Automation of Cold Chain Infrastructure Provides Benefits to Time Efficiencies, Documentation Rigor, and Sample Quality for Cryogenic Sample Management Workflows

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Introduction

Automation of the various aspects of manufacturing is a critical component in process development for advanced therapies. Efforts to date have largely ignored cold chain infrastructure as automated solutions are often viewed as an expensive alternative to manual solutions. However, groups who have chosen to automate this aspect of their processes are demonstrating real benefits in the form of time efficiencies and process rigor. This study, conducted by ARMI, will quantify the impact of using cryogenic storage automation produced by Azenta Life Sciences as compared to a manual solution in the form of time savings and sample quality after one month of storage. Time requirements will be captured and reported for each method of storage and the samples will be examined and compared post-thaw for differences in morphology indicative of repeated freeze-thaw cycles. Contrary to common assumptions, clear gains in time, data accuracy, and sample quality in cold chain infrastructure can be realized and should be considered a benefit of an investment in automation.

Methods

Using a typical workflow with a manual LN_2 dewar, samples in two different shelf locations in a standard rack were monitored during rack extractions (pulls) from the freezer. The process was repeated with an identical setup and locations using the CryoArc™ Pico -190 °C LN_2-Based Automated Storage System (Pico), from Azenta Life Sciences. The experimental racks were pulled out of cryogenic storage over a period of 16 days, for a total of 20 pulls. Post transactions, three vials of induced pluripotent stem cells (iPSCs) from the Pico and three vials of iPSCs from the LN_2 dewar were thawed, expanded, and examined for differences in cell morphology.

Conclusions

- Samples maintained in an automated storage environment maintain, at minimum, an equivalent level of sample quality to samples maintained in a manual access environment.
- Time required to access a sample in an automated system is less than the time required to access a sample in a non-automated system, as well as the number of personnel and the level of PPE required.
- Risk of sample exposure to ambient conditions can be reduced significantly through the implementation of automation as automated solutions do not require the full rack of samples to be removed from the storage environment to access a target location.

Automation is strengthening quality and documentation of cold chain related processes, providing multiple benefits to safety and accessibility of personnel in addition to reducing risks of sample exposure and subsequent detrimental effects on preservation and viability. Optimized process control and rigor through implementation of best practices, leveraged by automation can minimize transient warming events, which effect post-thaw cell functionality and product integrity, and should be considered when developing the cold chain infrastructure to support the manufacture and deployment of advanced therapies.

Experimental Setup and Cell Morphology Results

A Sample box placement within freezer racks

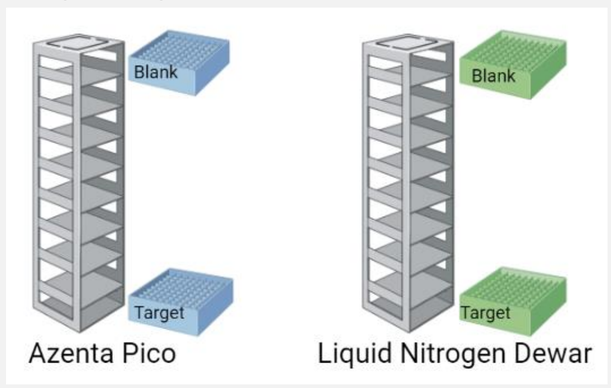


Figure made with Biorender.com.

B Cell vial locations within target box

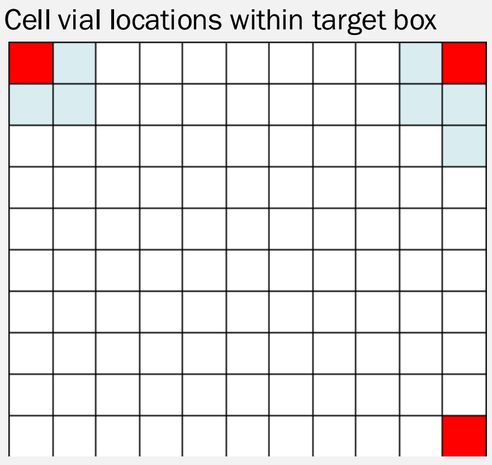
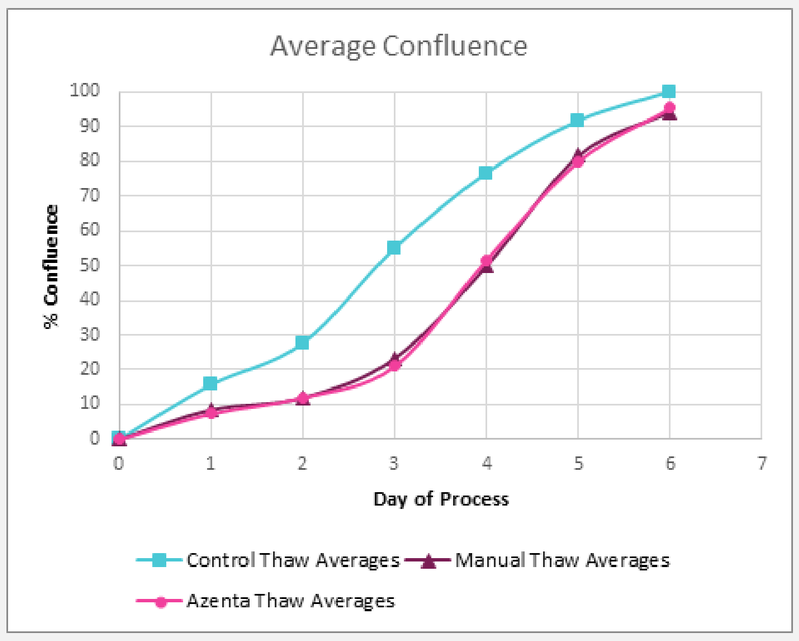


