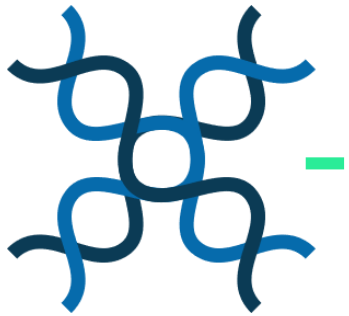

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Precise Storage and Storage Data as Critical Factors for CGT Cold Chain

Dr. Markus Albertini
Director & GM, Europe Region Services
Azenta Life Sciences

Problem statement

- **TEMPERATURE SENSITIVE THERAPIES LIKE CGT HAVE RIGOROUS COLD STORAGE STANDARDS TO PROTECT EFFICACY AND SHELF LIFE**

- **ACCORDINGLY CELLULAR THERAPIES ARE MOSTLY STORED BELOW -135°C**
  - The glass transition temperature of water (Tg), where enzymatic activity is believed to cease
  - Storage in liquid nitrogen vapour phase (LN2)

- **ROUTINE HANDLING IN AND OUT OF STORAGE CAN EXPOSES CELLS TO TRANSIENT WARMING AND EVEN TO TEMPERATURES ABOVE TG**

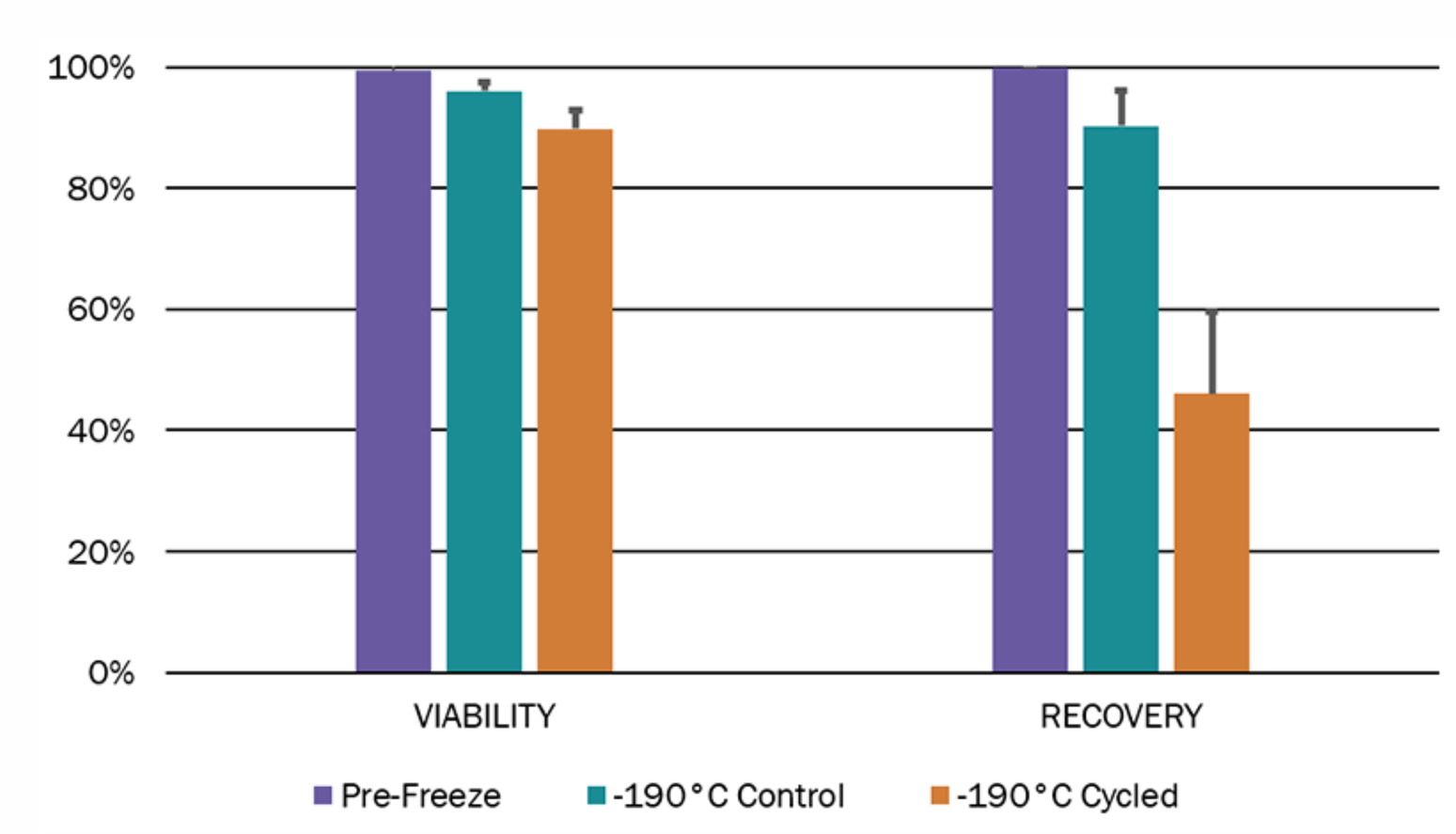
- **THE POTENCY AND FUNCTIONALITY OF THE CELLS POST THAW CAN BE IMPACTED**

- **PRECISE STORAGE AND MAINTAINING TEMPERATURE BELOW TG AND INTEGRITY OF CELLULAR PRODUCTS DURING ROUTINE HANDLING AND STORAGE IS PARAMOUNT**

- **ONLY WHEN UNDERSTANDING ENVIRONMENTAL VARIABLES AND OTHER CONTRIBUTORS TO TRANSIENT WARMING EVENTS (TGE) PRECISE AND REPRODUCIBLE STORAGE CAN BE ACHIEVED**

Transient warming reduces cell viability and recovery

- VIABILITY AND RECOVERY OF MESENCHYMAL STEM CELLS PRE-FREEZE AND POST-THAW



- Cells were stored for 3 months at -190°C with no exposure to warming events (control) or with 20 four-minute exposures to -110°C (cycled).

