

Best Practices in Cryopreservation

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About ATCC

- Founded in 1925, ATCC is a non-profit organization with headquarters in Manassas, VA
- World's premiere biological materials resource and standards development organization
- ATCC collaborates with and supports the scientific community with industry-standard biological products and innovative solutions
- Strong team of 400+ employees; over one-third with advanced degrees



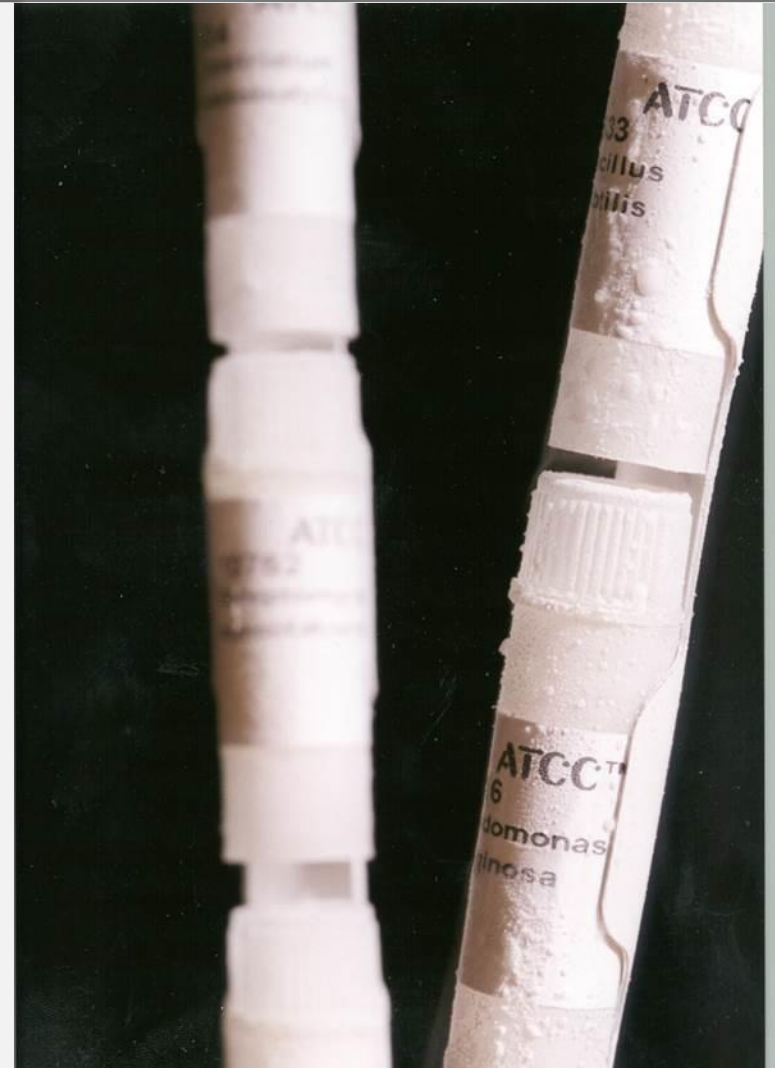
Established partner to global researchers and scientists



