0.5ml



## Packaging Considerations for Biopreservation

Transfus Med Hemother. 2011 April; 38(2): 149–156.

Published online 2011 March 16. doi: [10.1159/000326083](https://doi.org/10.1159/000326083)

PMCID: PMC3088737

[Erik J. Woods](#)<sup>a,b,\*</sup> and [Sreedhar Thirumala](#)<sup>a</sup>



## Storage Temperature

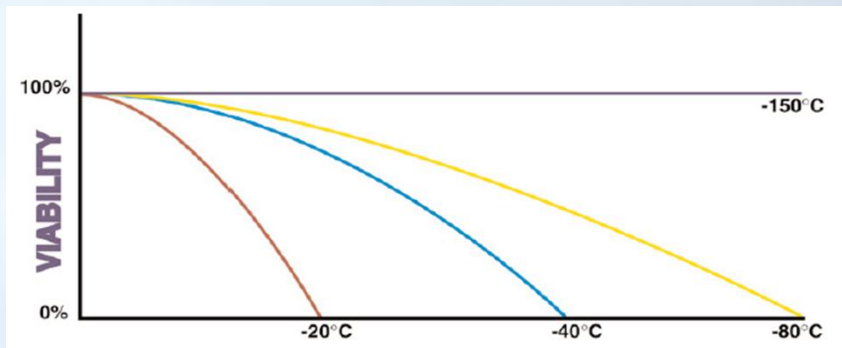
**“If you don't treat the sample properly, it can limit what you can do,”**

**“Researchers think 'freezing is freezing',”**

- but a typical freezer is not cold enough to stop degradative enzymes.

Most samples can be stored at  $-80\text{ }^{\circ}\text{C}$ ,  
but certain specimens, such as live cells,  
need to be kept at temperatures close to  $-200\text{ }^{\circ}\text{C}$ ,  
at which point enzymes are thought not to be able to function at all

Kristin Ardlie, Director of the Biological Samples Platform at the Broad Institute in Cambridge, Massachusetts.



*"The published temperature for the glass transition point varies slightly from researcher to researcher but is generally regarded as being  $-132\text{ }^{\circ}\text{C}$ ."*



## **Liquid nitrogen or vapor storage**

### **Liquid storage**

- **Concerns about safety**
- **Mainly IVF**
- **Storage dewar filled with liquid nitrogen**

### **Vapor storage**

- **Safe**
- **Storage dewar has platform and samples stored above in vapor**
- **Validated minus 190 storage throughout dewar**





# Safety in Liquid Nitrogen storage

## HAZARDS OF LIQUID NITROGEN CRYOVIALS

Edinburgh University <http://www.ucl.ac.uk/medicalschoo/msa/safety/docs/cryovials>



### Catastrophic failures of freezing bags for cellular therapy products: description, cause, and consequences.

[Khuu HM](#), [Cowley H](#), [David-Ocampo V](#), [Carter CS](#), [Kasten-Sportes C](#), [Wayne AS](#), [Solomon SR](#), [Bishop MR](#), [Childs RM](#), [Read EJ](#).

#### Source

Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, MD 20892, USA.





## Contamination in storage

*"There have been several documented cases of cross contamination in liquid storage, and although quarantine is an option, this only accounts for pathogens we know to look for now."*

### RISKS ASSOCIATED WITH LIQUID NITROGEN CRYOGENIC STORAGE SYSTEMS

Karen B. Byers  
Dana Farber Cancer Institute, Boston, Massachusetts

Tedder, R.S., M.A. Zuckerman, A.H. Goldstone, A.E. Hawkins, A. Fielding, E.M. Briggs, D. Irwin, S. Blair, A.M. Gorman, K.G. Patterson, D.C. Linch, J. Hepstonstall, N.S. Brink. 1995. "Hepatitis B Transmission from Contaminated Cryopreservation Tank." *Lancet* 346:137-139.

