BioCoR

Advancing the science, technology
and practice of bio-preservation

Using thermodynamic principles and practical realities to select a storage temperature

Allison Hubel, PhD

University of Minnesota

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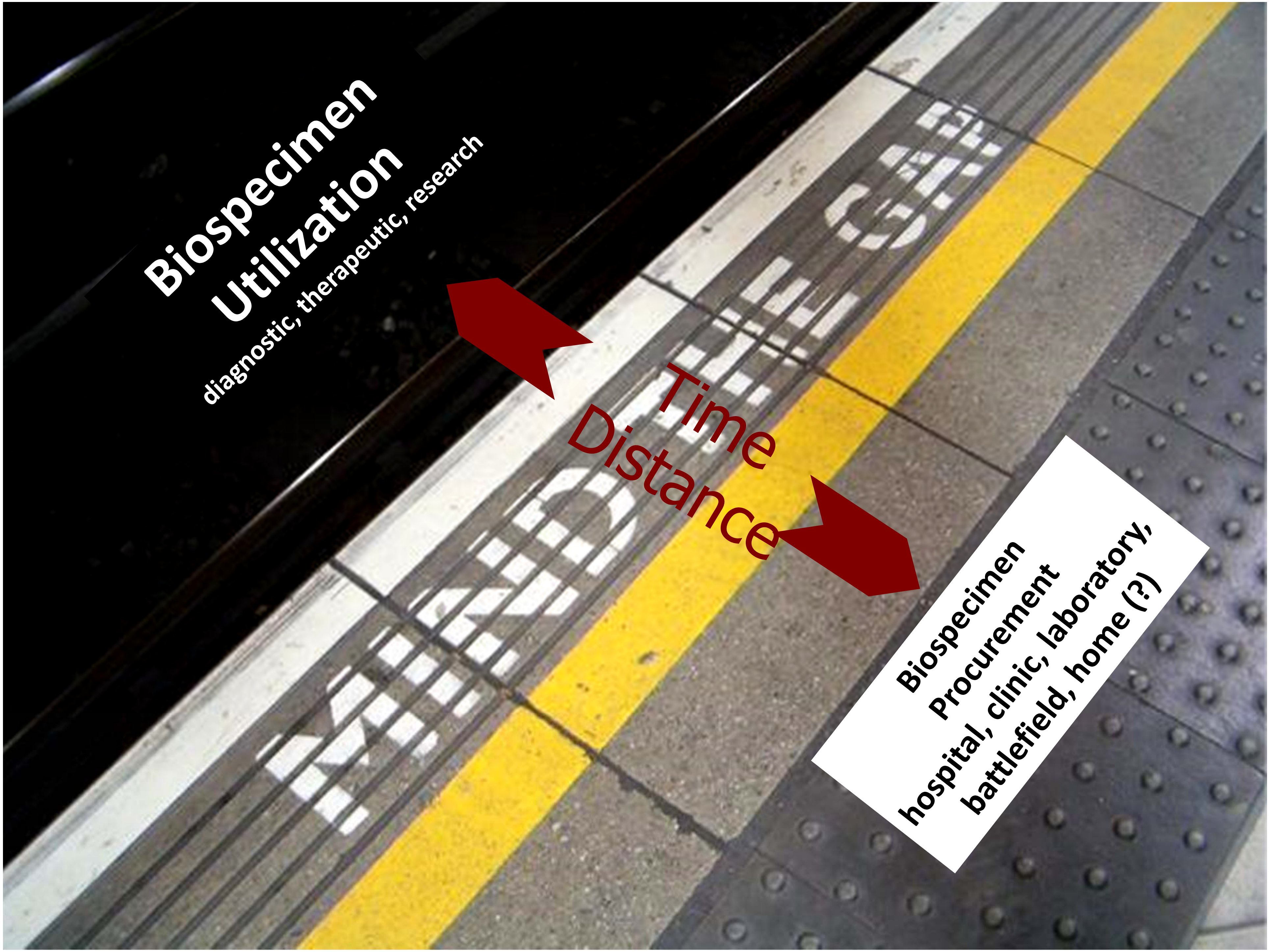
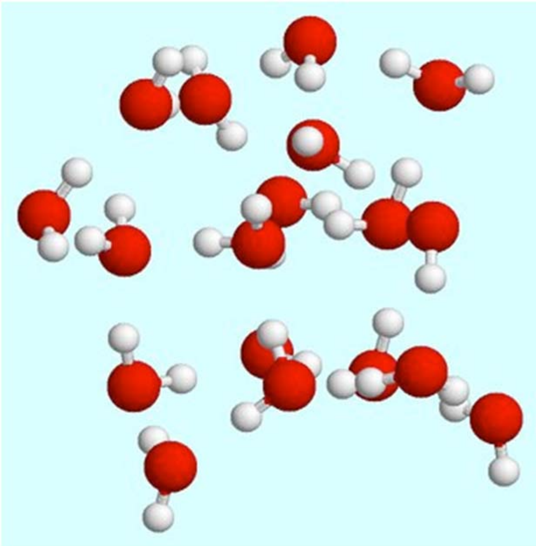
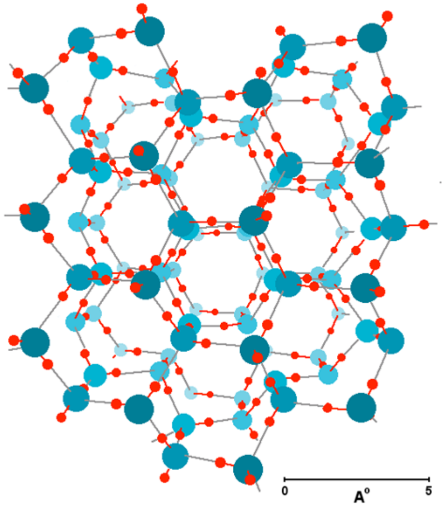

Biospecimen
Utilization
diagnostic, therapeutic, research
Time
Distance
Biospecimen
Procurement
hospital, clinic, laboratory,
battlefield, home (?)