Outline

Cryopreservation

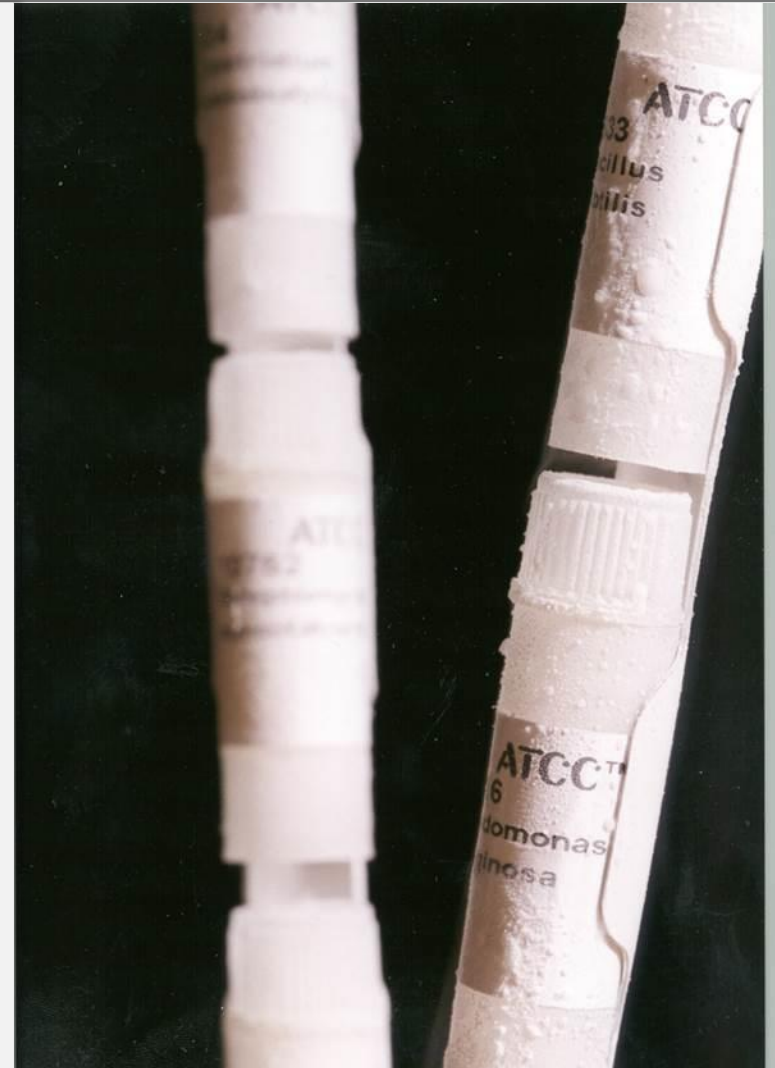
- Definition/benefits
- Cryoprotectants
- Procedures
 - Contamination check
 - Media preparation
 - Freezing cells/recovery
 - Post-thawing considerations



Outline

Inventory management

- Seed lot system
- Low temperature storage
- Biological materials management
- Inventory control
- Safety considerations



Cryopreservation defined

- The use of very low temperatures to structurally preserve intact living cells and tissue
- Unprotected freezing is normally lethal to cells while controlled cooling can be used to produce stable conditions that preserve life