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Understanding the variables and contributors of transient warming

Sources of transient warming/temperature variation

- **SAMPLE TRANSFER INTO THE STORAGE ENVIRONMENT**

- **STORAGE IN THE STORAGE ENVIRONMENT**
  - The risk of the innocent sample

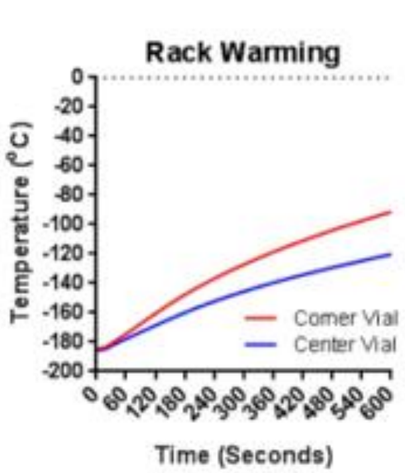
- **SAMPLE/MATERIAL RETRIEVAL OUT OF THE STORAGE ENVIRONMENT**

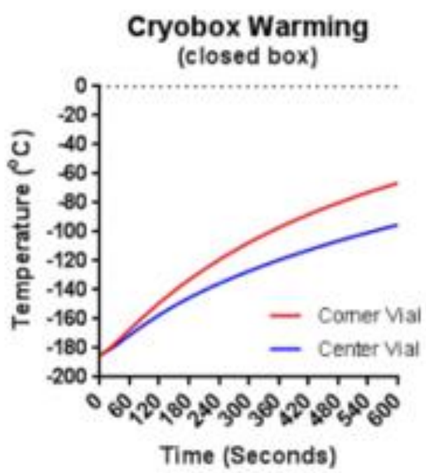
- **STEPS AND TIME REQUIREMENTS (HIGHLY TRAINED STAFF, MANUAL RETRIEVAL FROM LN2 TANK)**

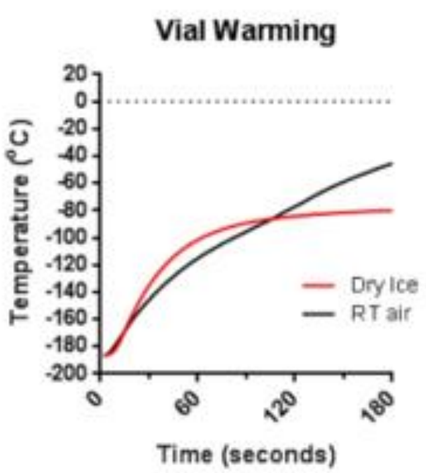
- 1. Open freezer and pull rack.	30 sec
- 2. Identify and pull the box.	30 sec
- 3. Identify and pull sample and transfer to transport device.	60 – 120 sec
- 4. Replace box into rack and return rack to freezer.	30 -45 sec

2.5 – 4 min in total

Time may vary depending on speed and accuracy of operator







A 2 ml vial cooled to -180°C will reach TG (-135°C) within ~30 sec when fully exposed to air.

Transient warming of the innocents

(2 ml vials, cryobox in shared rack, manual retrieval from LN2 tank)

