BioCoR

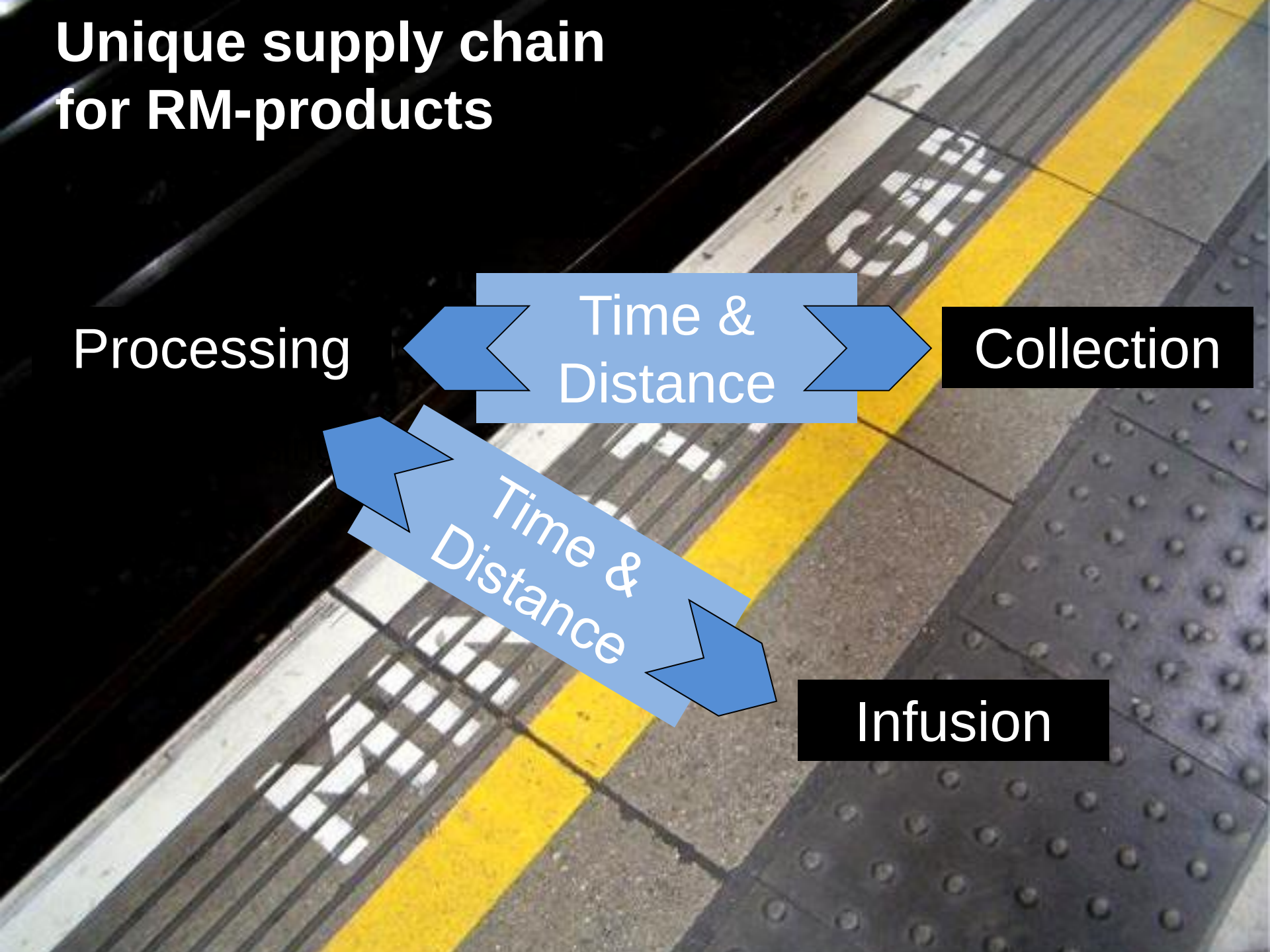
Advancing the science, technology and practice of bio-preservation

