Improving preservation practice

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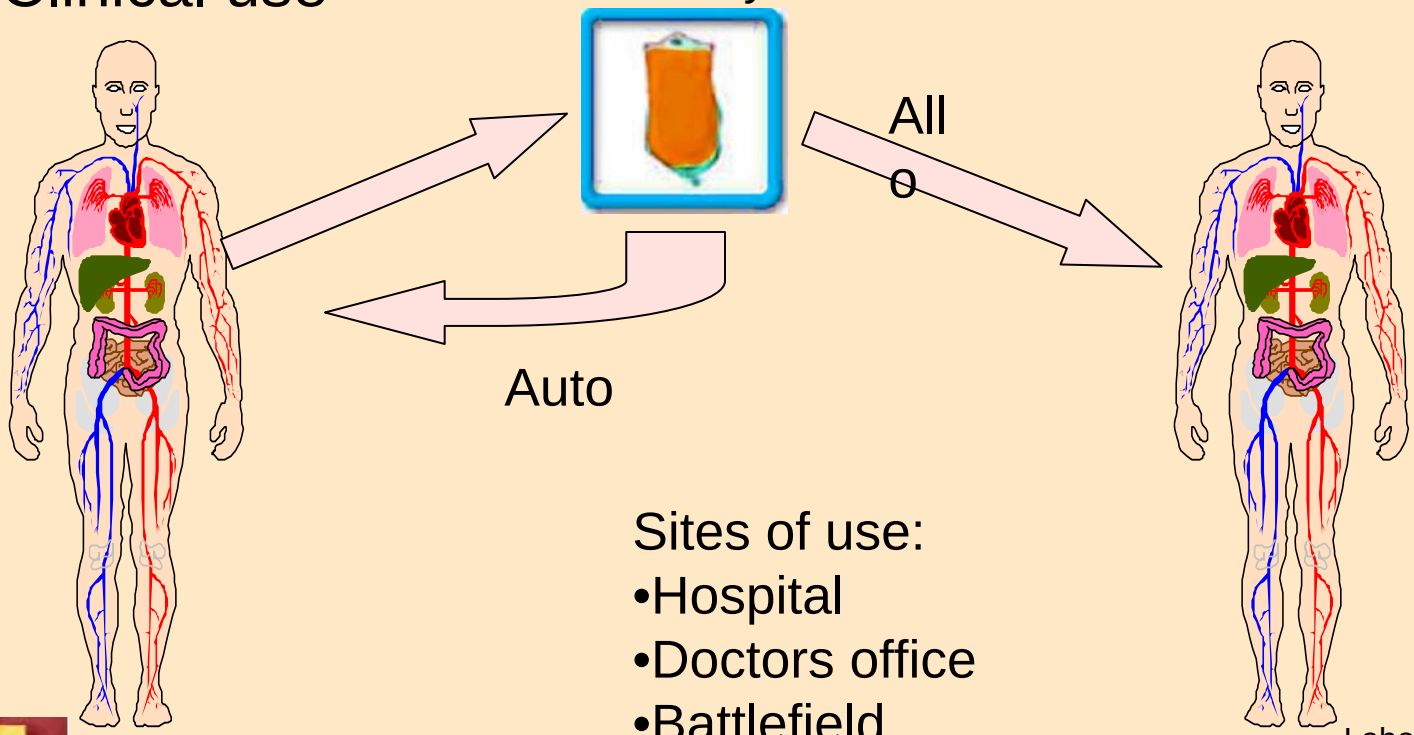
Cryopreservation and transport

Sites:

- Donation
- Cell processing
- Clinical use

Cells must be transported between sites and retain viability



Sites of use:

- Hospital
- Doctors office
- Battlefield

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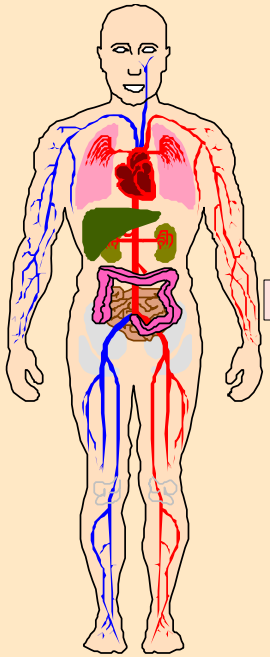
Cryopreservation and quality control

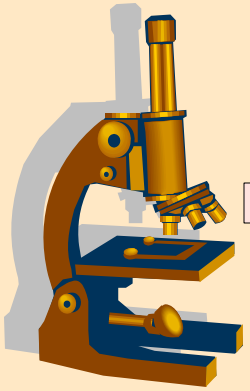
Harvest

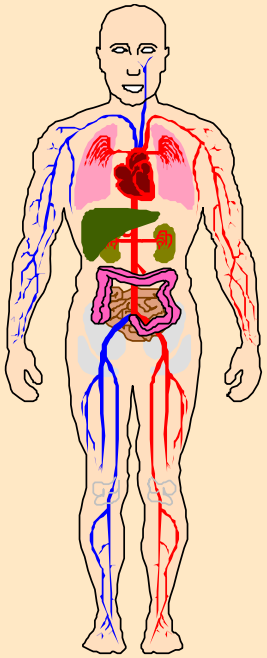
Processing

Quality Control

Administration







- Expansion
- Selection of subpopulations
- Genetic modification
- Biological modification

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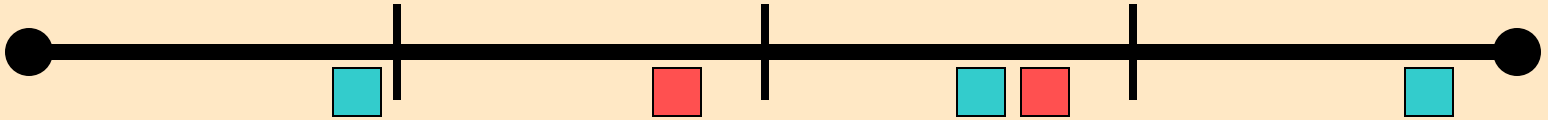
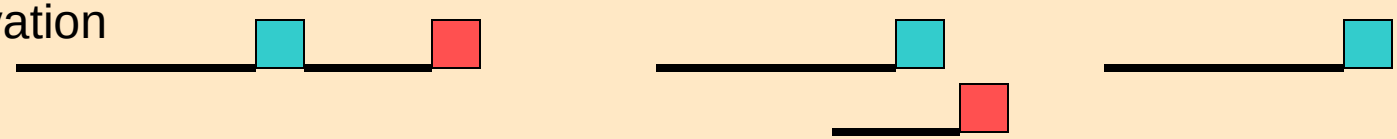
Manufacturing paradigm



With cryopreservation

Without cryopreservation



Product A

Product B

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Other benefits of cryopreservation

- Pooling of cells to reach therapeutic dose
- Increasing genetic diversity of cells

Components of a Cryopreservation Protocol

Liquid storage/
prefreeze processing

- Introduction of solution
- Cooling protocol
- Storage conditions
- Warming protocol
- Post thaw assessment

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Importance of preservation practice