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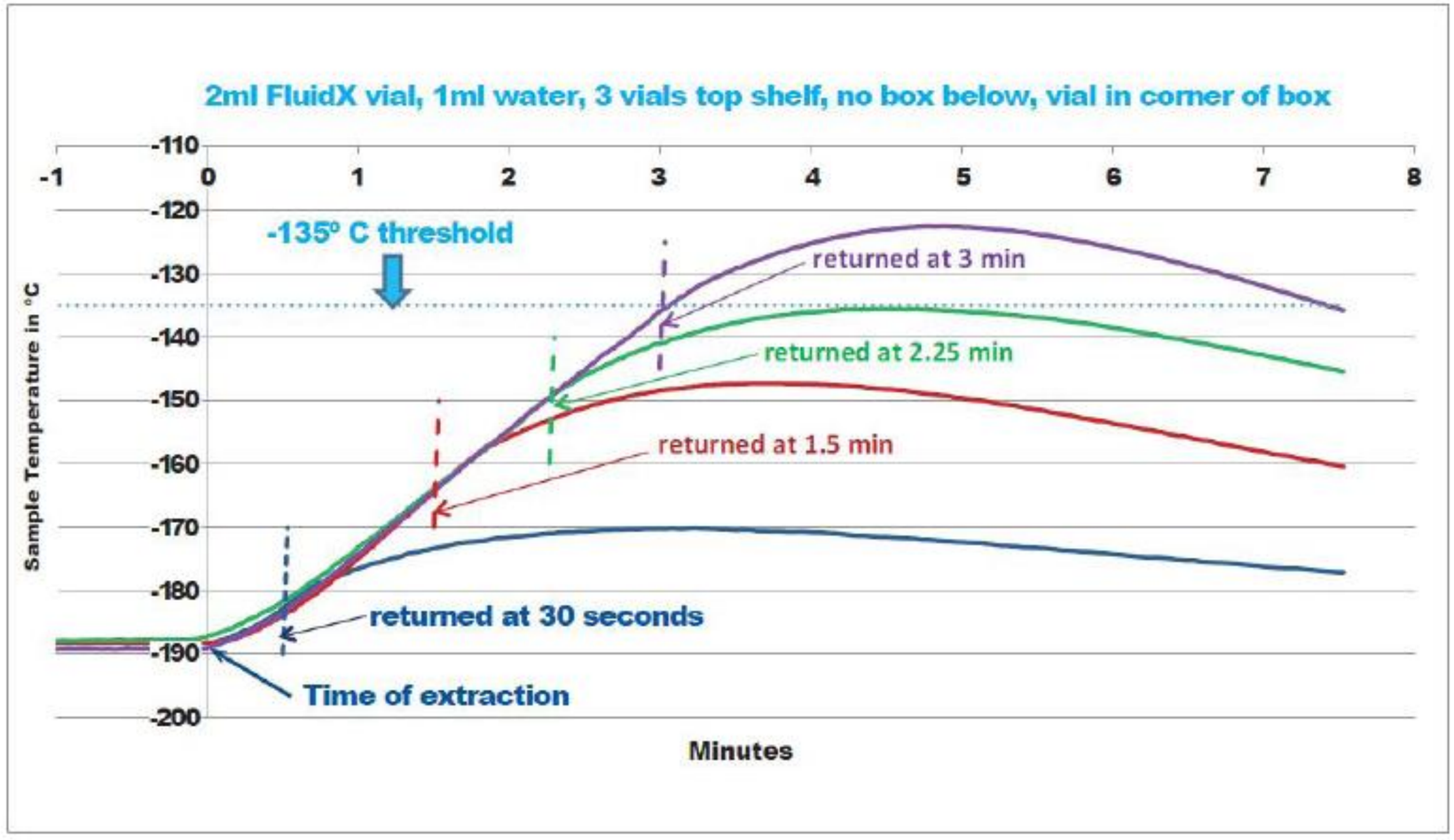
- **INNOCENT (NON-TARGETED) SAMPLES OFTEN UNDERGO TRANSIENT WARMING EVENTS (TWE)**



Racks pulled, cryobox stayed in rack and vial in cryobox until returned to LN2 tank..

- **BE AWARE OF THE ELASTIC WARMING AND RECOVERY TIME BACK TO -190°C**
  - Vials continued to be warmed by the heat from the surrounding rack and cryobox after replacement into storage environment.
  - Re-cooling of the vial did not begin until 2.5 - 5 minutes after re-insertion into the LN2 cryogenic environment.
  - If rack was pulled again1 hour after being returned to the freezer the sample may still be several degrees warmer than initially (data not shown); warming delta for innocents to be considered and compensated for when racks are pulled several times over day.

Transient warming of the innocents

(250 ml cryopreservation bags and cassettes in shared rack, manual retrieval from LN2 tank)

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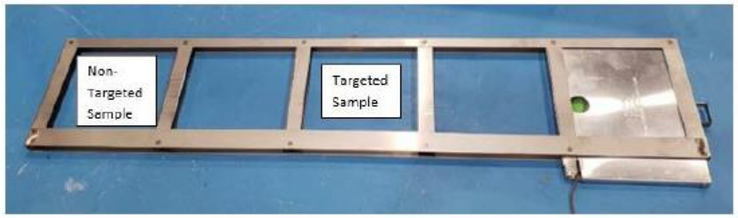
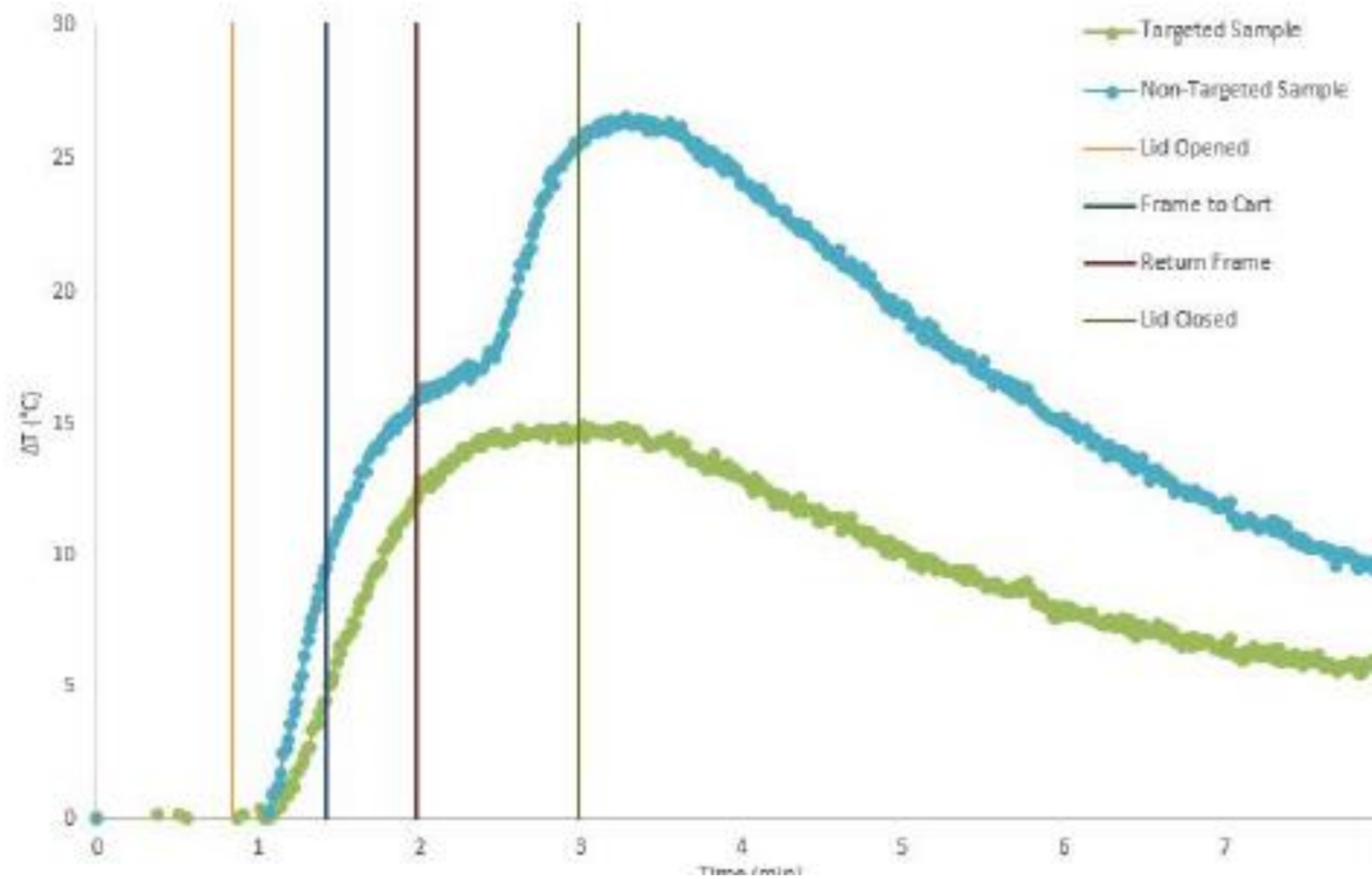
- **2 TRANSIENTS WARMING EVENTS (TWE)**
  - Rack removal and rack replacement
  - 30 sec at ambient to confirm that correct rack was pulled, +16,3°C
  - 30 sec at cryopreservation cart to identify and remove targeted sample from frame, +9,5°C
  - Elastic warming 1,1°C → +29,9° transient warming in total



