Clearing the Air: Adjusting practices to meet atmospheric conditions with automated freezers.

Li Zhou[1], Jeremiah Hawk[2], Jason Patterson[3], Rick Filonzi[3], Noel Hartigan[3], Natalie Gabrael[1], Aysen Yuksel[1], Dan Catchpoole[1]

1. The Tumour Bank, The Children's Hospital at Westmead, Westmead, NSW, Australia
2. Hamilton Storage Technologies Inc, Franklin MA, USA
3. Bio-Strategy Pty Ltd, Melbourne, Victoria, Australia

Introduction

- Automated freezer technology currently relies on barcodes to identify, locate and distribute sample tubes within an ultra-cold environment.
- However, build up of ice crystals in the form of frost must be avoided otherwise incorrect or failed barcode reads will create inventory gaps, errors and sample management inconsistency within the system. (Figure 2)
- Despite dry air purging within the system to create low humidity conditions during sample entry into a -80°C system, we encountered an unforeseen issue where the ambient humidity of the freezer room dramatically influenced frost build up during the introduction of snap frozen tubes into the Hamilton SAM HD system. (Figure 1 and 3)
- Lower freezing temperature alcohol-based solutions are often used break down ice formation. It has also been described that the interaction of different alcohols with plastics alters the strength of ice crystal anchoring to a surface.

Methods

- We compared 3 procedures where tubes were sprayed with various dilutions of alcohols, and mopped up with absorbent pads and/or brushing off the frost during the sample transferral procedure. (*see protocol below*)
- Twelve racks of pre-frozen (-80°C) tubes were processed according to the 3 chosen protocols prior to introduction into the SAM HD automated freezer.
- Quantitative method evaluation required daily systems audits over 7 successive days. Photographs were collected for qualitative evidence. The room environment temperature and relative humidity was monitored throughout.

Experimental Steps

1. The SAM was thawed and then re-commissioned to eliminate all existing frost from the system.
2. A Madgetech Data Logger was used to verify temperatures.
3. Room temperature and Humidity will be recorded at the start and finish of each day. (Table 1)
4. Twelve racks were prepared via the SOP definition described below.
5. The twelve racks were introduced into the SAM and then audited once a day for 7 days. (Table 2)
6. The job results and audit images from each rack will be captured for data presentation. (Figure 4)
7. Physical libraries were created to isolate audit test samples from method test samples.
8. 40 racks were audited in total each day to maximize the risk of added heat and moisture.
9. All tubes were filled with tap water to at least half way or more.
10. The tubes and racks were snap frozen in LN2 together.
11. Racks were all full.
12. LN2 level was about ½ way up the side of the rack.

Protocols Tested

SOP 1	1. Snap freeze each tube. 2. Place tubes in warm rack 3. Place rack into manual -80°C freezer until all daily tubes have been processed. 4. Relocate samples in rack into Esky with dry ice. 5. Racks are lightly sprayed with 100% Isopropyl Alcohol and wiped with dry paper towel. 6. Introduce into the SAM.
SOP 2	1. Snap freeze tubes in rack. 2. Place rack into manual -80°C freezer until all daily tubes have been processed. 3. Relocate samples in rack into Esky with dry ice. 4. Racks are lightly sprayed with 75% Isopropyl Alcohol and wiped with dry paper towel. 5. Introduce into the SAM.
SOP 3	1. Snap freeze tubes in rack 2. Spray liberally over entire rack with 100% Isopropanol 3. Store in manual -80°C freezer as necessary. 4. Transfer racks to Esky with absorbent cloth over dry ice to prevent ice from sticking to tube and rack bottom. 5. Spray liberally with 100% Isopropanol then brush bottom with yellow bristle brush and wipe excess Isopropyl off with dry towel. 6. Introduce into SAM.