Changes in molecular mobility



Water: liquid form

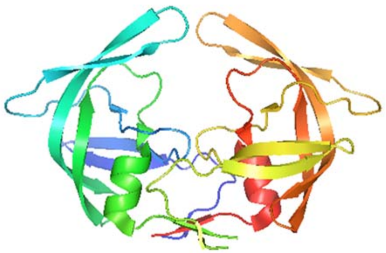
Reduced mobility



Water: solid form

Protein

+



Proteases

Reduced rates of reaction

Reduced activity

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Life-cycle of a biospecimen

Freezing
Storage
Shipping
Thawing

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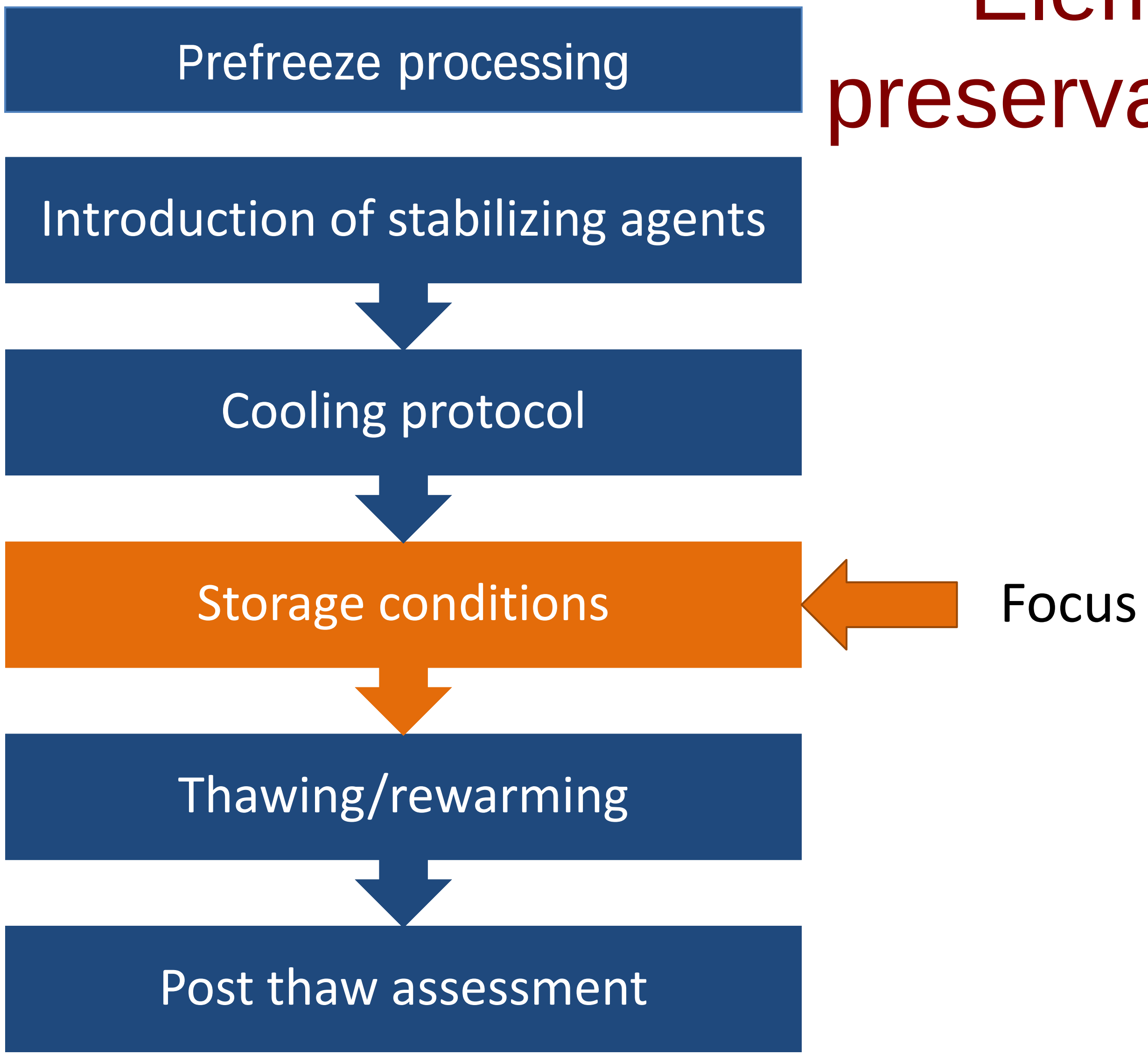
Elements of a preservation protocol