Manual sample frame

Sealed cryopreservation bag filled with 70 ml of liquid inside cassette



Key observations

- THE <u>WARMING RATE</u> VARIES WITH VOLUME (DATA NOT SHOWN)

- DEPENDING ON <u>VOLUME</u> AND POSITION IN THE CRYOBOX A VIAL/SAMPLE COOLED TO -190°C WILL REACH TG (-135°C) AS FAST AS WITHIN ~30 40 SEC (2ML VIAL) WHEN FULLY EXPOSED TO AIR.

- <u>ELASTIC WARMING:</u> TRANSIENT WARMING EVENTS CONTINUE AFTER THE RACK IS RE-PLACED INTO THE CRYOGENIC ENVIRONMENT

- <u>CONVECTION:</u> STANDARD HEATING AND COOLING (HVAC) HAVE A CONSIDERABLE IMPACT ON SAMPLE WARMING (DATA SHOWN IN NEXT SECTION)

- <u>TEMPERATURE RECOVERY TIME:</u> THE RECOVERY TIME OF A VIAL/BAG BACK TO 190°C CAN TAKE MORE THAN 1 HR.
  - e.g. depending on the position of the vial in the cryo box (center position); data not shown

- FAILURE TO MONITOR, PLAN AND COMPENSATE FOR THESE VARIABLES MAY RESULT IN FAILURE TO PROTECT INNOCENT SAMPLES FROM CROSSING TG, E.G. IN A SCENARIO OF SEVERAL RACK PULLS OVER A WORKING DAY

- MANUAL LN2 FREEZER AVAILABLE TODAY HAVE NO BUILT IN PROTECTION OR MONITORING OF INNOCENT SAMPLES

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Does automation make a difference?

Excample: BioStore III Cryo Storage System (B3Cryo)

Quality is assured by viewing user access, sample inventory, history, and audit-trail reports.

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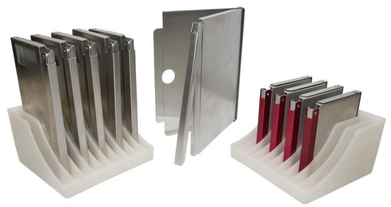
SAFER STORAGE WITH INVENTORY CONTROL

- Samples protected from excessive warming during retrieval
  - Automated cryobox/cassette delivery → timed, recorded and controlled
  - Insulating tower for temperature stability → slows warming by blocking convection
  - Sample integrity calculator predicts warming rates based on empirical data
  - Minimized warming of innocent samples
- Integrated software controls
  - Full vial or cassette level inventory tracking
  - 19 readily available reports to track temperature data and LN_2 levels
  - Improved ergonomics over manual workflows

Insulating tower



Transient warming; manual compared to automated rack pull (2 ml vials, centered in cryobox)