### **Viral Contamination of Embryos Cryopreserved in Liquid Nitrogen**

A. Bielanski,\* S. Nadin-Davis,\* T. Sapp,† and C. Lutze-Wallace†  
\**Animal Diseases Research Institute, Germplasm Centre of Expertise;*  
*and †Biologics Evaluation Laboratory,*  
*Nepean, Ontario K2H 8P9, Canada* *Cryobiology* **40**, 110–116 (2000)



## Biobanks – “The norm”





## Biobanks – “The ideal

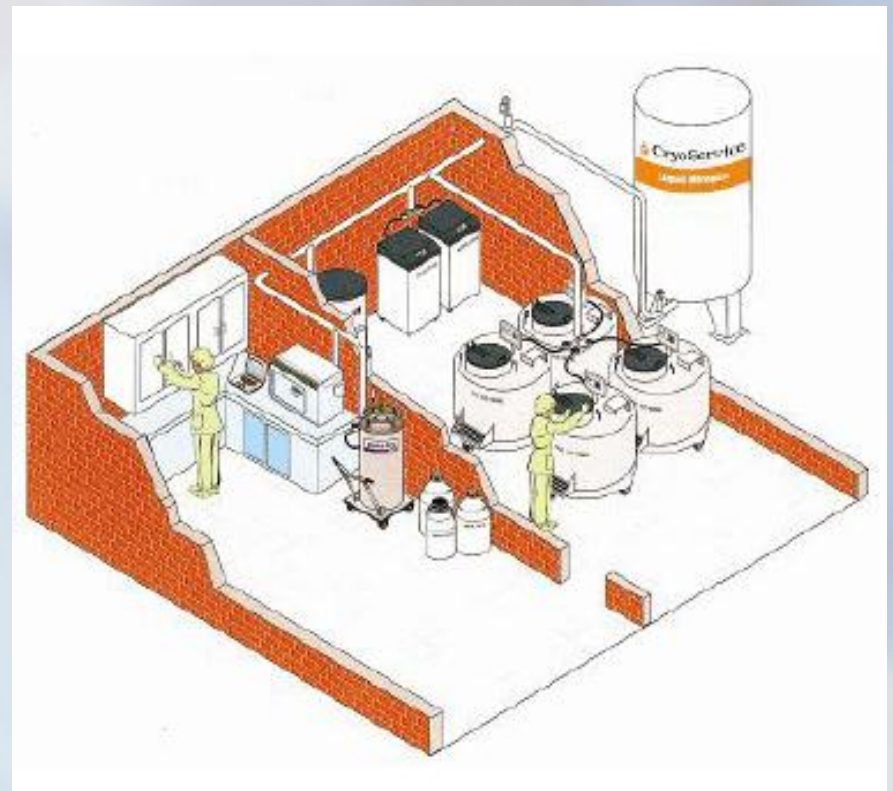




## Biobanks – The ideal lab location

Ground floor against an external wall

- Short SIVL line for LN2 delivery
- Reduced losses in line during fill process
- Simplifies ventilation
- No transporting of LN2 Storage Vessels in lifts
- No pressure drops due to rises in pipework





## Biobanks – The need to compromise

Above ground floor

- LN2 Storage Vessels in lifts (dedicated goods or restricted during use)

- Excessive LN2 consumption

- Pressure drop in pipework

- Basement and below

- LN2 Storage Vessels in lifts (dedicated goods or restricted during use)

- Gas accumulation. Forced extraction required





## Storage systems



### Chart Cryosystems

- Liquid storage
- Low liquid nitrogen consumption (normal working duration full days 38 – 104)
- Mid range vial capacity 750 – 6000
- Can be used with level alarm



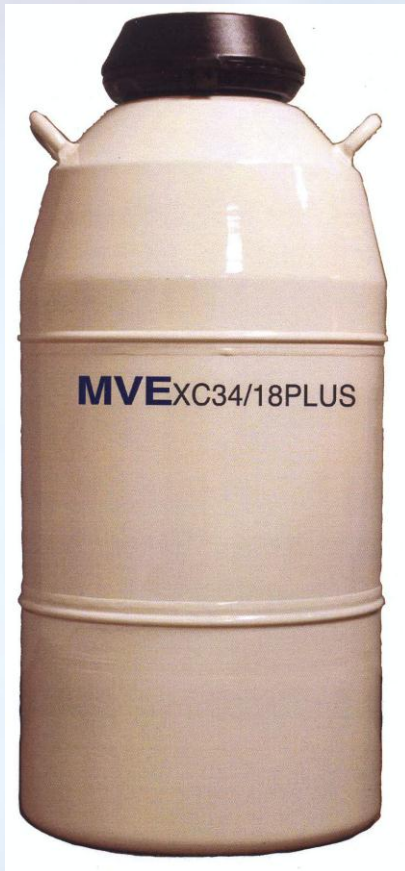
[www.chartbiomed.com](http://www.chartbiomed.com)