Storage Options

Mechanical Freezers
-20 C, -80 C, -153 C

Liquid nitrogen
storage (-196 C)
•Liquid phase
•Vapor phase

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The freezing process influences selection of the storage temperature

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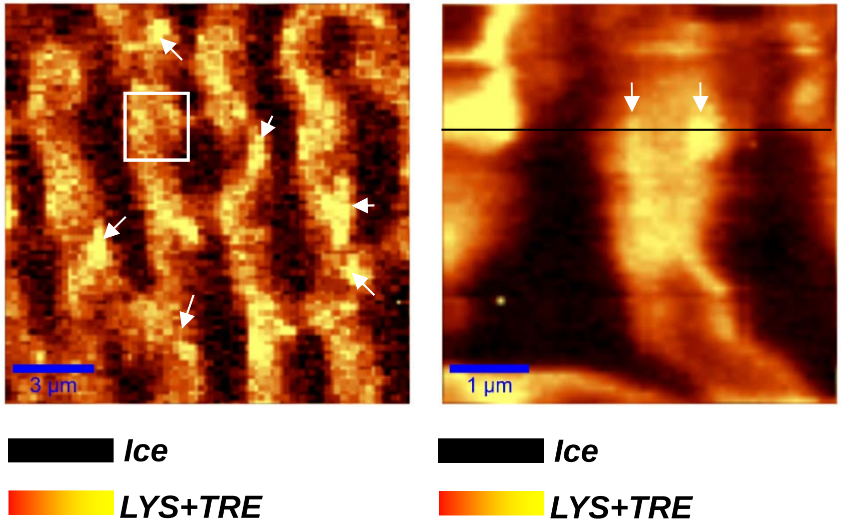
What do you know about storage of biospecimens because you have eaten a popsicle?

You can have solid and liquid together at the same time

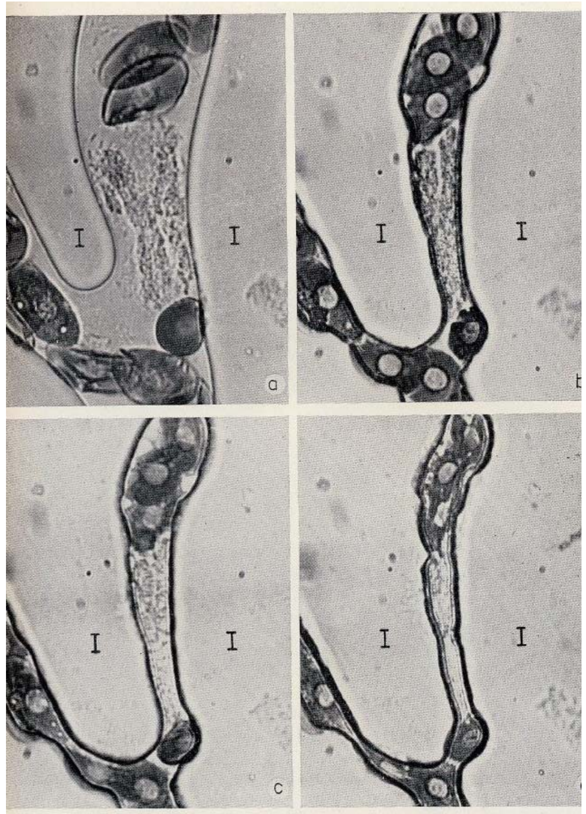
Solute is not incorporated into the ice

Complex mixtures freeze over a range of temperatures (not a single temperature)

Partitioning of biospecimen during freezing



- Water is removed in the form of ice
- Proteins/cells partitioned into gap
- Size of gap decreases with decreasing temperature
- Concentration in gap increases with decreasing temperature



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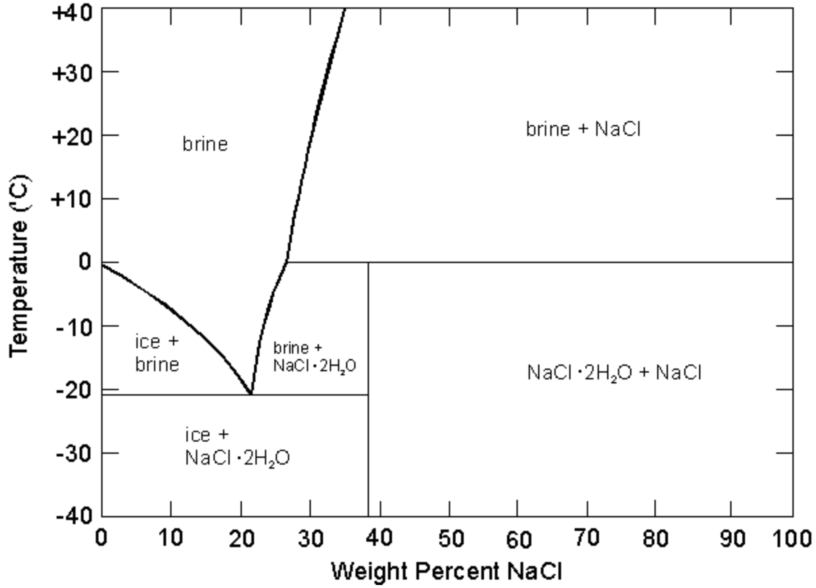
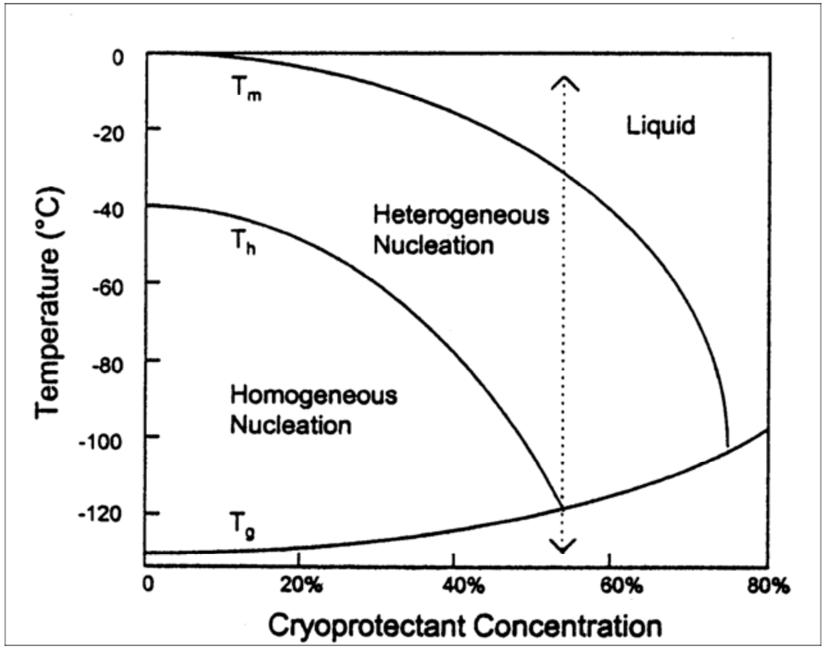
When does freezing end?





