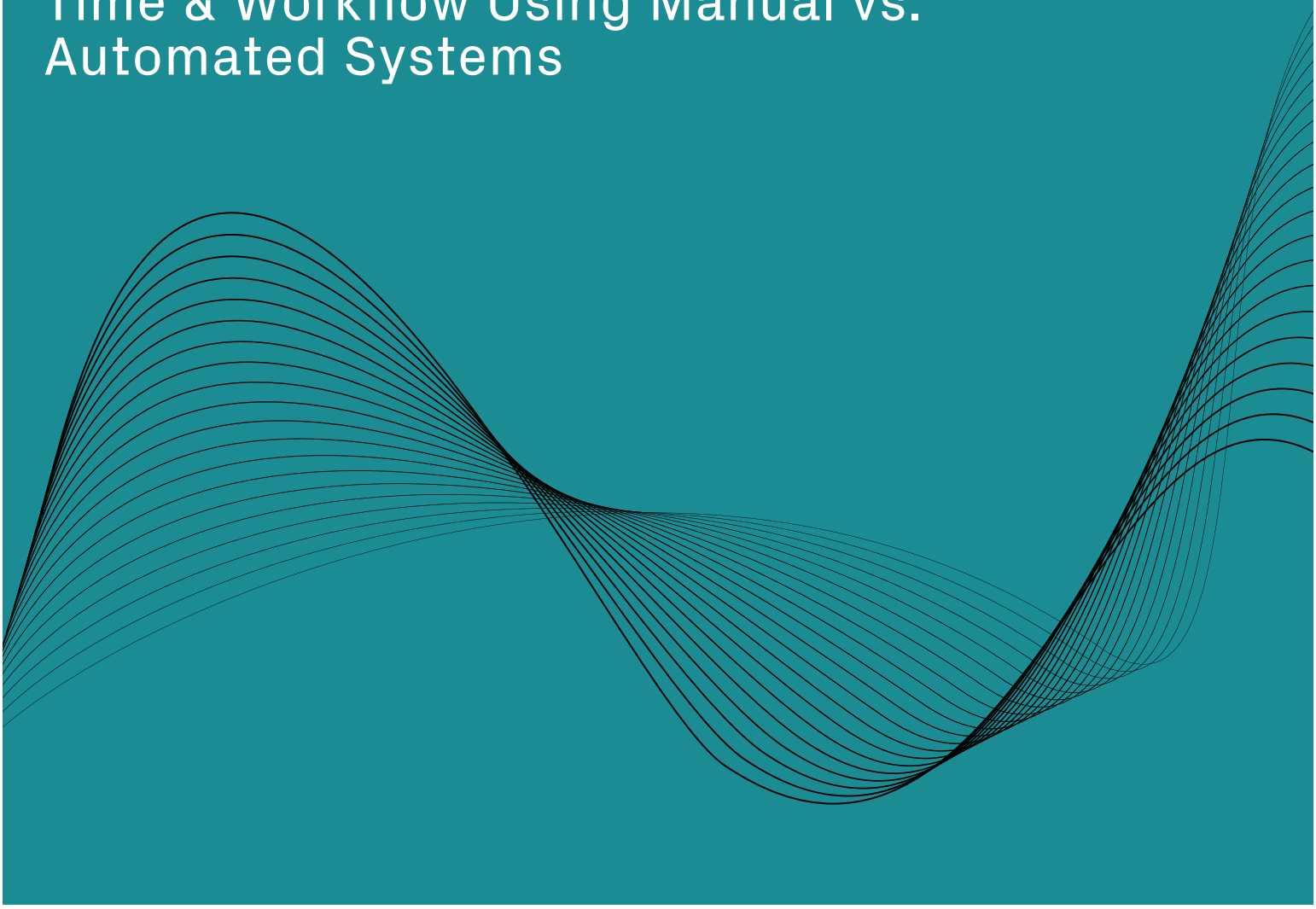
Storage, Automation & Logistics

WHITE PAPER

Sample Warming During Innocent Exposures From an LN2 Freezer:

Comparing Temperature, Time & Workflow Using Manual vs. Automated Systems



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Abstract

Storing samples in liquid nitrogen vapour phase freezers (LN2) is very common and performed throughout the biobanking and cell therapy industries. These freezers are chosen primarily because they maintain a sub -150°C storage environment and thus, keep the samples below -135°C, the glass transition temperature of water (Tg). This cryogenic storage practice preserves sample viability.

The concern is what happens to innocent samples (the ones not intended to be thawed) during routine rack exposures. Thousands of innocent samples may be exposed multiple times throughout their storage lifetime. Constant thawing/freezing through the glass transition phase may cause irreversible damage and affect sample functionality when thawed.