## Storage systems

### Chart MVE XC 34/18 Plus

- Vapor storage
- Low liquid nitrogen consumption (60 day hold time)
- Sealed canisters
- vial capacity 630 (in canes)
- Can be used with level alarm



[www.chartbiomed.com](http://www.chartbiomed.com)



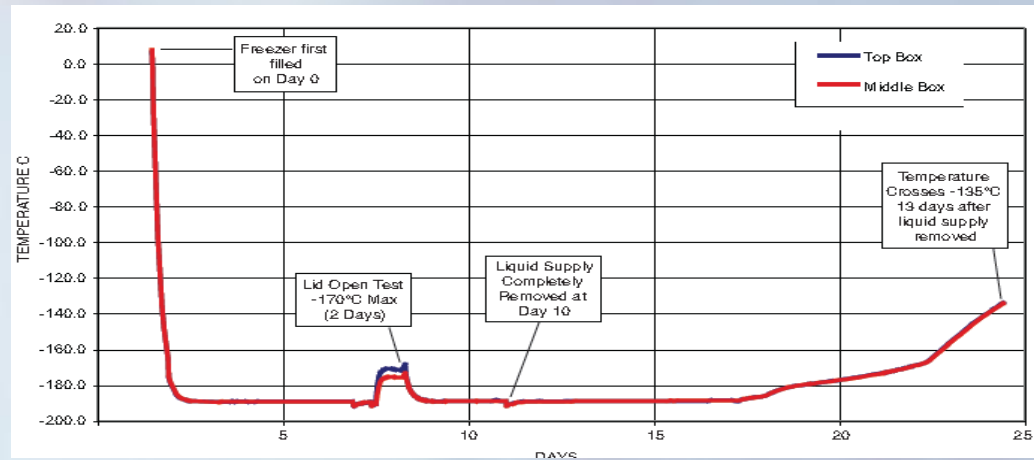


## Storage systems



### Chart MVE 1500 Series -190

- Vapor storage
- TEC 3000 controller
- 36000 – 42000 x 2ml vial storage
- High degree of security



[www.chartbiomed.com](http://www.chartbiomed.com)



## Storage systems

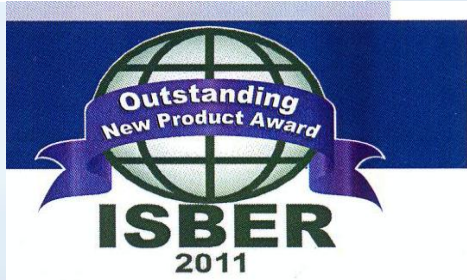




## Storage systems

### Chart MVE Variö Series Dewar

- Vapor storage
- Uses heat exchanger and LN
- 81000 x 2ml vial storage
- High degree of security
- User defined temperature  $-50^{\circ}\text{C}$  to  $-150^{\circ}\text{C}$
- Lower operating costs to mechanical freezer