The role of preservation in the variability of RM products

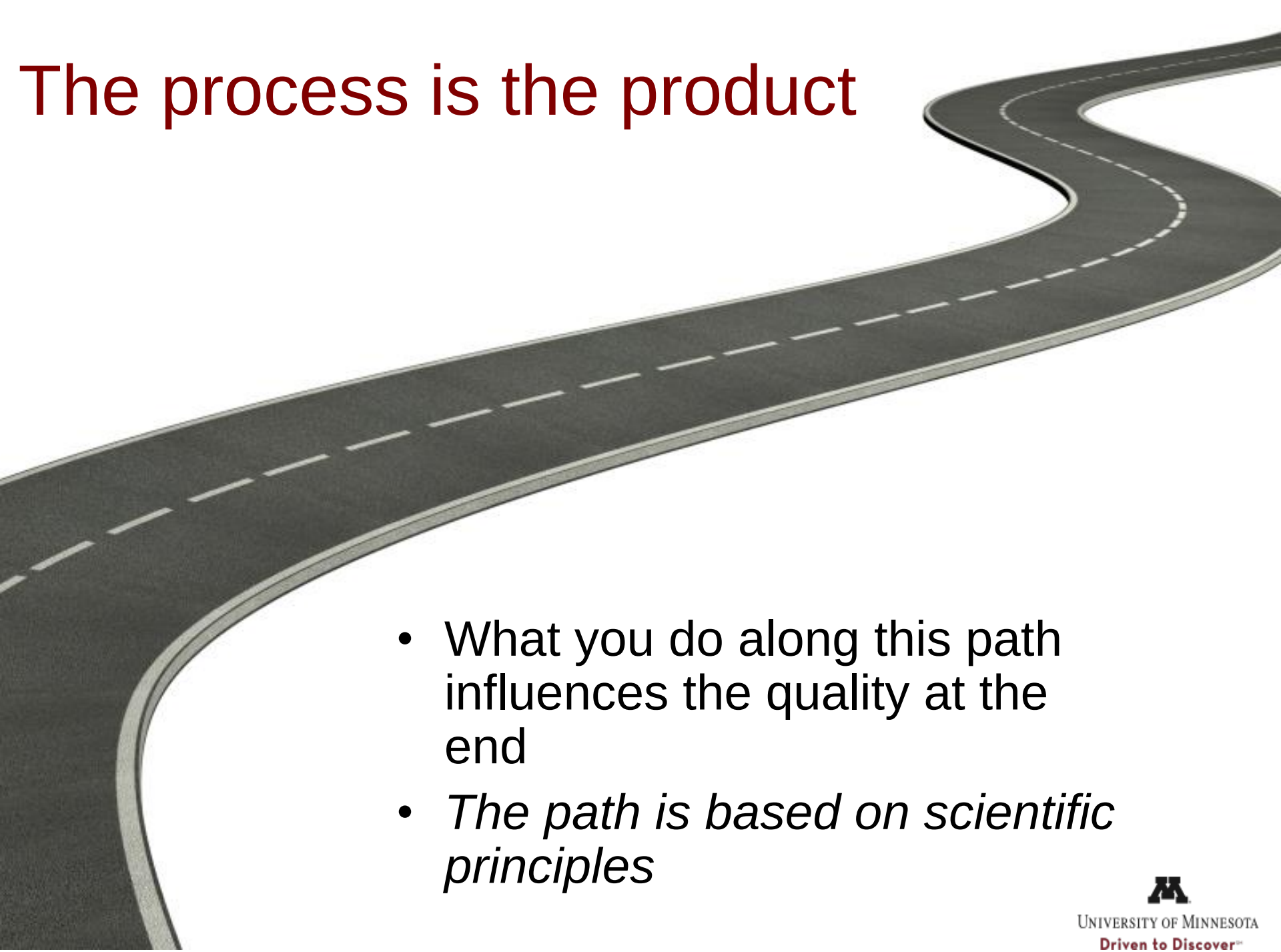
Allison Hubel, PhD

University of Minnesota

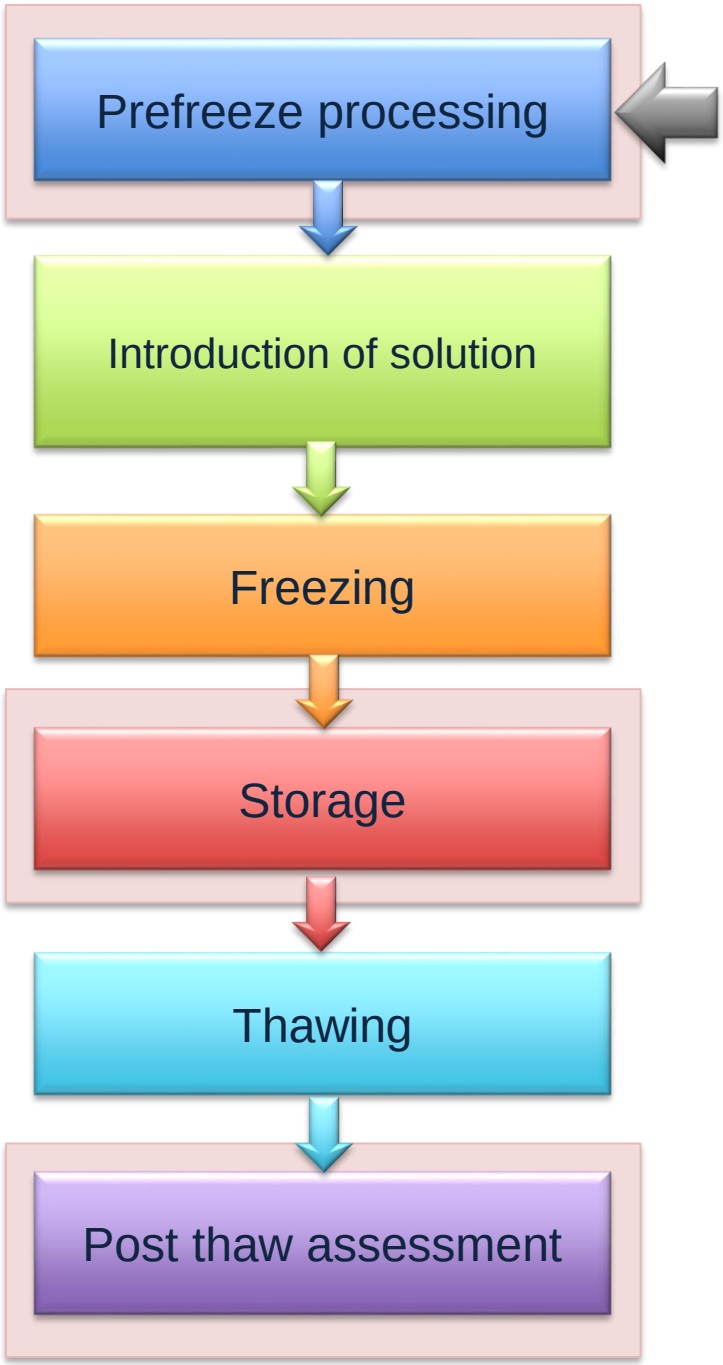
Unique supply chain for RM-products



The process is the product



- What you do along this path influences the quality at the end
- *The path is based on scientific principles*



Components of a Cryopreservation Protocol

- Each step can contribute to the variability
- Three steps will be briefly discussed.

Post thaw recovery is influenced by pre-freeze processing

Cell collection

Standard

- The source of the cells,
- Type of container,
- Anticoagulants or other additives in the collection tube,
- Delay time between collection and processing,
- Temperature at which the sample was held during the delay,
- Centrifugation speed,
- Post-centrifugation delay.