These experiments demonstrate and compare workflow, time and warm up rates of innocent samples using manual vs automated handling of cryoboxes.

Rack with cryoboxes in shelves 1, 4, 7, 10 & 13. Each cryobox has 1 vial in the center.

BioStore™ Cryo -190°C LN2-Based Automated Storage System (BioStore)

Introduction

Using a typical workflow with a manual LN2 freezer, samples (water) in five different shelf locations in a standard rack are monitored during rack extractions (pulls) from the freezer. The experiment is repeated with the identical setup and locations, but using the BioStore -190°C LN2-Based Automated Storage System (BioStore).

Materials

- BioStore -190°C LN2-Based Automated Storage System (BioStore)
- Chart MVE 1500 series LN2 -190°C vapour freezer (ARD) with Racks
- 5 of Azenta 10 ×10 cryoboxes with lids
- 5 of Azenta 2mL cryo vials, 1 centered in each cryobox
- All vials filled with 2mL of water
- 36 AWG Type T Thermocouples mounted midway up the water height
- Measurement Computing TC-Temp data acquisition unit sampling at 1.0hz using TracerDAQ software.

Procedures

- The 2mL vials were filled with 2mL of water and thin wire thermocouples were installed
- Vials were put into the center position of cryoboxes, lids put on and then the cryoboxes were inserted into shelves 1 (top), 4, 7, 10 and 13 of the rack
- Vials were monitored until the top box sample temperature was -180°C +/- 2°C
- 10 times the rack was extracted with the BioStore system
- 10 times the rack was extracted by manually lifting it out and to the side of the freezer
- 5 times the rack was extracted with the BioStore system to only shelves 1, 3, 5, 7 and 14, individually
- The cryoboxes were never removed, handled or touched.

Results

General Workflow Comparison

Workflows to remove a sample from a manual LN2 freezer may vary from lab to lab, user to user and even day to day. Since the entire process is performed by manual labour, innocent exposures, time extracted and inventory are not automatically monitored and recorded.

Typically steps to remove a cryobox from a manual LN2 freezer are:

1. Climb up stairs
2. Remove the lid
3. Reach inside to turn the carousel to locate the needed rack
4. Lift the rack up and out
5. Lay rack across the freezer or step down and lay across a bench or the floor
6. Remove the rack pin
7. Locate and remove the needed cryobox
8. Replace rack pin
9. Lift then lower rack back into the freezer
10. Replace the lid
11. Step down stairs

Typical steps to remove a cryobox from a BioStore system:

1. Input sample ID or Location into the PC and submit Order
2. (Rack raises) When prompted, open door and move cryobox
3. Rack automatically goes back down into freezer

Time Estimate Comparison

When performed manually, each cryobox removal may take a different amount of time, depending on its location in the freezer and the speed and accuracy of the operator. Below are estimates.

- 2 minutes - Time to find and remove cryobox
- 30 seconds to 2 minutes - Time rack is exposed outside of LN2 freezer

The workflow becomes standardised and controlled with automation. Retrieving the cryobox with the BioStore will vary based on the box location, but the rack exposure is controlled.

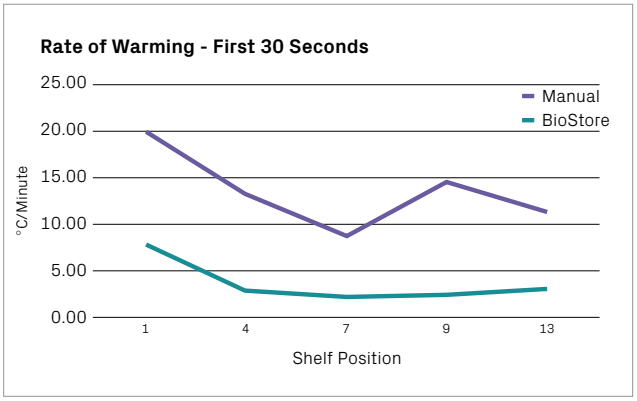
- 30 to 90 seconds - Time to find and remove cryobox
- 15 to 30 seconds - Time rack is exposed outside of LN2 freezer.

With the use of automation, manual labour is greatly reduced. Additionally, all actions and exposures are controlled, monitored and recorded.

Sample Rate of Warming

Minimising innocent sample warming is very important to ensure sample functionality post thaw. Therefore, users must do whatever is possible to limit the time innocent samples are outside of the LN2 cryogenic environment. This is normally done by working as quickly as possible after pulling a rack from the freezer. By using automation the rack exposure time can be reduced and by adding insulation around the rack, sample warming is also reduced.

