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Managing the Cold Chain to Minimize Pre-Analytical Variability

Dr. Alex Esmon
Senior Product Manager
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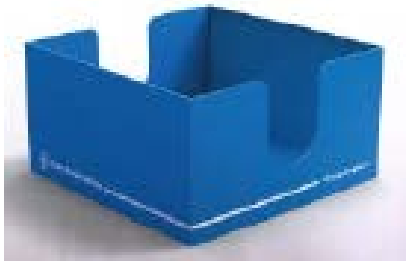
Today’s agenda

- We will discuss three scenarios
  - Importance of maintaining sample conditions
  - Concerns during Inventorying
  - Sample Transport
    - Unit transfer
    - Content transfer



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How Do I Maintain Sample Integrity?

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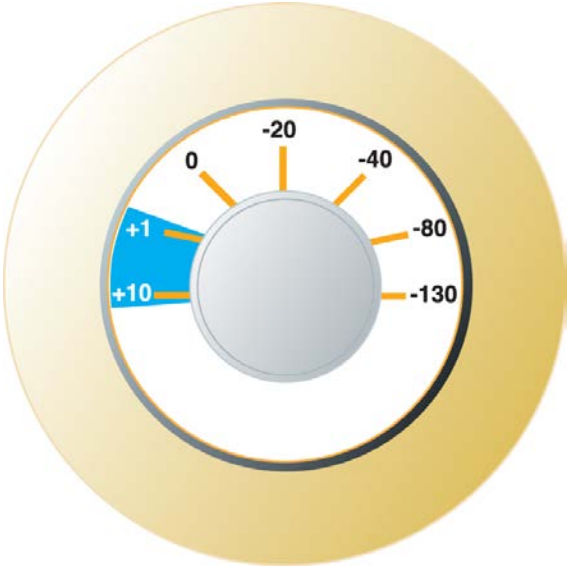
Inventorying ***and*** Maintaining Sample Integrity

- Ensuring sample integrity is paramount during a shipping or inventory - what's the point of shipping or inventorying samples that aren't worth saving?
- These processes can be accomplished in harmony
- Imperative to understand some basics about the freeze/thaw and ice phase transition process
- Follow practical tips for incorporation into lab SOP

Refrigerated Storage: the +4ºC Range

- ***Refrigerated samples need to avoid freezing!***
- ***Keep them in the +10ºC to +1ºC range if possible***



Going back and forth between frozen and thawed states is very bad for refrigerated samples - ice formation and cellular dehydration are very stressful and will cause mechanical damage to biological samples.

Some guidelines to follow:

- Guard against samples getting too warm
- Avoid dry ice for non-frozen samples
- A water/ice slurry is the best option for holding samples during the inventory process
- Minimize the duration and frequency of door openings - this will help the samples that are not actively being handled to maintain their integrity and keep the samples in the right temperature range

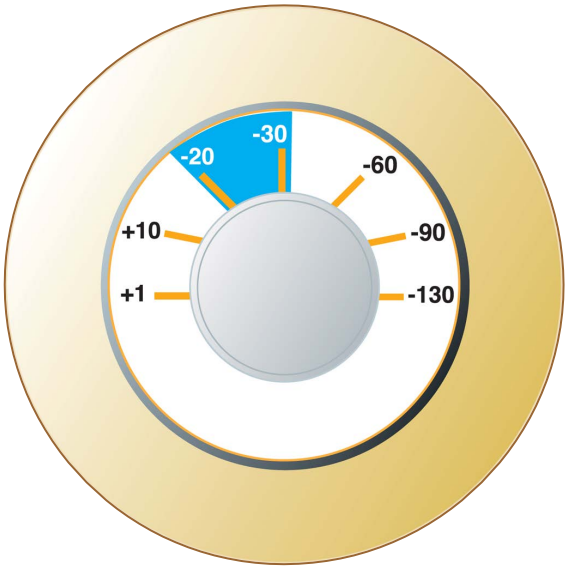
Frozen Storage: the -20ºC Range

- ***Frozen samples need to avoid thawing!***
- ***Keep them in the -20ºC to -30ºC range if possible***

The key here is to keep samples from thawing into the liquid state. The phase change from solid ice to liquid and back again is highly stressful for biological samples - ice formation can perforate cell walls or change the structure of compounds.