Additional elements for apheresis products :

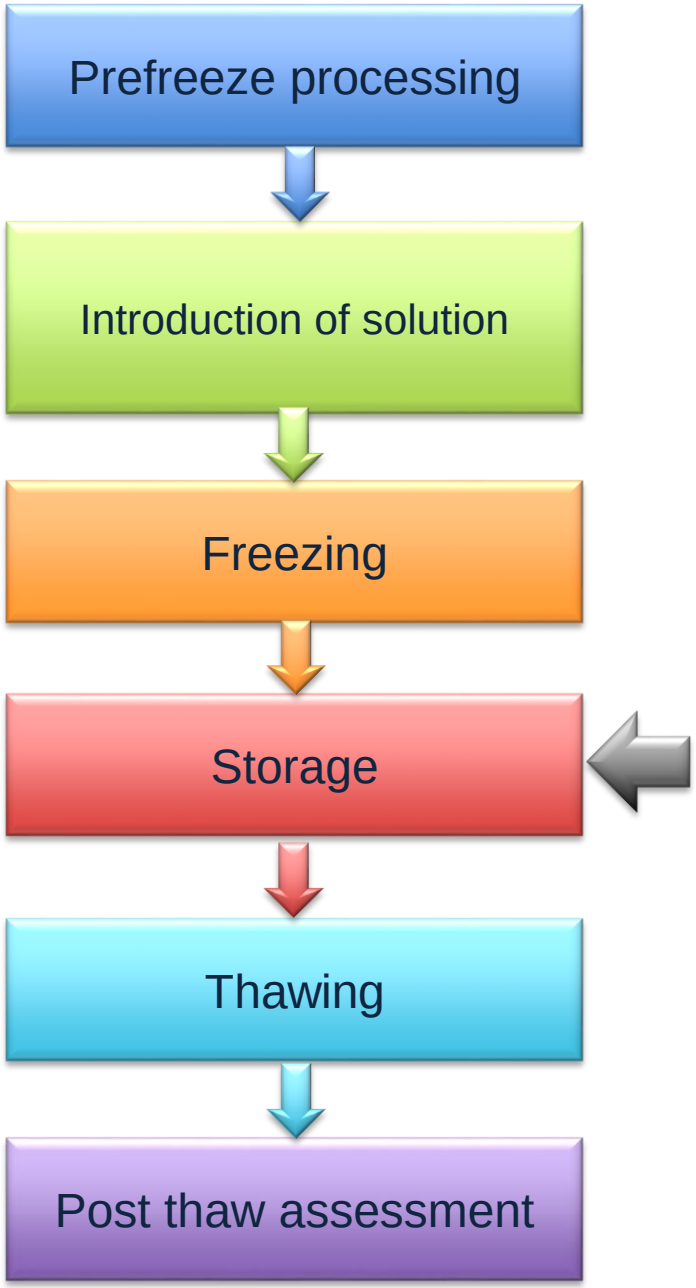
- Anesthesia used,
- Collection technique (location, etc.),
- Volume collected,
- Number of nucleated cells harvested,
- Filtration,
- Separation of mononuclear cells.

Annotation is critical

- *SPREC Standards from ISBER*
- *AABB Standards*

Pre-freeze processing

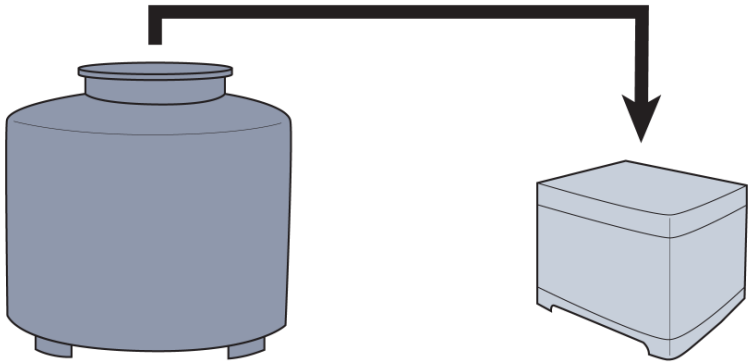
- Ex vivo manipulation
  - Isolation
  - Culture
  - Selection of subpopulations
  - Genetic modification
  - Centrifugation/washing
- Case studies:
  - Hepatocytes
  - MSCs