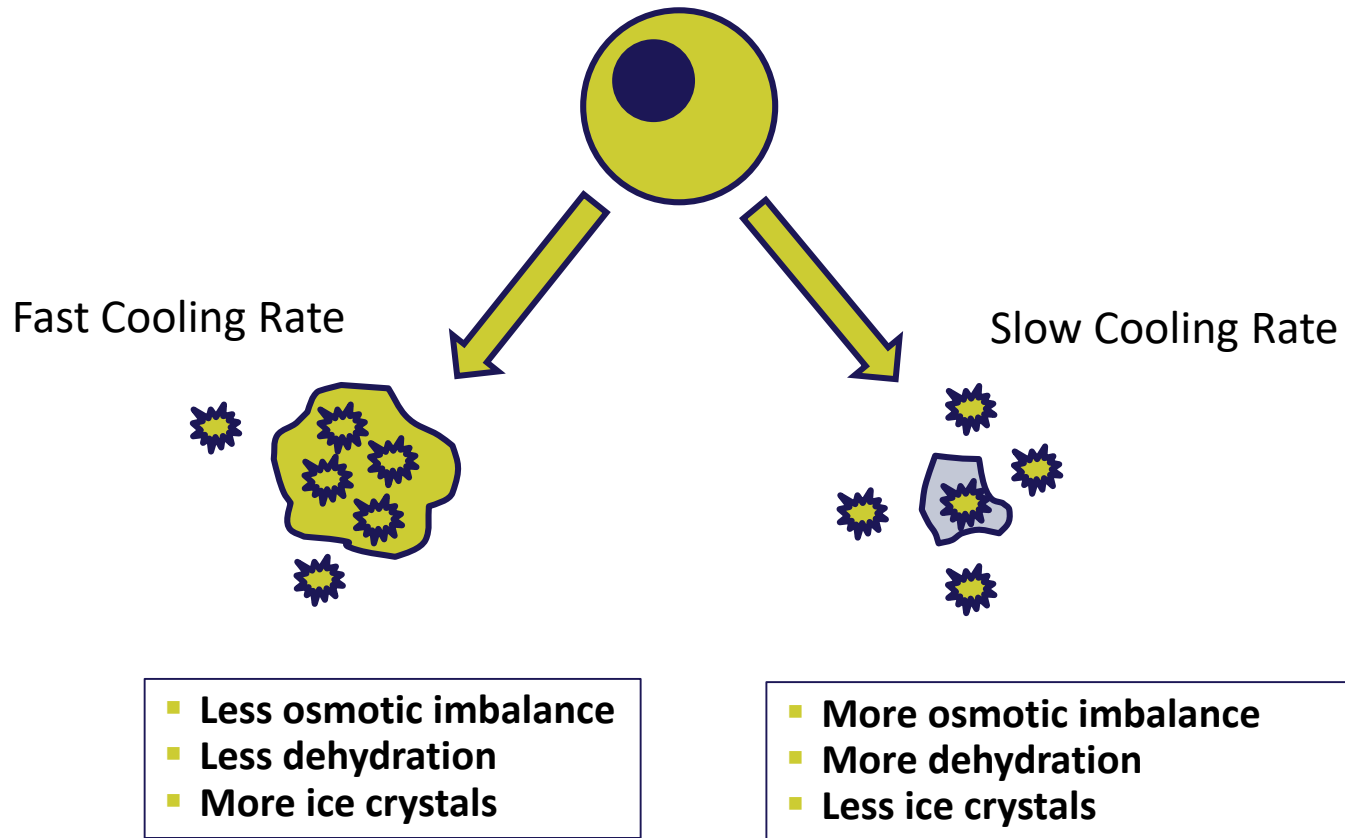


Benefits of cryopreservation

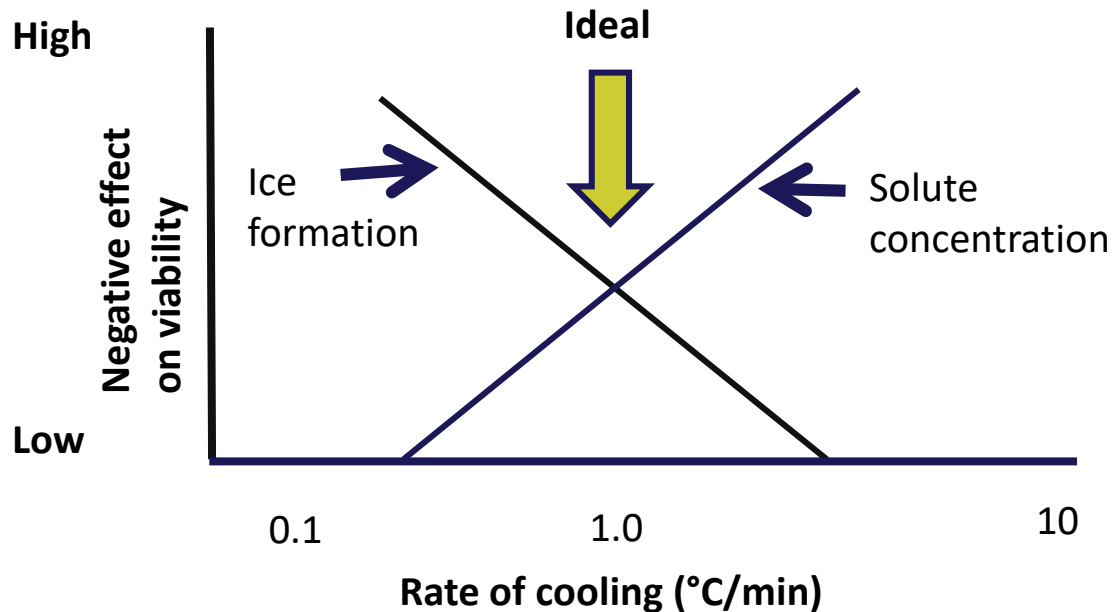
- Generation of safety stocks
- Saves time and money
- Preservation of cells
- Insurance against phenotypic drift
- Standard for experiments



Cryopreservation principles



Cryopreservation principles



- High levels of ice formation and increased solute concentration have a negative impact on cell viability
- Optimal cooling rate for cell viability is 1 to 3°C/min

Cryoprotectants

- Dimethyl sulfoxide (DMSO) and glycerol are the two most widely used cryoprotectants
- Aid in preserving cells
 - Encourage dehydration
 - Minimize solution effects