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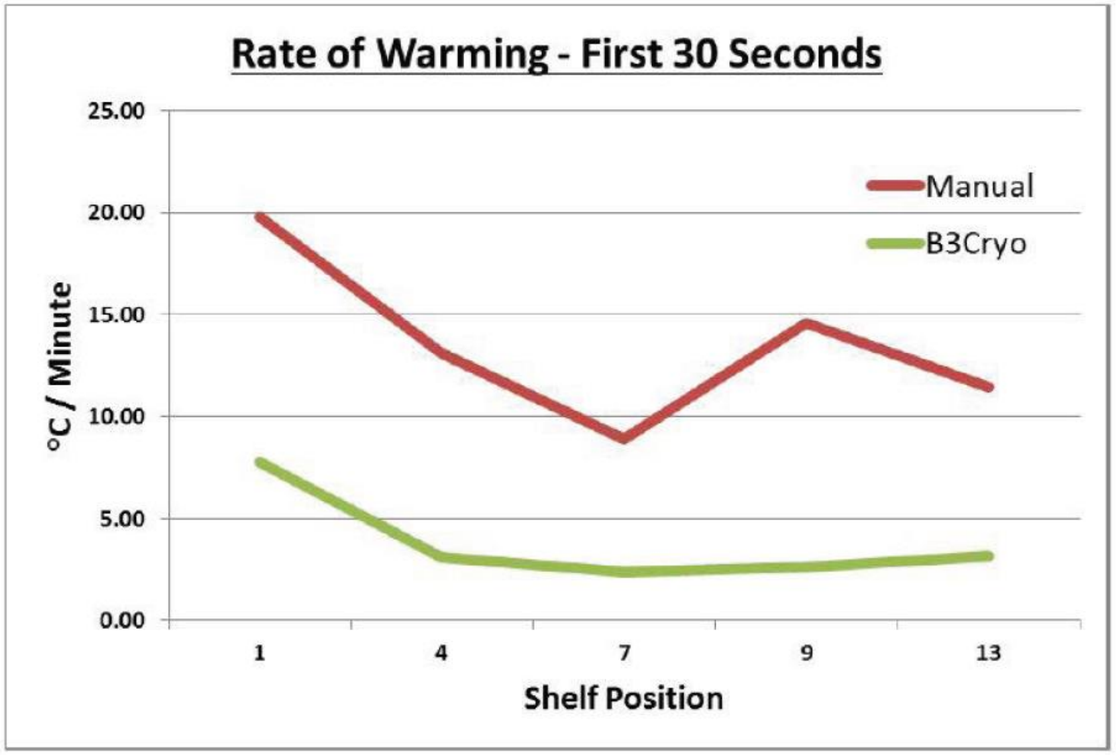
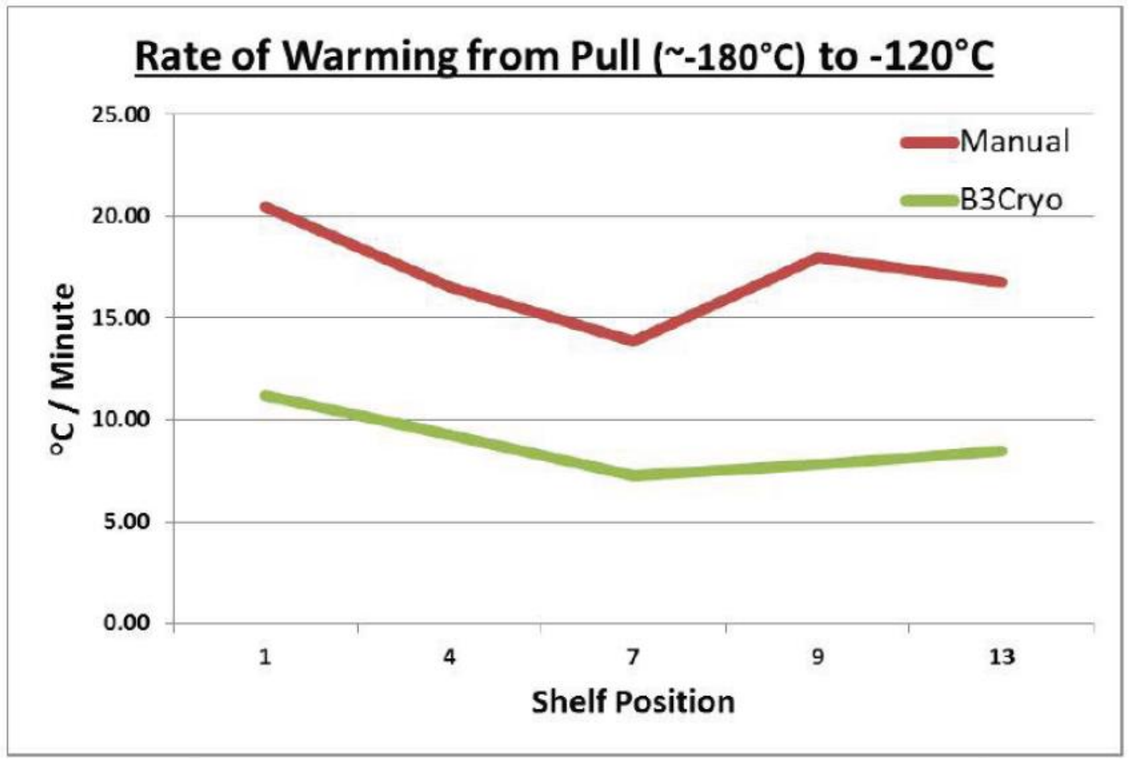
- AVERAGING ALL SHELF WARMING RATES THE B3CRYO SAMPLES (2 ML VIAL) WARM 70% SLOWER FOR THE FIRST 30 SECONDS

- THE MANUAL RACK HAS SIGNIFICANT TEMP DIFFERENCES BETWEEN DIFFERENT SHELFS, THE B3CRYO RELATIVELY LINEAR WARMING RATES FOR LOWER SHELFS