Components of a Cryopreservation Protocol

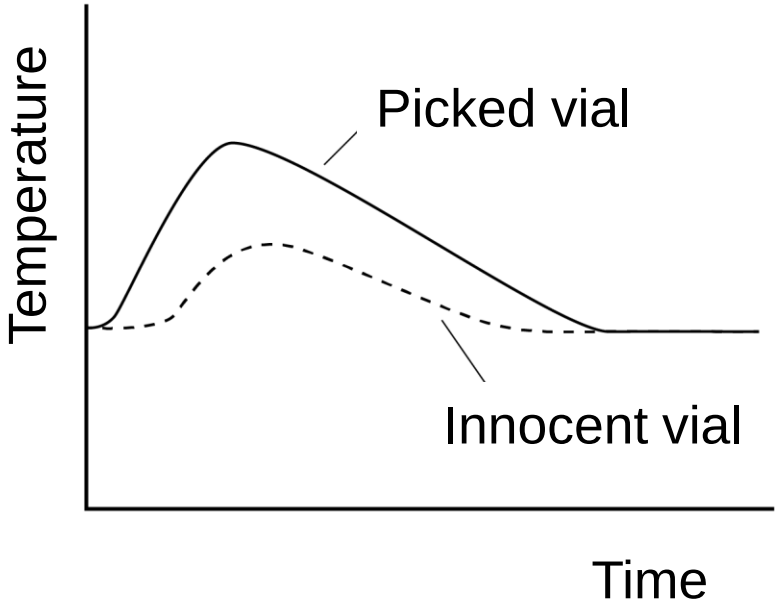
Poor practices result in diminished shelf life of the product

Stability in storage



Long-term storage unit

Sample carrier



Transient warming events (TWE)