Some guidelines to follow:

- With samples in -20º freezers you have some options, but *speed is the key*
- Dry ice or bench-top coolers around -20ºC are better than just an ice bucket
- If using dry ice, be aware that enzymes in glycerol are not recommended to be frozen, so work quickly
- If an ice bucket is your only option, use a water and ice slurry for better temperature transfer than ice alone



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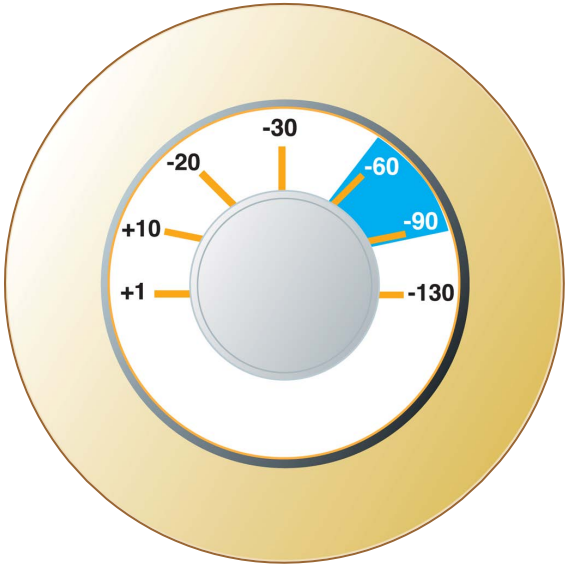
Ultra Cold Storage: the -80ºC Range

- ***Frozen* samples need to avoid *thawing*!**
- ***Keep them in the -60ºC to -90ºC range if possible***

Thawing refers to not only the change from ice to liquid but also the change from ice phase to ice phase. Just because it "looks frozen" doesn't mean changes aren't happening inside the sample.

Some guidelines to follow:

- Samples in the ultra cold storage range are riding the line between two forms of ice. As much as possible, these samples need to be handled using dry ice which has a temperature of about -70ºC. This will keep the samples from warming into a higher temperature range and will reduce undue stresses on the samples
- If these samples thaw, make sure you use a controlled freezing technique or equipment designed to control the rate of freezing (Mr. Frosty or a mechanical controlled rate freezer) to bring them back to the cryogenic range



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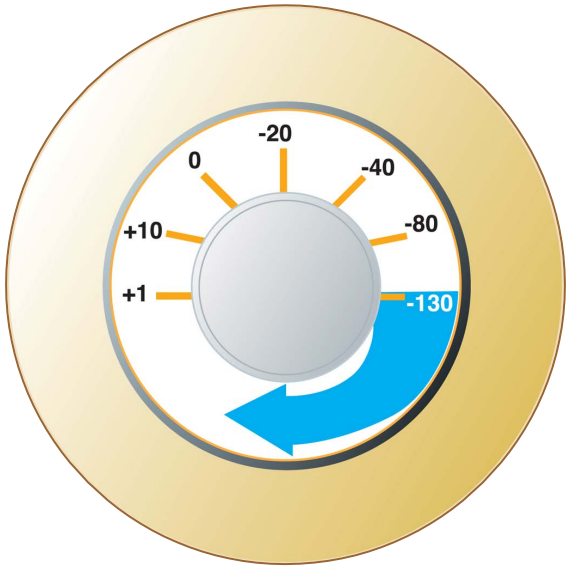
Cryogenic Storage: Frozen Means Frozen, Right?

- ***Frozen* samples need to avoid *thawing*!**
- ***Keep them below -130ºC if possible***

Thawing not only refers to the change from solid ice to liquid but also the change from ice phase to ice phase. Just because it "looks frozen" doesn't mean things aren't happening or changing.

Some guidelines to follow:

- Samples stored below -130ºC are in a "glassy state". They have crossed the *glass transition phase* and have taken on the properties of glass. Below this threshold, all known mechanical and metabolic activity within the cells stops.
- It is imperative to keep samples that are stored below the glass transition phase under this threshold at all times. Samples that toggle back and forth across this threshold can experience instantaneous ice nuclei formation which can seriously damage or destroy samples.
- If these samples thaw, make sure you use a controlled freezing technique or equipment designed to control the rate of freezing (Mr. Frosty or a mechanical controlled rate freezer) to bring them back to the cryogenic range.