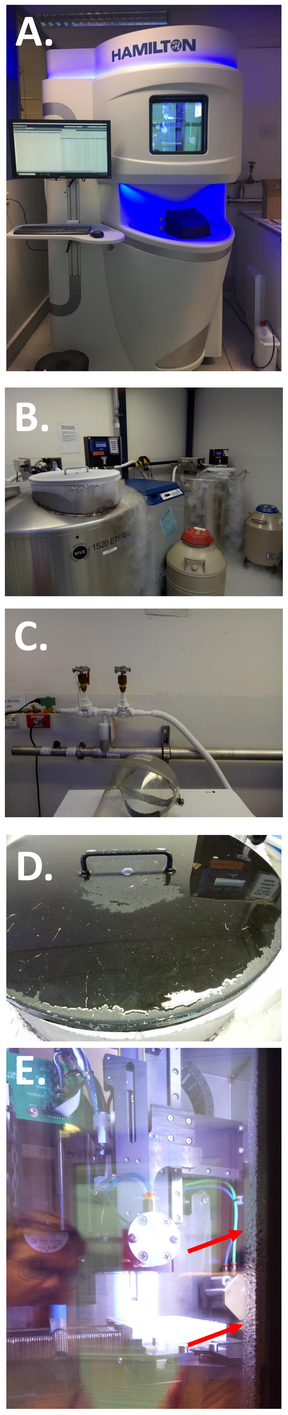


Figure 1

Freezer room environment showing (A) positioning of SAM HD, (B, C) frost on nearby liquid N equipment, (D) water pooling and (E) evidence of humidity inside SAM HD.

Table 1

Day	Temperature - In/Out	Relative Humidity (RH%) In/Out
1	23.3C / 26.1C	35.2% / 34.4% RH
2	22.3C / 24.2C	40.4% / 31.2% RH
3	24.3C / 26.2C	35.4% / 23.1% RH
4	23.1C / 25.7C	32.3% / 32.3% RH
5	22.5C / 23.8C	38.5% / 36.6% RH
6	22.63C	41.7% RH
7	24.1C	33.5% RH
8	21.75C	37.8% RH

Daily Temperature and Relative Humidity Values

Results

(1) Preliminary Observations

Figure 2 ***Problems Introducing Frozen Tubes to SAM HD***
(A) Unfrozen tubes, (B) frost build up, (C) isopropanol residue (D) fibres from wiping material, (E) highly reflective surfaces.

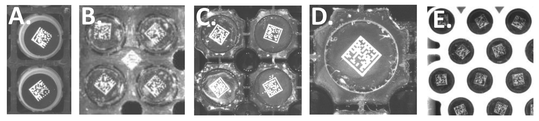
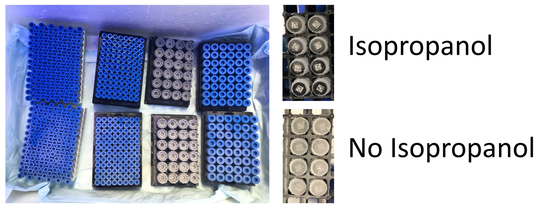


Figure 3 ***Frosting of Snap-Frozen Tubes During Transit.***
Pre-frozen racks and tubes sprayed with isopropanol before going on dry ice showed no frosting after 10minutes with Esky lid open. Untreated racks and tube demonstrated frosting.