Option #1: eutectic

- Assumes that sample fully solidifies
- T_{eut}= -21 C (isotonic saline)

Option #2: glass transition temperature

- Assumes sample partially vitrifies
- T_g= -120 C (10% DMSO solution)
- T_g= -132 C (pure water)

Summary: freezing of aqueous solutions

- Biospecimens are complex mixtures
- Complex mixtures freeze over a range of temperatures
- Specimen is partitioned into unfrozen solution
- Sample is not fully frozen until eutectic or glass transition temperature
- *Store at a temperature where sample is fully frozen*

What do I do if I do not know the phase diagram for my solution?

<u>Option #1:</u> Estimate glass transition temperature mathematically:

$T_g(\text{mixture})=T_{g1}\,(1\text{-}x)+T_{g2}x+k^*x\,^*(1\text{-}x)$

T_g=glass transition temperature
k=interaction parameter
X=weight fraction of component

Options, cont.

Option #2: Check scientific literature for stability studies

- Literature review being published in Biopreservation and Biobanking in June

Option #3: Use your QA/QC program for continuous improvement

- More on this later

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Selection of storage temperature: suppressing activity of degradative molecules

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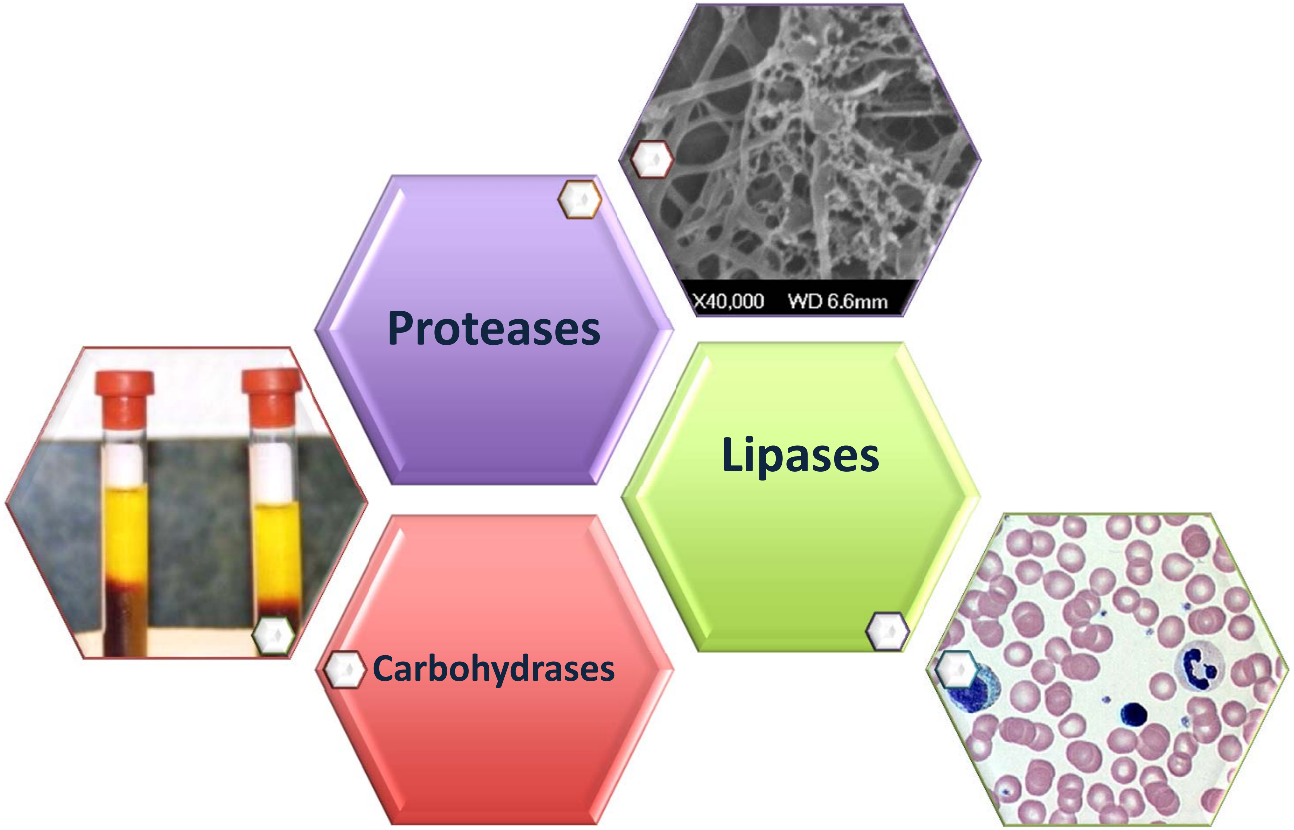
Degradative molecules are present in every sample