Cryoprotectants

Cell type	Cryoprotectant	Temperature	Number of cells
Animal cells	DMSO (5-10%) or Glycerol (5-10%)	-140°C	10 ⁶ to 10 ⁷ /mL
Bacteria	Glycerol (5-10%)	-80°C	10 ⁷ /mL
Yeast	Glycerol (10%)	-140°C	10 ⁷ /mL
Protozoa	DMSO (5-10%) or Glycerol (10-20%)	-140°C	10 ⁵ to 10 ⁷ /mL
Plant cells	DMSO (5-10%) and Glycerol (5-10%)	-140°C	3% to 20% cell volume
Animal viruses (free)	None	-80°C	NA
Animal viruses (infected cells)	DMSO (7%)	-10°C	10 ⁶ /mL

Cryopreservation procedure

- Check for contamination
- Media preparation
- Freezing cells in a controlled-rate chamber
- Recovering cryopreserved cells
- Post thawing considerations



Contamination

Sources

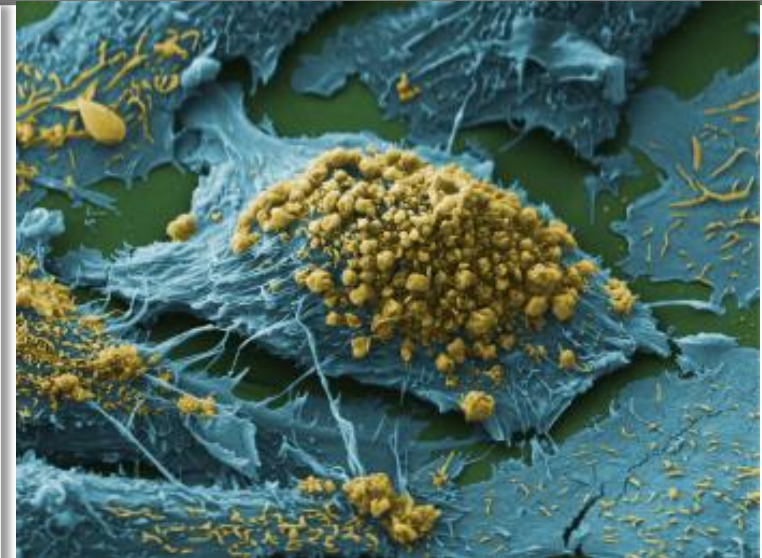
- Contaminated cell lines
- Improper aseptic technique

Types

- Microbial – Bacteria, mycoplasma, fungi, viruses
- Cellular – Cross contamination

Signs

- Turbid media
- Rapid decline in pH – color change
- Morphological changes
- Filamentous structures



Media preparation

Classical Cell Culture Media

Dulbecco's Modified Eagles Medium (DMEM) and Eagle's Minimum Essential Medium (EMEM)

RPMI-1640 (for suspension cells)

- 5-10% DMSO
- 20% fetal bovine serum (FBS) or bovine serum albumin (BSA)
 - Additional cryoprotectant properties
 - Necessary for post-thaw cell survival

ATCC Serum-free Freezing Media (ATCC® 30-2600™)