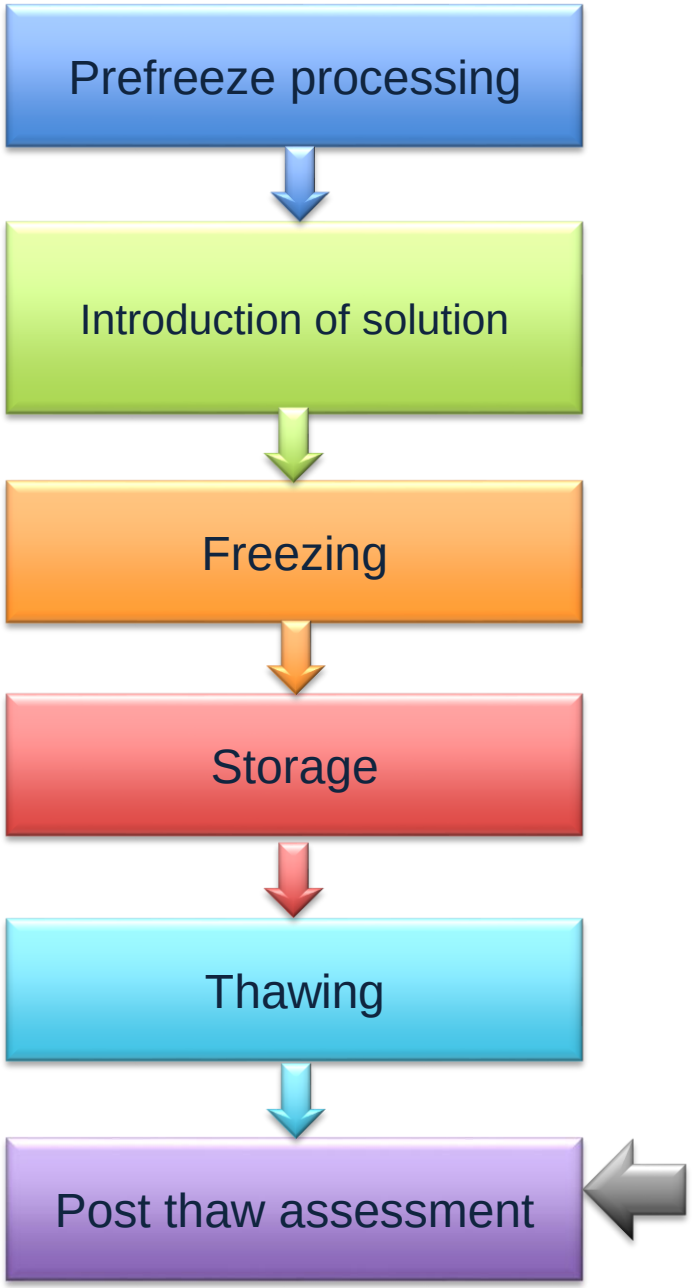
TWE and stability in storage

- Typically, sub-lethal damage
- Biomarker: apoptosis

Solutions:

- Training for individuals working in repository
- Limiting access and frequency of access
- Technology designed to reduce temperature excursions