(2) Audit Results - Quantitative

Table 2

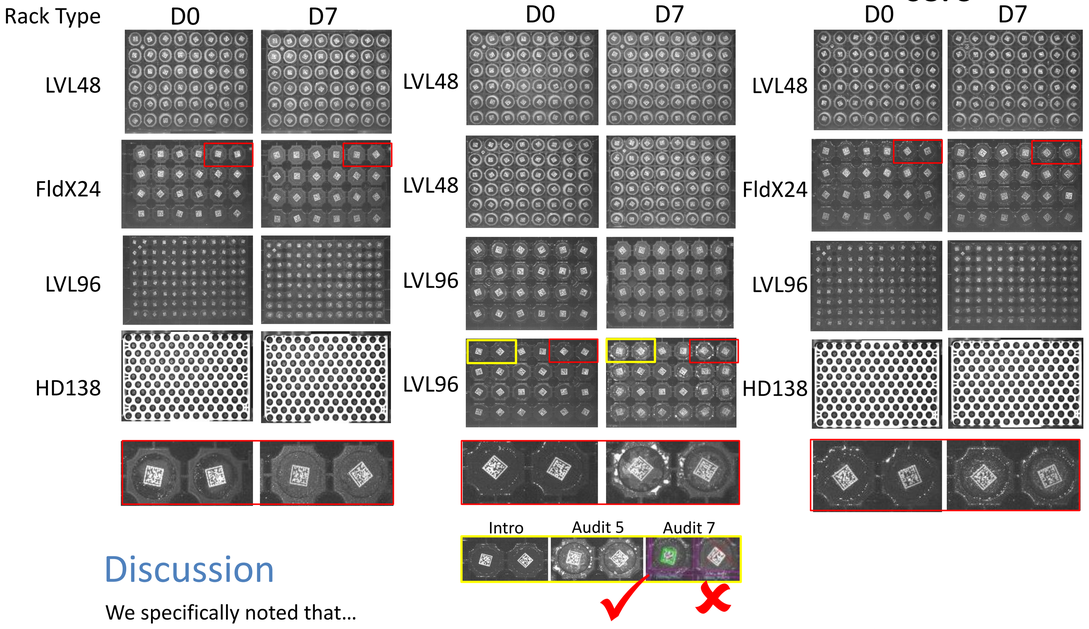
	SOP1	SOP2	SOP3
Retries	0	0	20
Errors	0	0	0
Misread Tubes	0	0	2
Frost	Low	low	low
Empty Spot Verification	Good	Good	Good

	SOP1	SOP2	SOP3
Ease of use	2	2	3
Cost	2	2	1
Time	2	1	3

Advantages with respect to the operator ease of use with each protocols.
(Scale of 1-5: 1 best – 5 worst)

(3) Audit Results - Qualitative

Figure 4 ***Comparison of images*** of racks and tubes taken during rack introduction (D0) and following audit 7 days later (D7) for each test protocol.. Close up view of equivalent tubes (red) are shown. An audit failure is highlighted with yellow box.



Discussion

We specifically noted that…

- Spraying snap frozen tubes with 100% iso-propanol early dramatically reduced frost build up during subsequent sample transfer steps through the humid environment. (Figure 2)
- Too much iso-propanol however caused handling errors due to slippery surfaces.
- Iso-propanol caused a film to form on some plastic reflecting different chemical reactivity between polycarbonate vs polypropylene which influences frost build up.
- Site specific operating procedures need to be considered when installing new automated freezers.

Conclusion / Recommendation

- SOP 1 had the best results. Recommendation continue with SOP1 but to snap freeze the tubes in the rack when applicable, use absorbent pad and or brushes to remove ice and frost build up during transit and rack introduction as required.
- SOP 2 showed more frost than the others affecting the reliability of the system to detect and read rack types, tube barcodes or rack barcodes. Other data from in house Hamilton frost testing support the use 100% over 75% Isopropanol.
- SOP 3 showed the least amount of frost but had several issues including the polycarbonate racks were susceptible to reflection, were slippery when brought back out of the SAM and carried a very strong odor when handling.
- The SAM HD was mechanically sound during testing and free of internal frost. When the room environment maintained 22-24°C on average and 30%-35% RH we have witnessed no frost issues in the SAM nor on the racks and tubes. Filling LN2 tanks and outside temperature and humidity have significant affect on room environment. Room air conditioning was insufficient.
- Investigations of specialized frost cleaning devices (eg Hamilton Defroster) is worth pursuing to bring consistency of rack handling into the standard operating practices when using automated freezers.