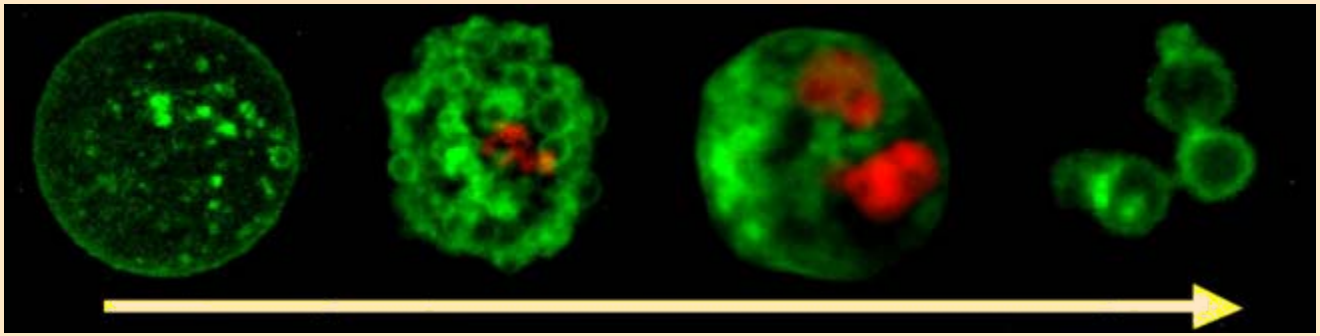
- Each element of protocol is important
- Subtle mistakes have profound results
- Improving preservation practice requires
  - Good practices
  - Ongoing research

Prefreeze processing

What happens to a cell before freezing influences post thaw recovery

Improving your practice: Monitor cell health (not viability) before freezing

- Apoptosis
- Shifts in metabolism



Van den Einden, Cytometry, 1997

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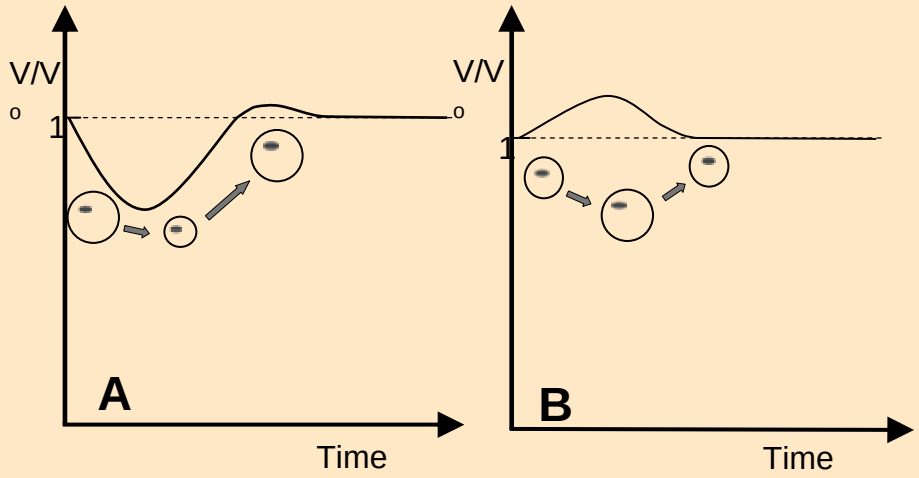
Introducing solutions

- Preservation requires the use of specialized solutions
- Solutions are not physiological
- Introduction and removal of the solutions can result in cell death
  - Osmotic stress
  - Biochemical toxicity



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Improving preservation practice

- Select an acceptable level of cell loss
- Measure losses from introduction and removal only (no freezing)
- Modify introduction protocol to reduce losses
  - Gradual/stepwise introduction
  - Introduction at reduced temperatures

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Improving practice

- Cell density during freezing influences post thaw recovery
- Freezing at cell densities > 20% cytocrit, results in reduced post thaw recovery
- Calculate the concentration at which you freeze
- Modify cell concentration, if needed

Cell Density, cont.

Relationship between cell density and cytocrit:

$$C = \frac{V_{totalcell}}{V_{total}}$$

Sample calculation: Estimate the maximum cell concentration for a cell with diameter of 20 mm

$$V_{cell} = \frac{4}{3}\pi r^3 = \frac{4}{3}\pi(10x10^{-6}m)^3 = 4.2x10^{-15}m^3 = 4.2x10^{-9}ml$$

$$0.2 = CV_{cell} \qquad C = 48x10^6 cells/ml$$

Controlled rate freezing, cont.

In search of the perfect curve…….

- Common deviations:
  - Delayed latent heat of fusion
  - Failure during controlled rate freezing
- Problems during CRF can reduce viability

QuickTime™ and a
decompressor
are needed to see this picture.