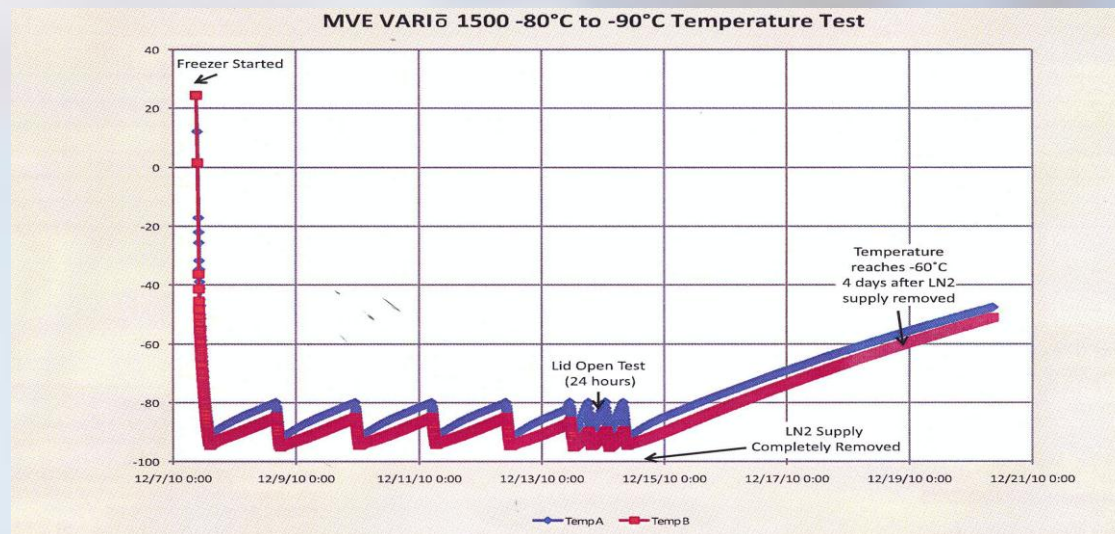




## Storage systems



### Chart MVE Variö Series Dewar





## Safe handling of frozen samples



### Chart MVE Cryocart

- Ensure viability of samples maintained
- Loading and retrieval of samples from storage
- Hold time of 18 hours with lid on
- Temperature monitor



[www.chartbiomed.com](http://www.chartbiomed.com)



## Transport of Frozen Samples

### Chart MVE Vapor Shippers

- Vapor shipping of samples
- Hold time 10 days (upright)
- IATA compliant



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# Transport of Frozen Samples

## Planer ShipsLog

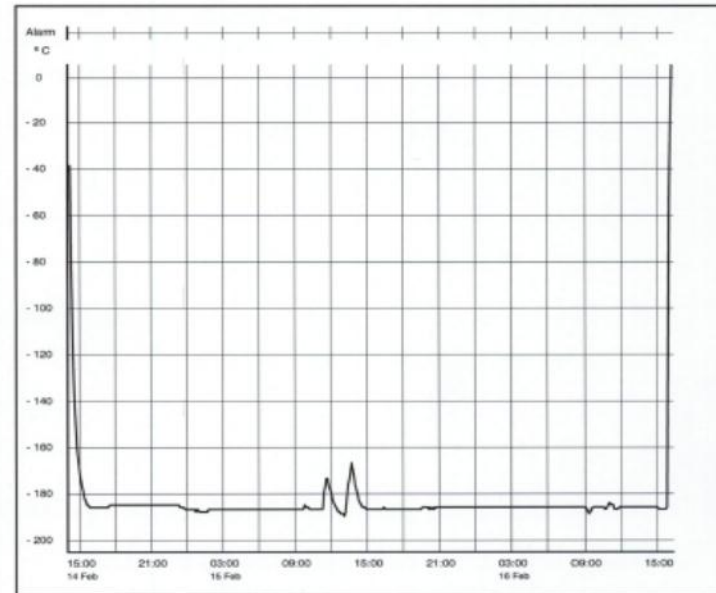
Temperature logging of shipment



SHIPSLOG ID: 3122  
Record Number: 1 of 1  
Title: Transport SA 16 02 2012

SHIPSLOG Settings  
Log Interval: 6 min  
High Alarm Level: No Limit Set  
Low Alarm Level: No Limit Set  
Alarm Delay Period: Not Applicable

Printed Period  
Start: 14:00 14 February 2012  
End: 18:12 16 February 2012  
Log Entries: 503



Temperature Statistics  
Maximum: +5.6 °C Error  
Minimum: -189.6 °C Error  
Average: -183.5 °C Error  
Variance: +16.2 °C Error

Alarm Periods  
High Alarm: No Alarms  
Low Alarm: No Alarms  
Data Smoothing  
No Data Smoothing

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## Supply and storage of liquid nitrogen



### Euro Cyl Vacuum Cylinders

- Low pressure
- 50 – 1000 litre options



[www.chartbiomed.com](http://www.chartbiomed.com)





## Safety

### Scilabub

\* Frosters aprons and gloves



**scilabub**®  
protective hand wear



### Analox

\* Oxygen deficiency monitors





**Thank you**



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