Components of a Cryopreservation Protocol

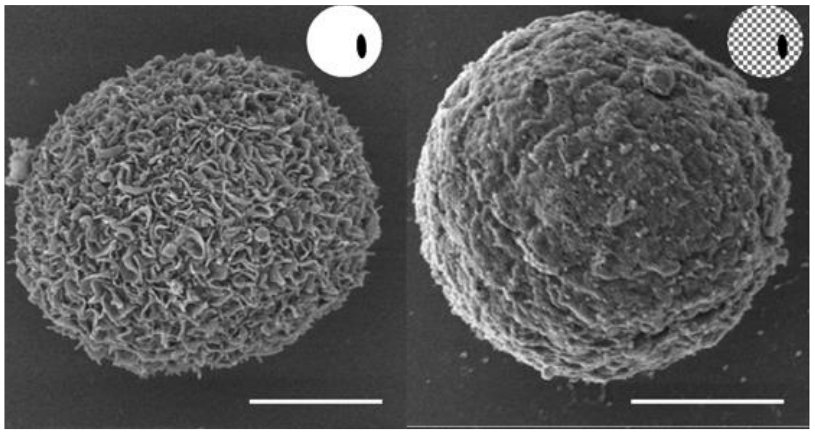


It is easy to do post thaw assessment poorly

Use measures that matter

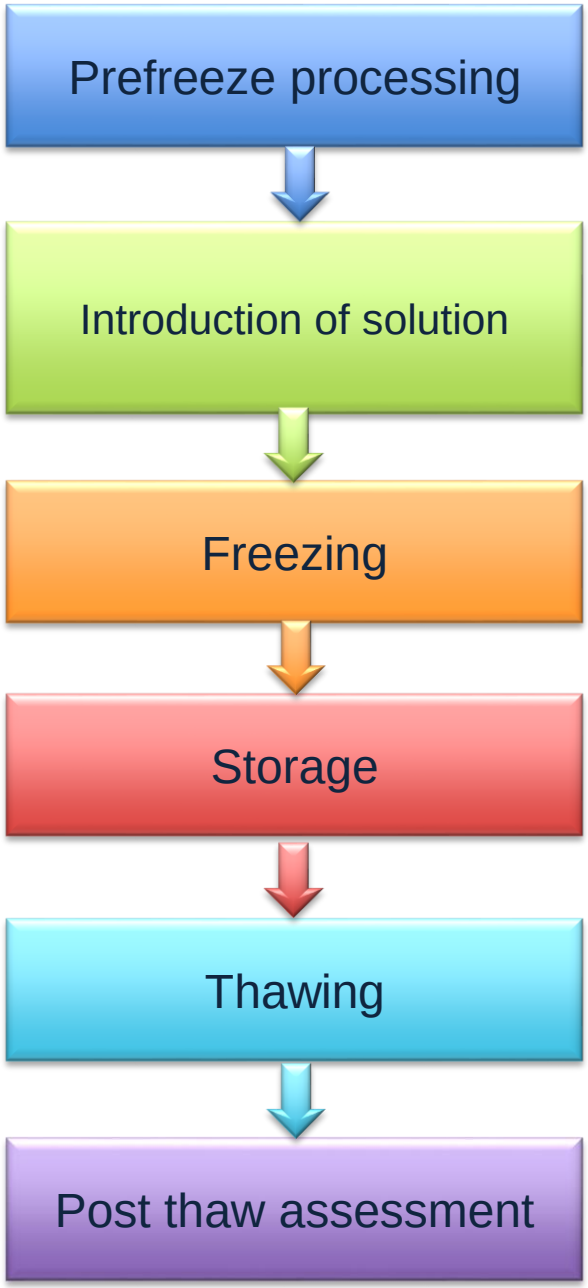
Assessing the viability of a frozen and thawed cell is not the same as assessing the viability of a cell

Pre-freeze Post-thaw



Sources of variation in post thaw assessment

- Failing to calculate cell losses due to lysis,
- Failure to account for the effects of post thaw apoptosis,
- Failure to optimize assay for frozen and thawed cells,
- Using a single measure or one that does not correlate with function.



Summary

- *Seemingly subtle changes can have a profound effect on post thaw recovery,*
- *Understanding the scientific basis for each step is critical in preventing poor outcomes.*

Physics vs. the FDA

- Case study: mapping CRF, temperature deviations when freezing 80 versus 40 vials,
- Larger ‘lots’ are desirable,
- More mass increases heat transfer requirements,
- Larger lots may > variability.

Protocol Drift

"We do it this way because that is the way that we do it"

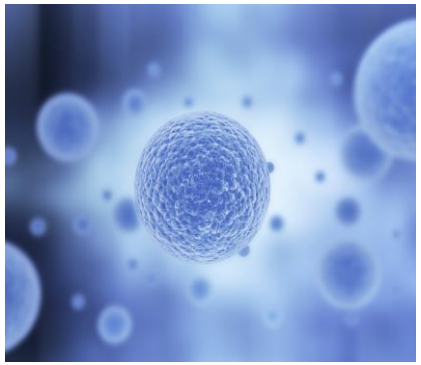
Case study: thawing of LN_2 units

QA/QC systems

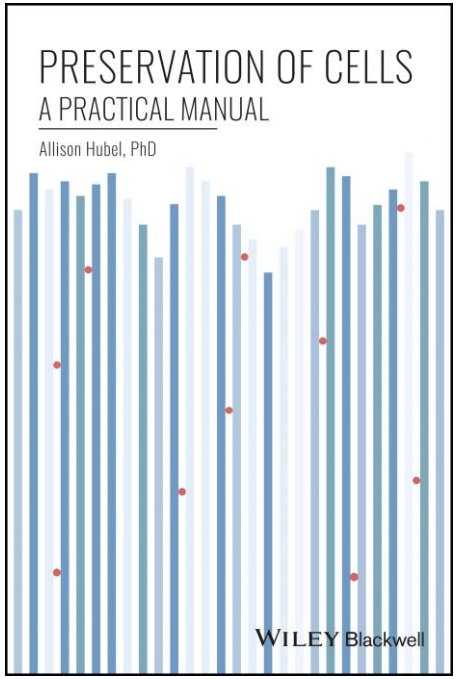
- *Protocols*
- *Training*
- *Auditing*
- *Proficiency testing*

Help with the fundamentals

Short courses



Newsletter



Hands on training

BioCoR library

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