McCullough, Transfusion, to be submitted

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Cooling protocol

Temperature history during controlled rate freezing varies with:

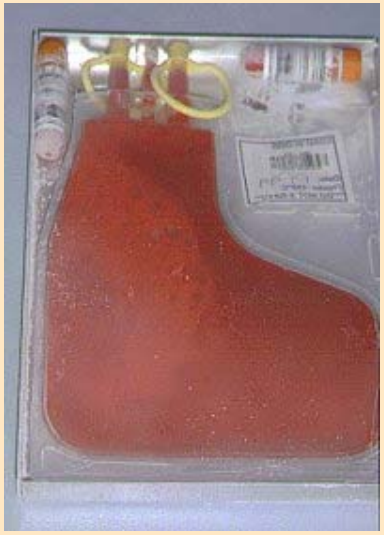
- Sample volume
- Sample container
- Location in freezer

Cooling protocol cont.

Fundamental problem: *actual temperature history of every sample is not known.*

Actual freezing curve *is not known*

"Optimal curve" cannot be determined.



Improving preservation practice: new technology

- Temperature as a function of time must be monitored:
  - Freezing
  - Storage
    - ‘rewarming’
- In development: a temperature sensor for each sample being frozen

Storage/repositories

Samples that are frozen are stored in a repository

Key questions:

- What is being stored
  - Volume
  - Container (vial, straw, bag)
- Why is it being stored
- How many samples are being stored

Design of repository should reflect those issues

Repositories, cont.

- LN_2 usage is strongly influenced by
  - Frequency of access
  - Line losses (delivery system)
  - Physical layout
  - Valve configuration
  - Number of freezers/storage units

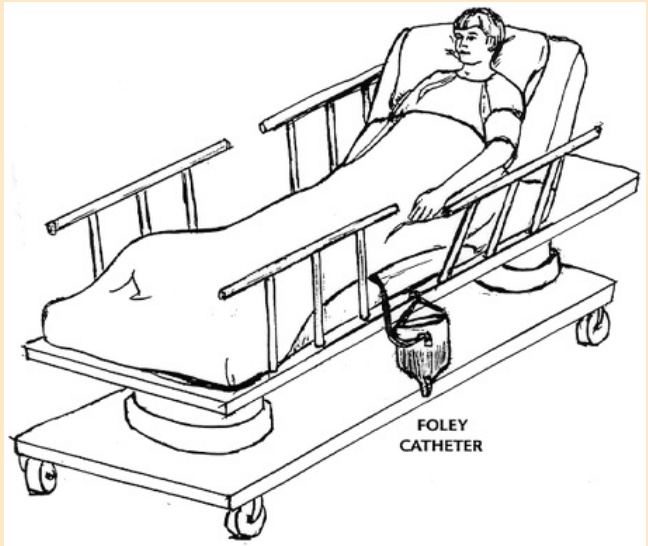
Improving your practice

- Develop a central repository for multiple users
- Train personnel on accessing a repository
  - Minimizing thermal excursions for repository and sample
- Times for accessing repository are limited

DMSO Disconnect

Medicate patient to attenuate adverse effects

Removing DMSO takes too much time and there are too many cells lost



This is an awful experience

DMSO: adverse effects

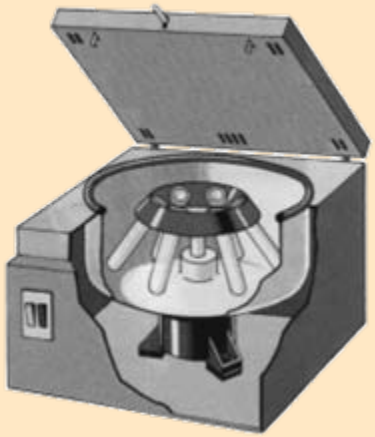
- DMSO is not approved for human infusion
- Adverse effects are well documented
  - Common effects (nausea, dyspnea)
  - Less common (cardiac, renal)
  - Recent studies document neurological effects
- Needed technology investment: removing DMSO with minimal cell losses