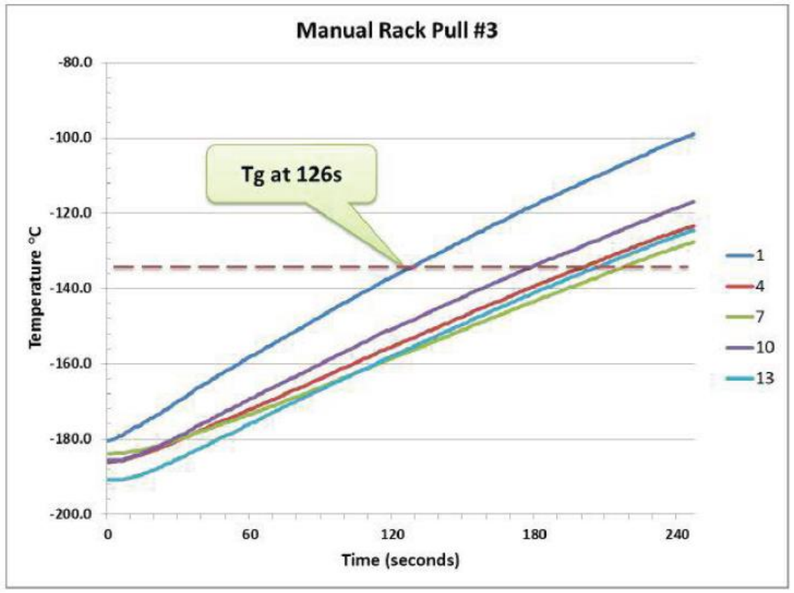
- WARMING RATE OF EACH SHELF IN A RACK FROM EXTRACTION TO -120°C



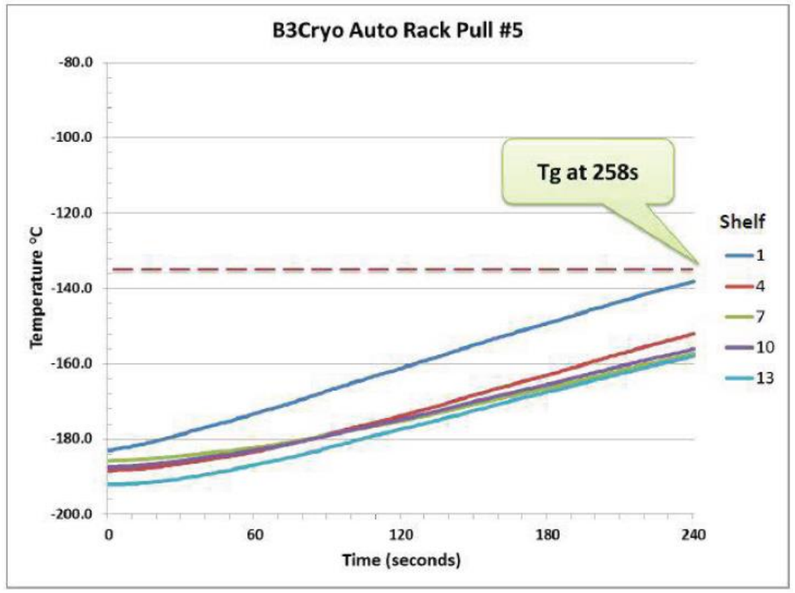


Average Time to warm to -120C	
Manual	184 seconds
B3Cryo	343 seconds

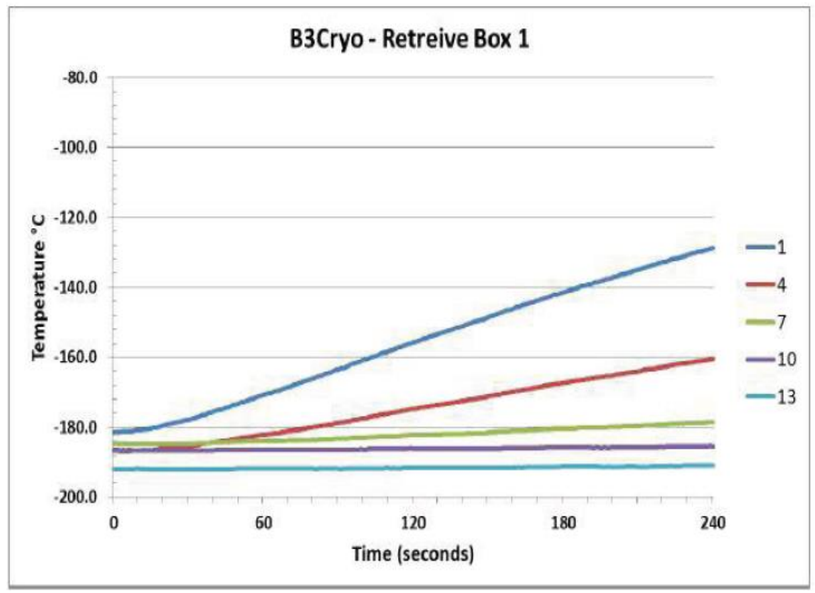
Transient warming; manual compared to automated rack pull (2 ml vials, centered in cryobox)