Activity of degradative molecules

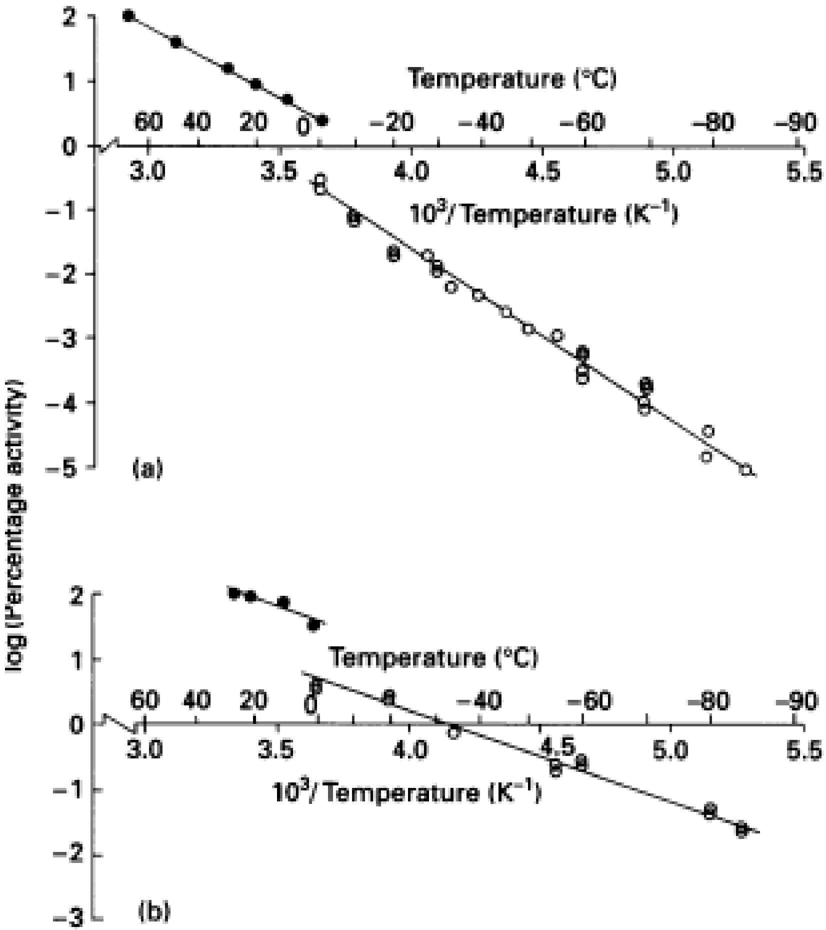
Protein activity is a function of its dynamics

Reducing temperature
reduces activity of molecule

Activity can be described by
Arrhenius Equation

$$k = A \cdot e^{-Ea/(R \cdot T)}$$

There is a threshold temperature below which no activity is observed β-glucosiadase activity

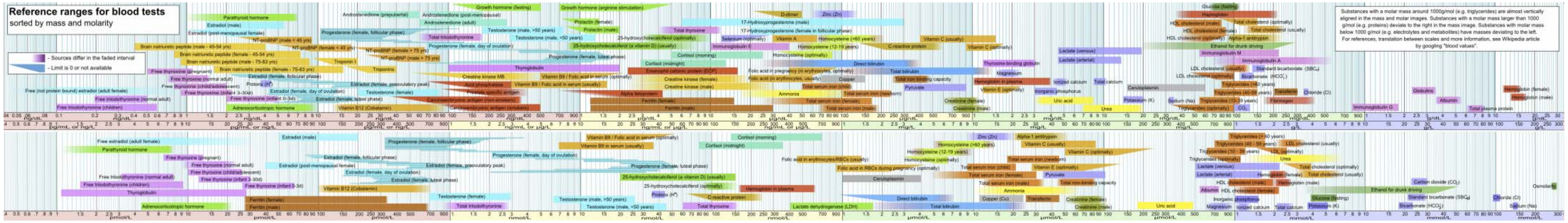


More, Biochem J, 1995

Enzymatic activity with temperature

- Reduced temperatures reduces activity of enzymes
- Measurable activity observed at low temperatures