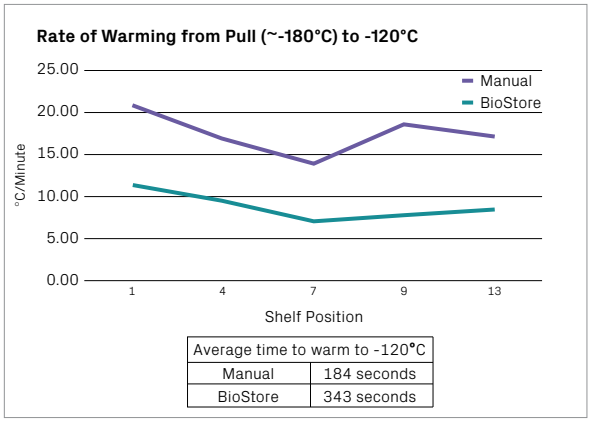
Typically during a manual workflow the rack is outside of the freezer for more than 30 seconds, but for the sake of equal comparisons the graph below represents the rate of warming of each innocent sample for the first 30 seconds of ambient exposure for both manual and automated rack pulls.



Averaging all the shelf warming rates, the BioStore samples warm 70% slower for the first 30 seconds of exposure vs. manual.The rate of warming in shelf 1 is the highest, but whereas the BioStore has relatively linear warming rates for lower shelves, the manual rack has significant differences between different shelves.

Note: Manual rack warming can be further influenced by the environment. Shelf 13 (and likely shelf 14) is affected by the surface the rack is rested on, we found insulation foam to provide the lowest transmission of heat. Room airflow from a typical HVAC vent can also affect manual rack warming by approximately 30%[1]

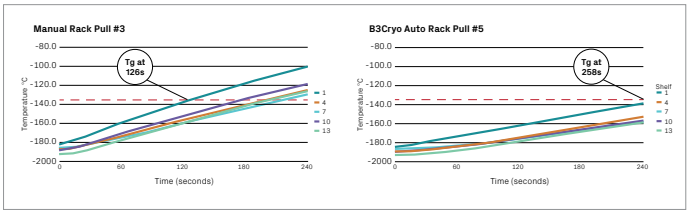
The following shows the rate of warming of each shelf from extraction to -120°C (past the glass transition temperature) and the average time to reach -120°C.



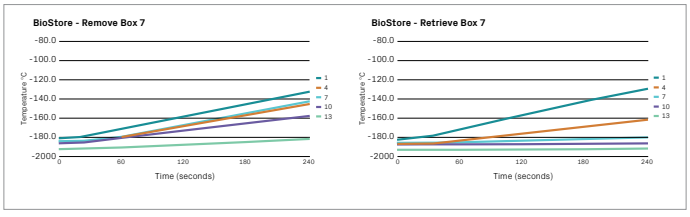
Average time to warm to -120°C	
Manual	184 seconds
BioStore	343 seconds

Sample Temperatures

Below are the temperatures of all 5 innocent samples during a 2 minute exposure of both manual and BioStore workflows.



Sample Temperatures



Above shows the temperature of all 5 innocent samples when retrieving a cryobox from shelf 1 (top) and shelf 7 using the BioStore system. Notice how the lower shelf samples warm much less than if the entire rack were removed from the LN2 freezer. To protect innocent sample warming, it is better to only lift the rack to the minimum height needed to remove the needed cryobox.

Conclusions

- Workflow steps to remove a cryobox are greatly simplified when using the BioStore system vs. Manual
- During the first 30 seconds after rack extraction, the BioStore has a 70% lower sample warming rate than Manual
- Innocent samples take 51% longer to warm across Tg with the BioStore than vs. Manual
- Samples in lower shelves are protected from excessive warming in the BioStore system by remaining inside the freezer during access of higher shelf boxes
- If performing rack extractions manually, thoroughly understand rates of warming, environmental variables and contributors. Develop SOPs and audit periodically to ensure valuable samples always stay safely below Tg.
- Understand that after any rack is returned to the LN2 freezer the samples will continue to warm1 and different sample volumes will warm at different rates, i.e. 1mL vials will warm ~60% faster than 2mL.[2]

References:

1 Warhurst, J., Fink, J., Holmes, T. et al. (2015 May).Protection of innocents: continued sample warm up after return to a cryogenic environment (below -150°C) following a transient ambient picking operation. Oral presentation presented at annual International Society of Cellular Therapy conference, Las Vegas, NV.

2 Salvetti, M., Fink, J. Barlett, A. et al. (2015, May). Thermal excursions of cryogenically frozen vials (below -150°C) and the risk of rising above Tg,H2O: analyzing warm-up rates from cryogenic storage to both dry ice and ambient temperature environments. Poster presented at annual International Society of Cellular Therapy conference, Las Vegas, NV.

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