- TEMPERATURE OF INNOCENT SAMPLES IN DIFFERENT SHELFS RELATED TO TIME OF EXPOSURE

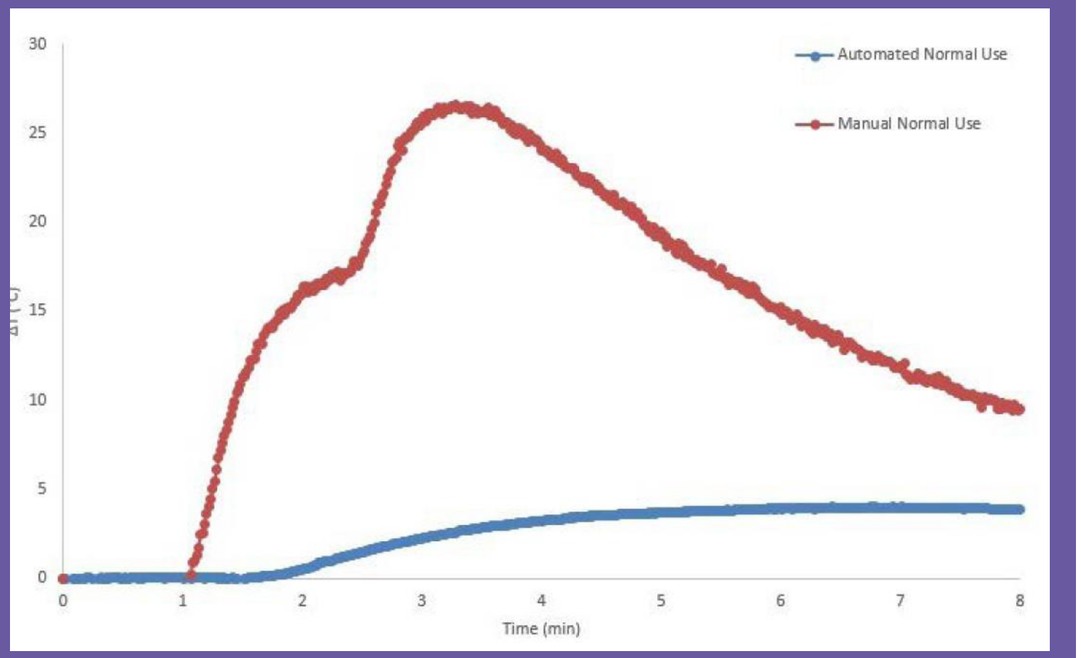


- TEMPERATURE OF INNOCENT SAMPLES WHEN RETRIEVING A CRYOBOX FROM LOWEST SHELF (13)



- TEMPERATURE OF INNOCENT SAMPLES WHEN RETRIEVING A CRYOBOX FROM HIGHEST SHELF (1)

TRANSIENT WARMING: MANUAL COMPARED AUTOMATED RACK PULL (250 ML CRYOPRESERVATION BAG IN CASSETTE)



IT TAKES A SAMPLE EXTRACTED USING AUTOMATION MORE THAN 16 TIMES AS LONG TO REACH TG THAN A SAMPLE USING STANDARD MANUAL METHODS

- LOWER SHELFS WARM MUCH LESS WHEN NOT ENTIRE RACK IS REMOVED FROM LN2 (AUTOMATED RETRIEVAL)

Warming of samples when exposed - with and without sleeve/convection (2 ml vials, centered in cryobox)

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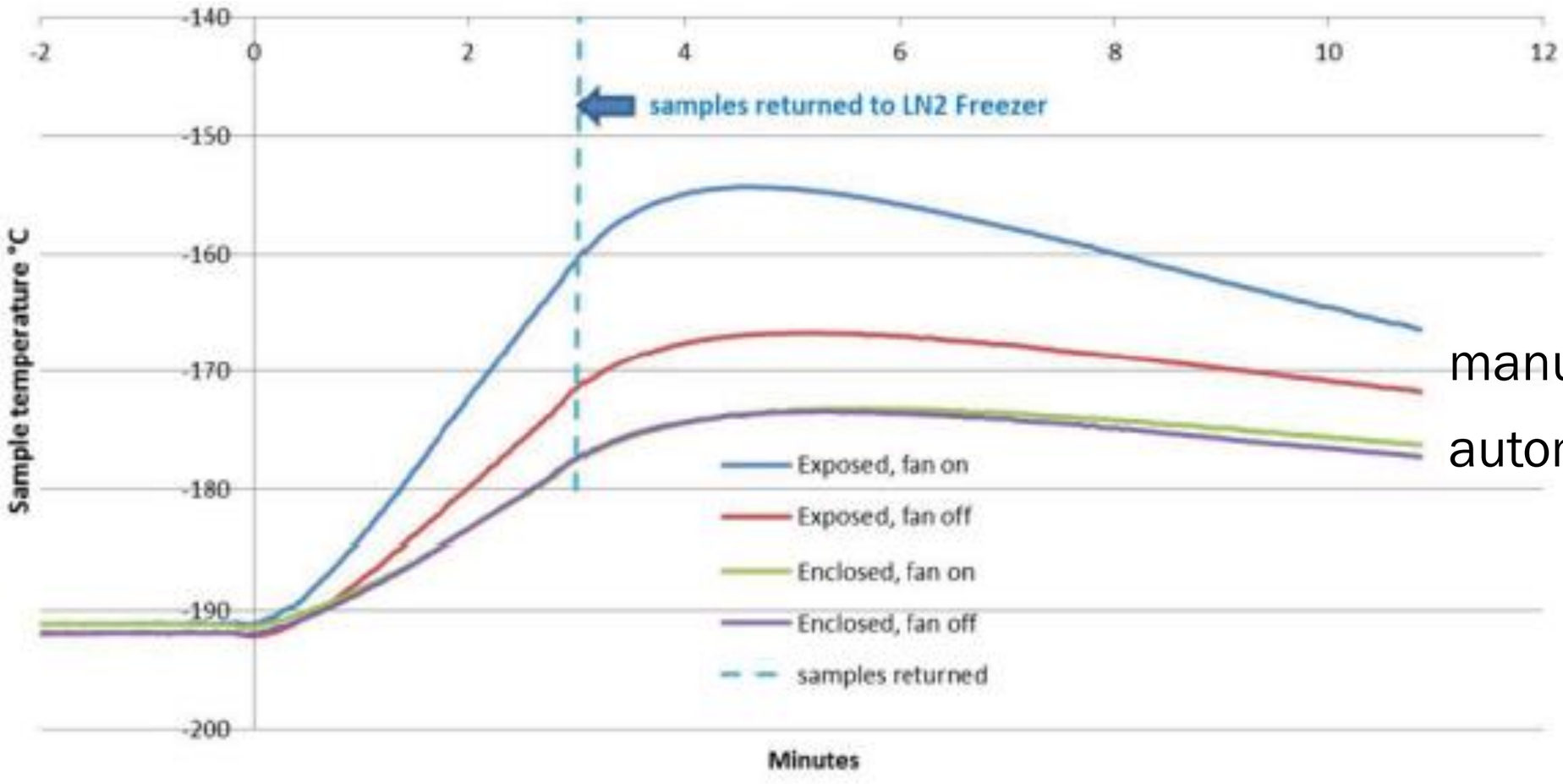
- BESIDES TEMPERATURE OTHER ENVIRONMENTAL FACTORS CAN CONSIDERABLY IMPACT TRANSIENT SAMPLE WARMING