Plasma Proteome



Low temperature activity

- Conventional wisdom: inactivation by ~-53 C for most proteins
  - ➢ RNAase activity has been measured down to -93 C
  - ➢ β-glucsoidase activity down to -70 C
- Low temperature activity of many enzymes have not been measured
- *Samples should be stored at temperatures below which degradative molecules are inactivated*

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Factors that influence sample stability when in storage

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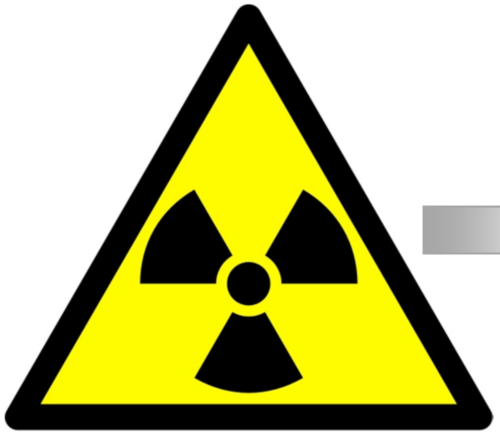
Temperature Fluctuations

Result from accessing of working repositories

Amplitude and frequency vary with location in the repository and methods used for retrieving samples

Background ionizing radiation



- Accumulated damage
- DNA scission
- Cell death

This damage mechanism has been studied for cells.
Not clear if other biospecimens experience the same

Summary

- Minimizing temperature fluctuations is also desirable
  - Training of biorepository workers
  - Controlling access to repository

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The role of QA/QC on selection of storage temperature and continuous improvement

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Potential sources of degradation

- Collection
- Extraction
- Storage

Monitor using QA/QC program

Continuous improvement

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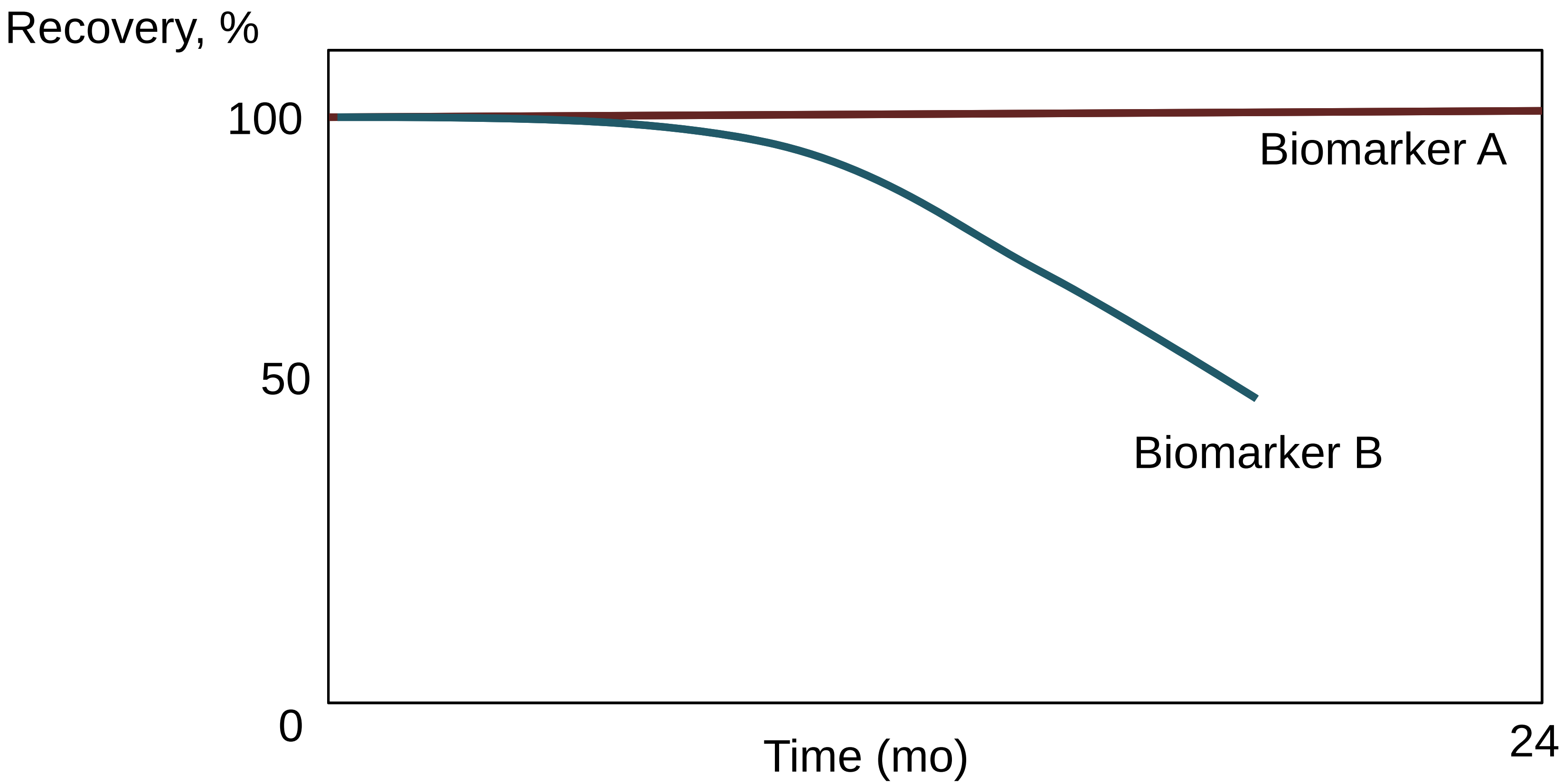
Testing sample stability