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Summary

- ***Frozen* samples need to avoid *thawing*!**

  Follow the suggested techniques

- ***Refrigerated* samples need to avoid *freezing* and *thawing*!**

- If thawing occurs, be prepared with a suitable re-freeze plan that accounts for the needs of the samples

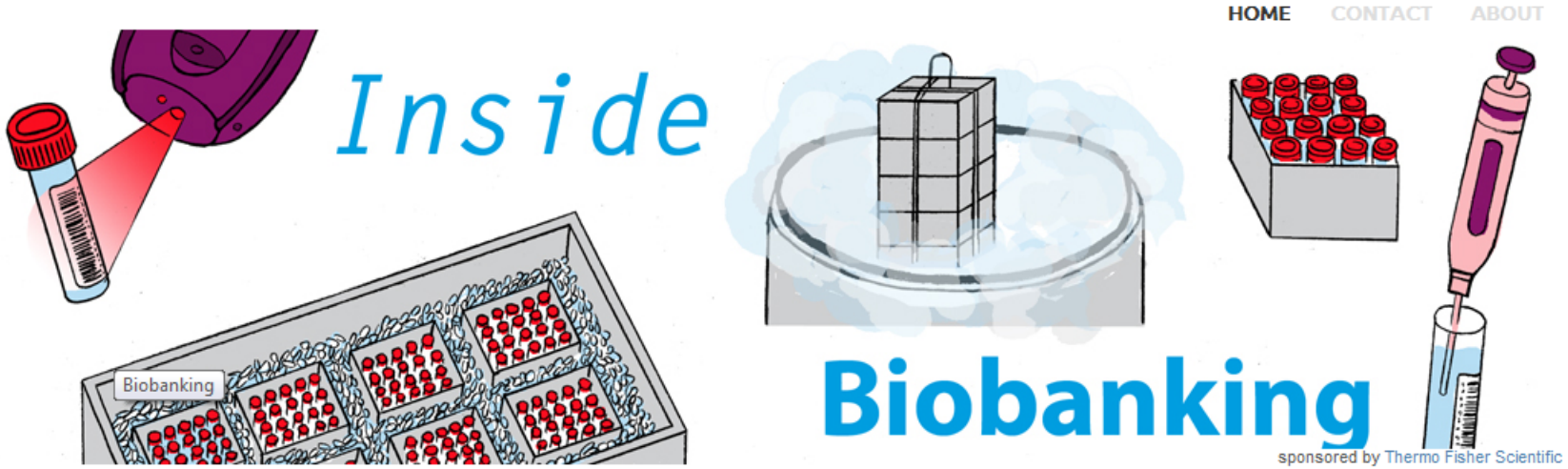
- Know where to find the tools you need

Useful Links and Resources:

Ice formation and Biological Impact:
http://www.crcpress.com/product/isbn/9780415247009

Cryopreservation theory and practice:
Thermo Scientific Cryogenic Preservation Guide

Accelerating Science: Biobanking



Biobanking

Biobanking for Global Prostate Cancer Research Collaboration

By **Amanda Maxwell** on April 30, 2015

During an educational workshop held at the Science of Global Prostate Cancer Disparities in Black Men Conference in Nassau, Bahamas in 2012, attendees discussed a range of biobanking and biorepository strategies that might help with collaborative research efforts into the disease.1 Prostate cancer in black men of African origin presents not only a different course *Read the rest of this article*

0 Comments

ACCELERATING SCIENCE

BIOBANKING

- **Collection (18)**
- **Preparation (7)**
- **Sample Analysis (6)**
- **Storage and Transport (28)**
- **Management (24)**
- **Case Studies (34)**

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Preparing for an Inventory Event

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It's Time to Inventory…*What Do I Need to Know?*

- Be aware of and familiarize yourself with the Biological Safety Level (BSL) of your samples and make sure you are in compliance with your organizational safety requirements
  - http://www.ors.od.nih.gov/sr/dohs/BioSafety/Pages/bio_chem_safety.aspx
- Understand what you will need to inventory your samples in various environments
- Have a plan for maintaining your log - Excel™, LIMS, logbook, database, sample tracking documents, etc
- Have a plan for "unidentified sample vials"

Refrigerated Storage: +1ºC to +10ºC

Remember: refrigerated samples need to avoid freezing!

- Avoid dry ice for non-frozen samples

- **What you need to have ready:**
  - Ice bucket/cooler with dry ice
  - Tube storage boxes
  - Conical tube storage racks
  - Absorbent pads
  - Markers for wet surfaces
  - Labels for wet surfaces
  - Logbook or database

Pro Tips

- If you silence your local alarm during the inventory, remember to turn it back on when you are done
- If you have alarms at a centralized location, make sure you advise facilities what you are doing
- Try to limit door openings to 60 seconds or less, and space them out by at least ten minutes
- Post a copy of the log you generate on the outside of the refrigerator
- When returning tubes to the refrigerator, try to avoid door storage – consider storing only your daily solutions and plates in the door (if applicable)

Frozen Storage: -30ºC to -10ºC

Remember: frozen samples need to avoid thawing!