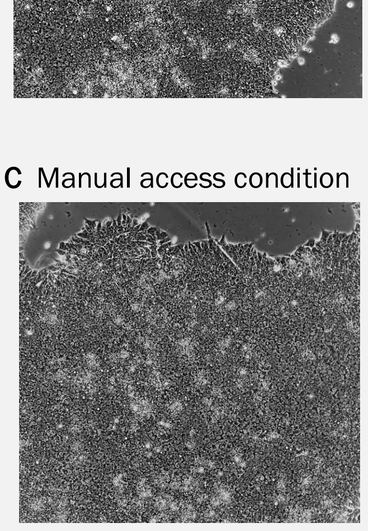
Figure 1. Experimental Setup. (A) The two experimental conditions: One rack in the Pico and one in an LN_2 dewar. Each rack had two boxes, a blank box that does not contain cells, and the target box containing the cells to be thawed at the end of the experiment. The blank box was used during each transaction with the target box being removed from the freezer at the beginning and end of the experiment. (B) Positioning of cell vials removed from the freezer box and thawed at the end of the experiment. The most vulnerable position at the corners of the group of cells were chosen to observe the cells most likely to be impacted by transient warming events. Populated positions are highlighted in blue and selected vials to thaw are highlighted in red.

A Sample box placement within freezer racks



B Control condition

C Manual access condition



D Automated access condition

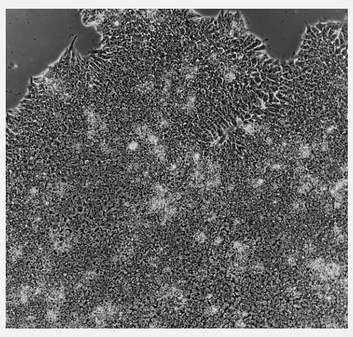


Figure 2. Average Confluence and Cell Morphology. (A) Average confluence of three different thaws from each condition. Representative image of cell morphology on Day 04 at 10X magnification in (B) the control condition, (C) the manual LN_2 dewar condition, and (D) the Pico condition. The morphology from each condition looked similar, with each having healthy iPSC morphology characteristics (large nucleus with prominent nucleoli, high nucleus to cytoplasm ratio, densely packed cells, and defined borders.)

Time to Access Samples and Sample Exposure Time Results

A Manual access time estimates

Process Step	Description	Time (minutes)
Create pick list	Prior to retrieving samples, users will need to access the LIMS system or storage manifest to locate the precise location of the targeted materials. A list is created and physically brought to the system on a clipboard or folded up paper in the lab coat.	2:00
Don Personal Protective Equipment (PPE)	Users of manual LN2 systems require special PPE including LN2 safety gloves, apron, and face shield.	1:00
Pick time (2 person for safety and accuracy)	Total pick time	5:20
	Finding the right rack	*(0:08)*
	Lift rack – remove locking pin – pull box	*(0:35)*
	Identify and confirm target vial	*(0:47)*
	Replace box – pin – rack	*(1:10)*
	2 person effort requires doubling time	*(2:40)*
Wrap up	Updating inventory, removing PPE	3:00
	TOTAL TIME FOR 1 VIAL (MANUAL)	11:20

B Automated access time estimates

Process Step	Description	Time (minutes)
Create pick list	User accesses onboard inventory with username and passcode	1:00
Don Personal Protective Equipment (PPE)	Limited PPE required (gloves, eye wear)	0:10
Pick time (1 person required)	Total pick time	0:45
	Automated box retrieval	*(0:35)*
	Visual inventory with targeted vial highlighted	*(0:05)*
	Replace Box – Process is simple with rack lifted to desired height and no pin to replace	*(0:05)*
Wrap up	Updating inventory, removing PPE	0:10
	TOTAL TIME FOR 1 VIAL (AUTOMATED)	2:05

Figure 3. Time to Access Samples Results. Both manual (A) and automated (B) sample pulls were measured using a stopwatch to provide time estimates for completion of each step in the sample access process. Clear time savings were achieved using automation to manage sample access, including the elimination of the requirement for a second person for safety and verification purposes.

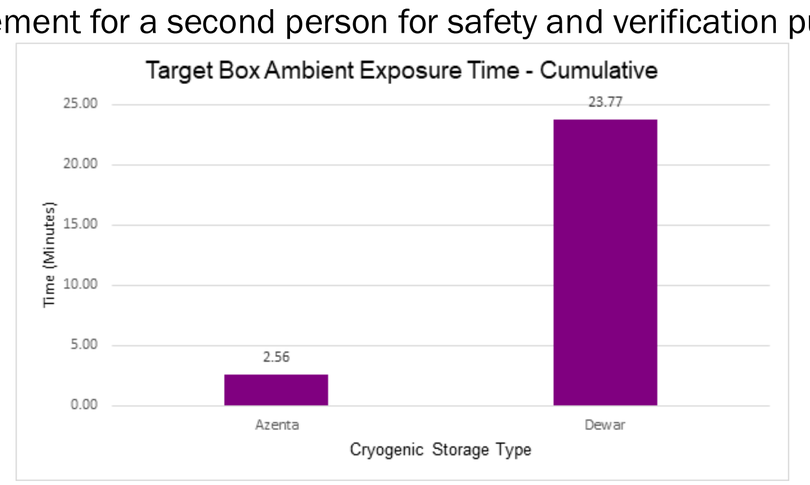


Figure 4. Cumulative Ambient Exposure. Total amount of time target samples were exposed to ambient conditions was measured during the course of the experiment. Due to the nature of the automated retrieval process, target samples maintained in the automated Pico system were exposed to ambient conditions over 9X less than target samples maintained in the manual LN_2 dewar.

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