- SEEMINGLY MINOR FACTORS AND OFTEN OVERLOOKED FACTORS SUCH AS HVAC CAN MAKE A SUBSTANTIAL DIFFERENCE IN THE SAMPLE WARMING RATE



manual

automated

- DATA FROM 2 ML VIAL IN CENTER CORNER OF CRYOBOX

Key observations

- STANDARD LN2 FREEZER AVAILABLE TODAY HAVE NO BUILT IN PROTECTION OR MONITORING OF INNOCENT SAMPLES THAT MAY BE REPEATEDLY EXPOSED DURING ROUTINE RACK PULLS

- AUTOMATED DEVICES PROVIDE PRECISION REQUIRED THROUGH TIMED, RECORDED AND CONTROLLED PROCESSES

- AUTOMATED METHODS REDUCE EXPOSURE TO TRANSIENT WARMING AND THE RISK OF CROSSING TG CONSIDERABLY (DEPENDING ON VOLUME AND POSITION 2 – 16 TIMES)
  - Insulating tower protects against warming through convection
  - Lower shelfs stay in the cooling environment when vial/cassette is pulled from lower rack
  - Temperature differences between shelfs during rack pulls are flattened
  - Time of rack being outside the LN2 environment reduced and controlled

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Conclusion

Conclusion

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- A CONTROLLED APPROACH INCL, AUTOMATED STORAGE SYSTEMS IS RECOMMENDED TO GUARANTEE COMPLETE THERMAL CONTROL OF THE SAMPLES

- HIGHER QUALITY IS ASSURED BY VIEWING USER ACCESS, SAMPLE INVENTORY, HISTORY, AUDIT-TRAIL REPORTS AND TEMPERATURE PREDICTION

- TARGETED AND NON-TARGETED SAMPLES ARE BETTER PROTECTED FROM WARMING ABOVE TG (GLASS TRANSITION) THROUGHOUT SAMPLE RETRIEVAL

- TRANSIENT WARMING SIGNIFICANTLY SLOWED DOWN FOR INNOCENT SAMPLES THROUGH ENGINEERING CONTROLS BASED ON BEST KNOWLEDGE OF ENVIRONMENTAL VARIABLES AND CONTRIBUTORS TO TRANSIENT WARMING

- FOR MANUAL APPROACHES AND SYSTEMS REGULARLY AUDITED SOPS ARE RECOMMENDED

- NOT TO FORGET: EMPLOYEES TRAINED ACCORDING TO BEST PRACTICES AND KNOWLEDGE ARE A KEY SUCCESS FACTOR

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About us

CGT Therapy Sample and Material Management

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Global sample/material storage and management with automation capabilities is one of the core competencies of Azenta Life Sciences. Transfer the risk associated with managing your cell and gene therapy assets to us to ensure secure storage, auditing, tracking, and delivery when you need it within one global, interconnected platform.

Complete Sample and Material Life Cycle Management

- Project management
- Clinical sample bioprocessing services (pre-analytical and analytical)
- Storage temperature capabilities:
  - — Electromechanical: Ambient to -80°C
  - — LN_2-based: -20°C to cryogenic (-196°C)
- Licenses, accreditations, and certifications include (but are not limited to): CAP, CLIA, FDA, GDP, GTP, GLP, ISO 9001, NABP, EMA, PMDA, and state licenses for tissue storage and distribution of materials
- Processes that protect the identity, integrity, and chain of condition, from the moment of receipt until a retrieval is requested

Experts in cold chain maintenance below glass transition temperature

Thank you!

For more information, please contact me:

markus.albertini@azenta.com

https://web.azenta.com/cell-and -gene-therapy

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JOINTLY CREATING THE FUTURE OF CELL & GENE THERAPY ORCHESTRATION