QA/QC program finds Biomarker B is not stable

- Samples are not stored correctly

Continuous Improvement

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Upcoming article

BIO-2013-0064-ver9-Hubel_1P
Type: review-article

REVIEW ARTICLE

BIOPRESERVATION AND BIOBANKING
Volume 12, Number 3, 2014

DOI:10.1089/bio.2013.0064

Storage of Human Biospecimens: Selection of the Optimal Storage Temperature

Allison Hubel,[1,2] Ralf Spindler,[1,2] and Amy P.N. Skubitz[1,3]

Millions of biological samples are currently kept at low tempertures in cryobanks/biorepositories for long-term storage. The quality of the biospecimen when thawed, however, is not only determined by processing of the biospecimen but the storage conditions as well. The overall objective of this article is to describe the scientific basis for selecting a storage temperature for a biospecimen based on current scientific understanding. To that end, this article reviews some physical basics of the temperature, nucleation, and ice crystal growth present in biological samples stored at low temperatures (−20°C to −196°C), and our current understanding of the role of temperature on the activity of degradative molecules present in biospecimens. The scientific literature relevant to the stability of specific biomarkers in human fluid, cell, and tissue biospecimens is also summarized for the range of temperatures between −20°C to −196°C. These studies demonstrate the importance of storage temperature on the stability of critical biomarkers for fluid, cell, and tissue biospecimens.

- More complete discussion of these scientific principals

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Literature review: storage stability studies

Biofluid specimens

- Blood
- Plasma
- Serum
- Urine

Cell Specimens

Tissue Specimens

Temperature (subzero)

Duration

Analyte(s)

Stability

Summary

- Store at temperatures below which the sample is fully solidified
- Store at temperature below which the activity of all degradative molecules are inactive
- Verify stability

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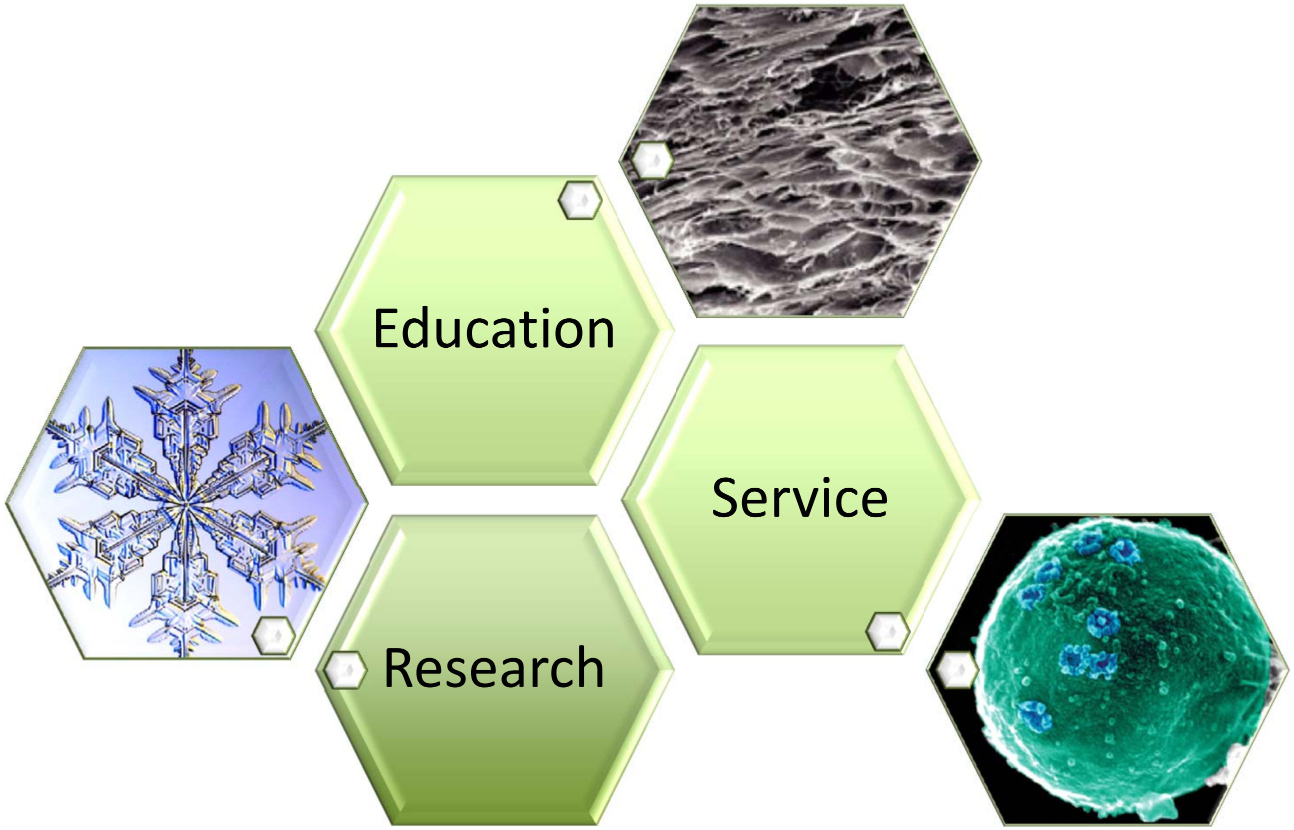