- Dry ice or bench-top coolers are better than just an ice bucket

- **What you need to have ready:**
  - Dry ice containers or bench-top coolers
  - Tube storage boxes
  - Absorbent pads
  - Markers for wet surfaces
  - Labels for wet surfaces
  - Liquid transfer devices (manual or automatic)
  - Log book or database

Pro Tips

- If you have a local alarm on your freezer, make sure you silence it
- If you have alarms at a centralized location, advise those facilities what you are doing
- Try to limit door openings to 30 seconds or less, and space them out by at least ten minutes
- Post a copy of the log you generate on the outside of the freezer
- When returning tubes to the freezer, try to avoid door storage

Ultra Cold Storage: -40ºC to -80ºC

Remember: frozen samples need to avoid thawing!

- Dry ice is best; ice buckets not recommended

- **What you need to have ready:**
  - Two (2) dry ice containers
  - Tube storage boxes
  - ***Cryo Cradle***
  - Freezer racks
  - Absorbent pads
  - Cryo markers
  - Labels for wet surfaces
  - Liquid transfer devices (manual or automatic)
  - Log book or database

Pro Tips

- Consider adjusting your local alarms during the inventory
- If you have alarms at a centralized location, advise those facilities what you are doing
- Try to limit door openings to 30 seconds or less, and space them out by at least ten minutes
- Post a copy of the log you generate on the outside of the ULT unit

Cryogenic Storage: Below -130ºC

Remember: frozen samples need to avoid thawing!

- These samples need to spend a minimum of time away from cryogenic temperatures

- **What you need to have ready:**
  - Two (2) bench-top LN_2 containers
  - CryoCart (if available)
  - Cryo tongs or long forceps
  - Cryo tubes
  - Tube storage boxes
  - Liquid transfer devices (manual or automatic)
  - Heat block/water bath
  - Cryo markers rated for -200ºC
  - Log book or database

Pro Tips

- Deactivate your LN_2 level alarm during the inventory, but remember to turn it back on when completed
- Inventory one box at a time
- Stage your samples: cryo storage → bench-top LN_2 container #1 → **log information/re-label**→ bench-top LN_2 container #2 → cryo storage system (this will allow you to keep the samples in LN_2 for most of the process)

Summary

- Take a deep breath – this process can be accomplished!
- Make sure you have the tools you need for each situation
- Logging is key to risk mitigation
- Know where to find the tools you need
- Fisher Bioservices offers a complete range of services for inventory management

Useful Links:

www.fishersci.com

www.fisherbioservices.com

www.thermoscientific.com/content/tfs/en/products/cold-storage.html

www.thermoscientific.com/en/products/lab-consumables.html

Sample Tracking Documents

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How to Maintain Sample Integrity During Sample Transfer

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Product Management Team

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making connections winning together

Why Might You Need to Transfer Samples?

Sample Unit Transfer

- Consolidation of sample types, reagents, or other custom categories
- Maximizing storage density during re-inventory and organization
- Replacing old sample boxes
- Disposing unknown samples (temporary storage location)

Temperature Ranges:

•Ambient, refrigerated, -20ºC, -80ºC, < -130ºC (vapor phase LN_2)

Sample Content Transfer

- Compromised sample container
- Aliquot to improved storage format
  - Tube size
  - Improved writing space or label compatibility
  - Desirable format for future inventory requirements

Temperature Range

Ambient, refrigerated, -20ºC, -80ºC, < -130ºC (vapor phase LN_2)

Sample Unit Transfer - to a New Box

Transferring samples to a new box

- Inspect for mold, moisture buildup, aging, structural integrity
- Label box before pre-conditioning
- Pre-condition box
  - **+1 to +10ºC:** Use wet/slurry ice, cold packs, or walk-in refrigerators
  - **-10 to -30ºC:** Use dry ice (DI) station
  - **- 40 to -80ºC:** Use dry ice station
  - **< -130ºC:** Use Cryocart/LN_2 stations or DI (box by box)
- Transfer tubes using picker/tweezer tool; don't touch the tube body

Maintaining Temperature

- Use a metal sleeve to hold the boxes, for example CryoCradle™
  - Acts as a heat sink to remove heat from the specimen
  - Helps to maintain temperature closer to that of the storage temp
  - 12ºC colder vs without a sleeve
  - Serves as a physical barrier between box and dry ice

Avoid freezing refrigerated samples and thawing frozen samples!

Sample Unit Transfer - to New Storage

Limit time of exposure and frequency of door opens

- Transport vessels:
  - **+1 to +10ºC:** Insulated cooler with ice or cold packs
  - **-10 to -30ºC:** Dry ice (DI) cart or insulated cooler with DI
  - **- 40 to -80ºC:** DI cart or insulated cooler with DI
  - **< -130ºC:** Cryocart or dry shippers with temperature monitor (for temporary hold time)
- Depending on the process, if you are transporting right after re-inventory, then transport samples rack by rack

Avoid freezing refrigerated samples and thawing frozen samples!