Improving preservation practice: post thaw processing

Current methods:
centrifugation

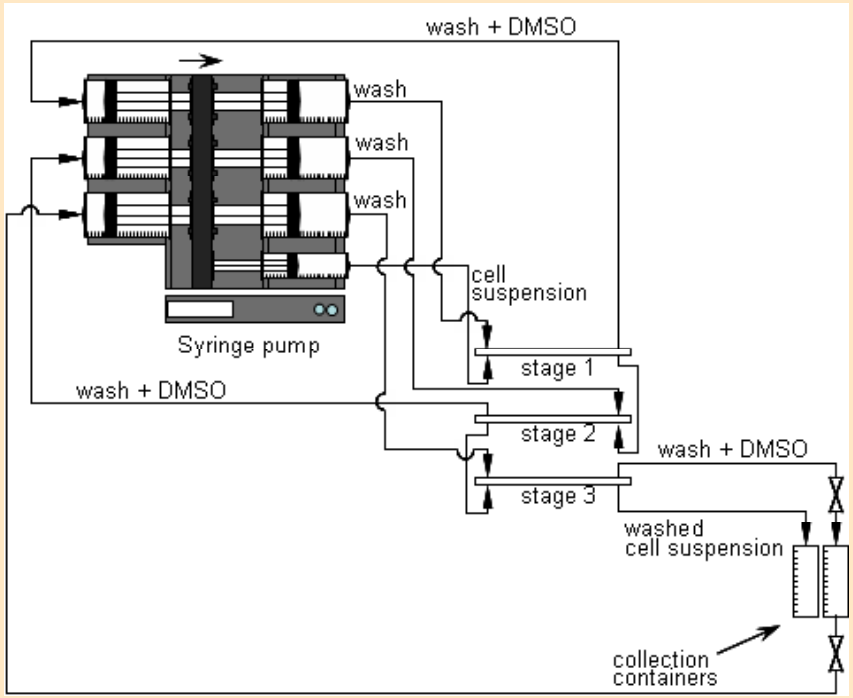
<u>Problems:</u>

- Labor intensive
- Operator dependent
- Cell losses are significant (25-30%)



Microfluidic technology

- Low power requirements
- Control of cell motion
- Reduced labor
- Reduced stresses on cells



Mata, Lab on a Chip, 2008

Microfluidic device, cont

CVF	**(1/**Pe**)*(*L*/*d*)**		**Cc*[a]**
%		ml/min	exp
0	1.2E-01	0.85	0.03
0	7.3E-02	1.41	0.05
2	7.3E-02	1.41	0.09

- *>95% DMSO removed*
- *Cell recovery ~90%*
- *1.5 ml/min processed*

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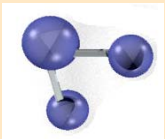
Post thaw assessment

It is easy to perform post thaw assessment badly

Kansas Stem Cell Program Sued

October 2006

A group of patients has sued a Kansas City stem cell transplant program alleging that it used shortcut methods to prepare the cells, resulting in the deaths of one-fourth of 40 patients treated between 1998 and 1999. Those patients died within 100 days of the treatments from complications including hemorrhages, infections and the return of their cancer. In two years, half of the patients died -- a much higher death rate than other centers.



Post thaw assessment

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

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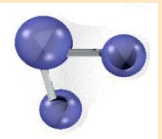


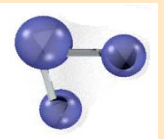
Post thaw assessment

Freezing and thawing produces:

- Changes in membrane integrity
- Metabolic function
- Tendency toward apoptosis

Implying that:

- Membrane integrity tests ≠viability
- Viability may change with time post thaw



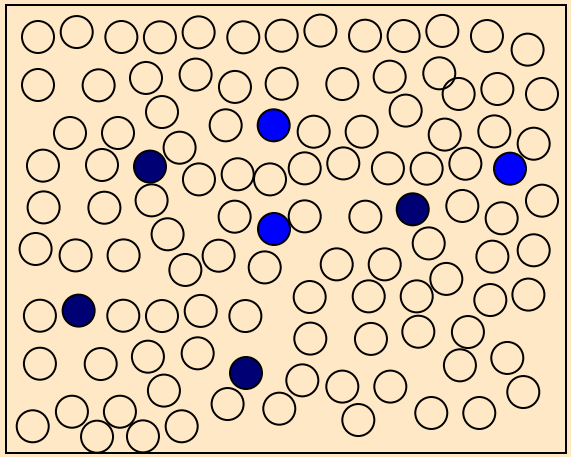
Measurement Bias

Prefreeze Assay

Total number of cells =100

Number of target cells =7

Frequency of target cells=7/100=7%

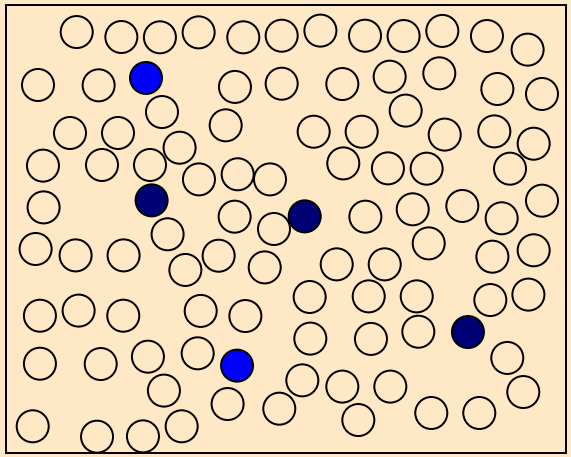


Post thaw Assay

Total number of cells =71

Number of target cells =5

Frequency of target cells=5/71=7%



Measurement Bias, cont.

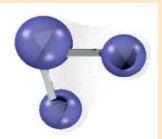
Current method of accounting

Recovery of target cell= frequency of target cell post thaw/frequency of target cell prefreeze= 0.07/0.07=100%!!!

⇨Measurement Bias

You have not accounted for the cells that were lost

Recovery of target cell=total number of target cells post thaw/total number of target cells prefreeze



Summary

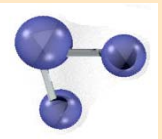
Operator-based improvements:

- Testing each element of protocol
- Developing good practices for repositories
- Validating post thaw assessment methods

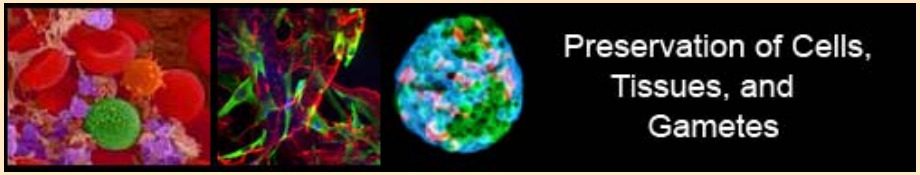
New developments:

- Technologies that reduce labor and operator dependence
- Appropriate monitoring technology
- Nontoxic alternatives to DMSO

The ability to preserve cells is integral to cell therapy. Advances in the field are necessary to improve outcome.



Professional short course



Topics covered:

- Liquid storage of cells and tissues.
- Fundamentals of cryopreservation
- Protocol development
- Quality systems
- Clinical cell cryopreservation
- Repository design
- Gamete preservation
- Tissue preservation
- Regulatory issues for cell/tissues

Lecturer

- Allison Hubel, University of Minnesota
- Charles Lee, University of North Carolina, Charlotte
- Ian Pope, CoreCryolab
- Ken Roberts, University of Minnesota
- Marilyn Waxberg, RCRI
- Alptekin Aksan, University of Minnesota
- David McKenna, University of Minnesota
- Diane Kadidlo, University of Minnesota

www.me.umn.edu/education/shortcourses/preservation

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