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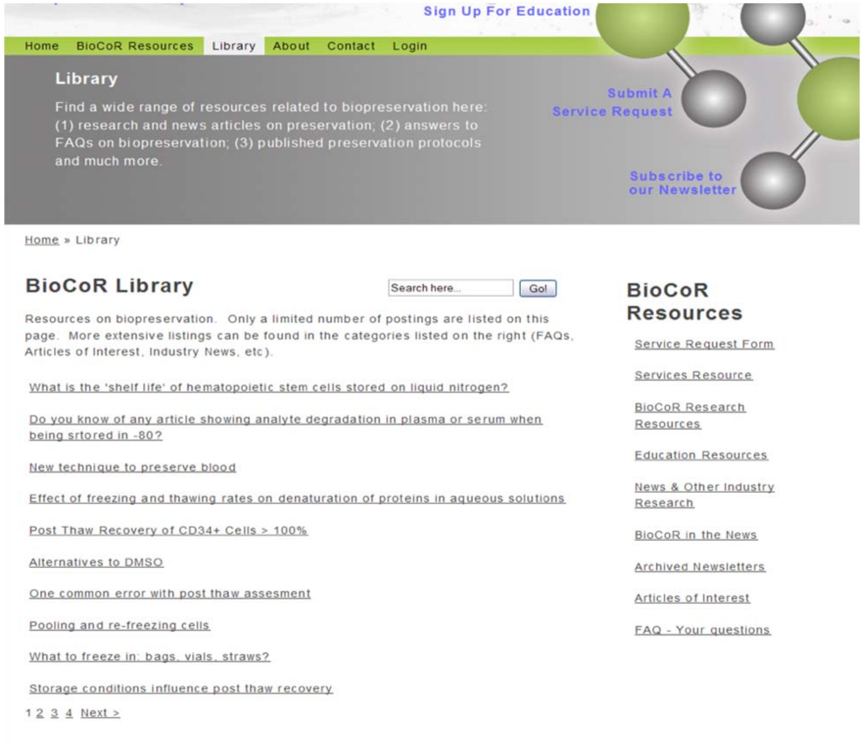
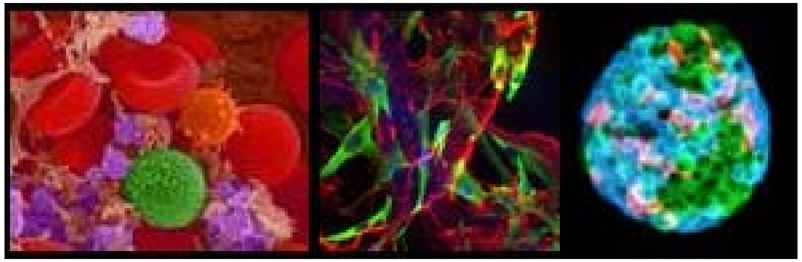
Education Resource

BioCoR library





Preservation of Molecular, Cellular and Tissue Biospecimens

Endorsed by ISBER

Preservation of Cellular Therapies

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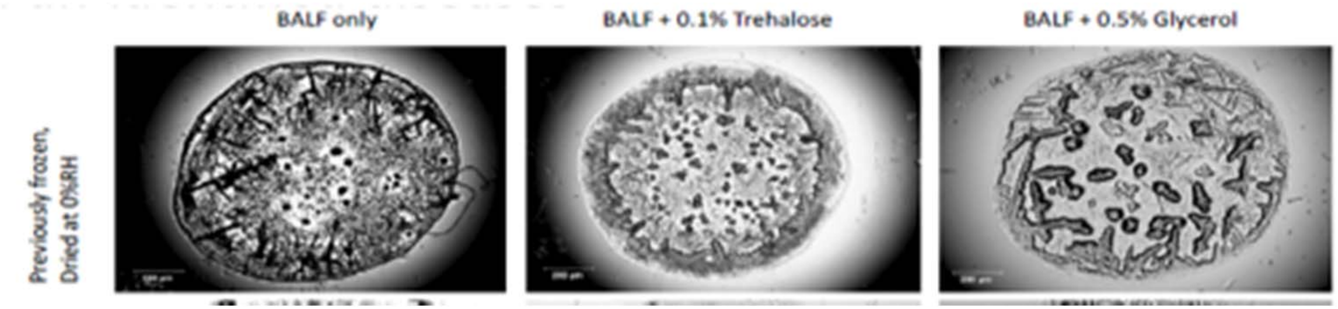
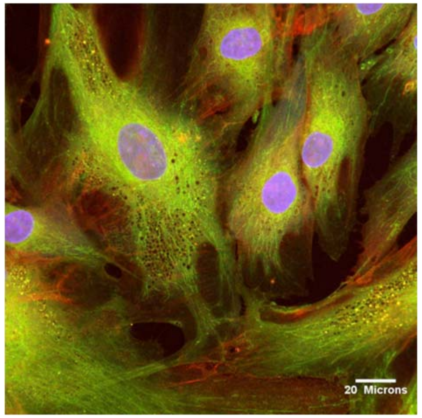
BioCoR Research

Dry State Storage of Plasma



QC tests for UCB



Improving preservation of MSCS

All projects are NIH funded

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Acknowledgements

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