Sample Unit Transfer - to an Off-site Facility

: Transporting refrigerator, freezer, or LN_2 tanks

shippers

per

vice provider

- What is the shipper hold temperature?
- What is the IATA/DOT biohazard policy

LN_2 Trucks

- Cooled by LN_2 pipes - units not powered
- Multiple temperature in one trip
- Design has exemption for infectious material?
- Multiple solenoid for control and monitoring
- Back-up & redundancies

Generator Trucks

- Mechanically powered - units have power
- No cryogenic temp
- Mechanical power risks on hot days
- Stability of storage unit parts
- Not recommended to run during transport (oil, motor, etc).

Avoid freezing refrigerated samples and thawing frozen samples!

Sample Unit Transfer - to an Off-site Facility

- Shipping via LN_2 vs. Generator Trucks: Transporting refrigerator, freezer, or LN_2 tanks
- Service provider-assisted pre-qualified shippers

  +1 to 10ºC: Gel pack shipper
  -10 to 30ºC: Dry ice (DI) or phase change shipper
  - 40 to -80ºC: DI or phase change shipper
  < -130ºC: DI shippers of various sizes

- Things to note if shipping without a service provider

  Are samples category A or B?
  What is the shipper hold temperature?
  What is the IATA/DOT biohazard policy

Dry Shipper (Vapor phase LN_2)

Selection of dry shipper
Canister or Racking options for boxes
Hold times for the shipper
Temperature monitoring
Orientation and labeling factors

http://connect.fisherbioservices.com/10-things-about-dry-shippers
http://blog.fisherbioservices.com/

Avoid freezing refrigerated samples and thawing frozen samples!

Fisher BioServices Blog—an excellent resource

Fisher BioServices
A Thermo Fisher Scientific Brand

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Cell Therapy Logistics: Beyond the Basics

Posted by Dan H. O'Donnell on Apr 30, 2015 11:02:58 AM

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My previous eBook, *Commercially Successful Cell Therapies: Navigating the Ultra Cold Chain Distribution Minefield*, and several of my blogs, have discussed the basics of building a successful logistics strategy for

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Sample Content Transfer - When Thawing is Required

- ***Gently*** agitate if needed to accelerate thawing
- When sample is thawed, gently wipe exterior of container with disinfectant such as alcohol to prevent contamination during sample transfer process

Carefully following good thawing practices will help you maintain sample quality!

R
p

- YouTube Video: Thawing Cells

Why Thaw quickly?

Thawing and re-freezing is the most stressful series of events a frozen biological sample can undergo. Thawing quickly provides the best chance to “outrun” the formation of harmful ice nuclei during the warming process. Generally speaking, The faster the better.

Sample Content Transfer - Sample Transfer

Sample Transfer Recommendations

- Sample transfer procedures should be performed only in an environment that is in accordance with safety practices and procedures
- Ensure that the liquid transfer device is properly calibrated and ancillary products are within reach at the

It is critical to maintain sample quality during the transfer process!

Resources for sample transfer best practices:

- Biological Safety & Compliance http://www.ors.od.nih.gov/sr/dohs/BioSafety/Pages/bio_chem_safety.aspx
- Cell Culture Cafe Increase Pipette Efficiency Webinar

Sample Content Transfer - Sample Identification

Sample Identification

- Ensure the new sample container is appropriately labeled for future identification
- Remember to log that the sample was thawed and transferred to a new sample container
- Cool your sample to refrigerated

Log not only the sample identification information, but transfer history

Resources for sample identification best practices:

- Thermo Scientific Cryopreservation Guide
- ATCC http://www.atcc.org/

Summary

Avoid freezing refrigerated samples and thawing frozen samples!

- Sample unit transfer – to a new box or new location
- Sample content transfer
  - Thaw samples as quickly as possible using a warming bath at 37ºC and gently agitate if necessary
  - Disinfect the container before the sample transfer process to prevent contamination
  - Transfer samples using calibrated pipettes and appropriate plastic products
  - Securely cap or seal new sample containers and ensure labeling for future identification is complete and logged
  - Properly control freezing rate appropriate for sample type
  - Record sample transfer details, source location, and new container identification and location

Content Presented Today

http://acceleratingscience.com/biobanking/

Please visit this content anytime!

A full team is here to support you—please stop by!

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Erik Steinfelder

We are here to help improve your bio-banking experience