- All in one media
- 10% DMSO with proteins and additives for cell survival

Cell Suspension

- 3×10^6 to 5×10^6 cells/mL
- 1 mL total volume



Freezing cells

-70°C

Controlled rate freeze chamber

-1°C/min cooling rate

A few hours to 24 hours



-140°C

Liquid nitrogen tank



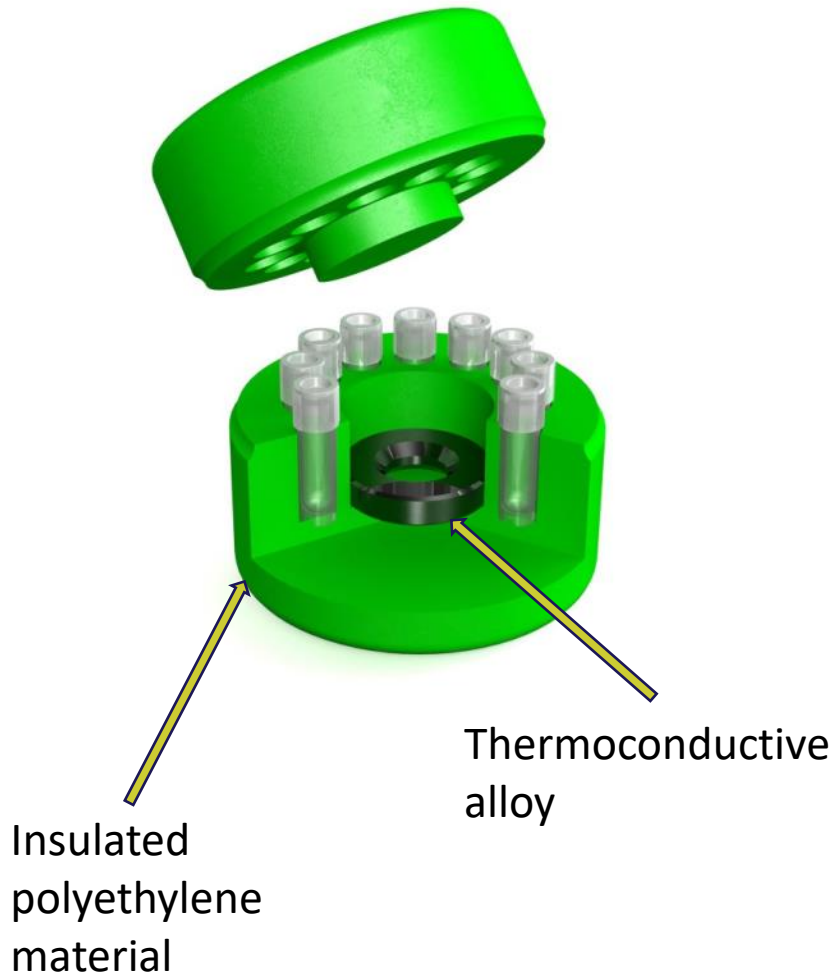
Freezing cells



Controlled rate freezer

- Programmable electronic freezing unit
- Reliable, consistent rate of cooling
- Expensive, maintenance cost

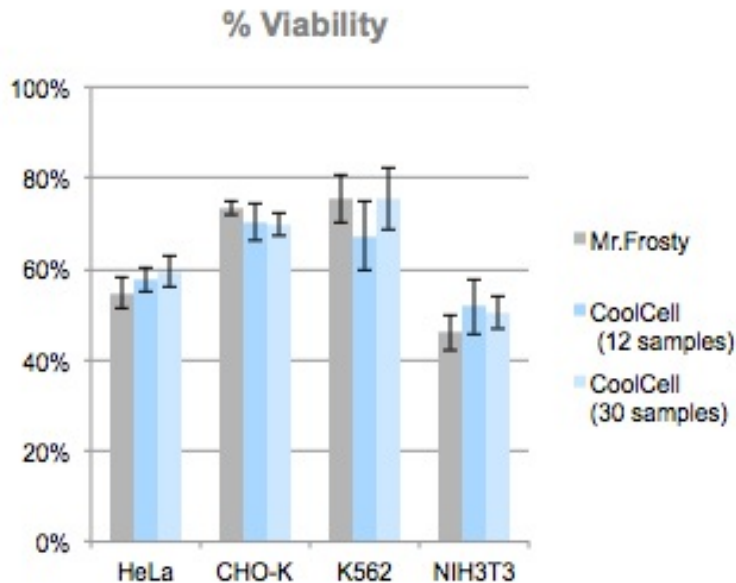
Freezing cells



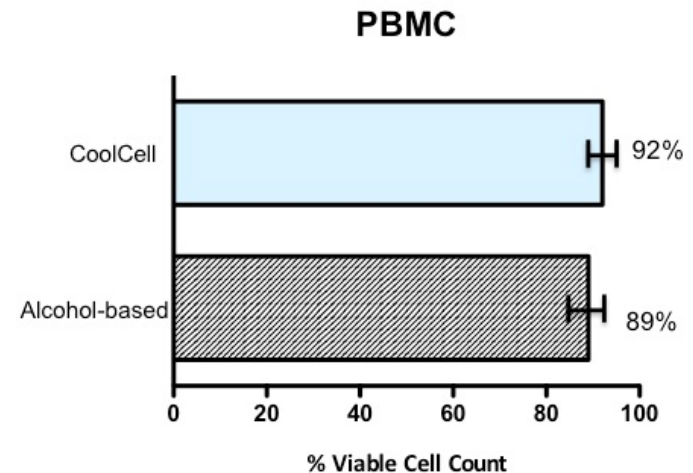
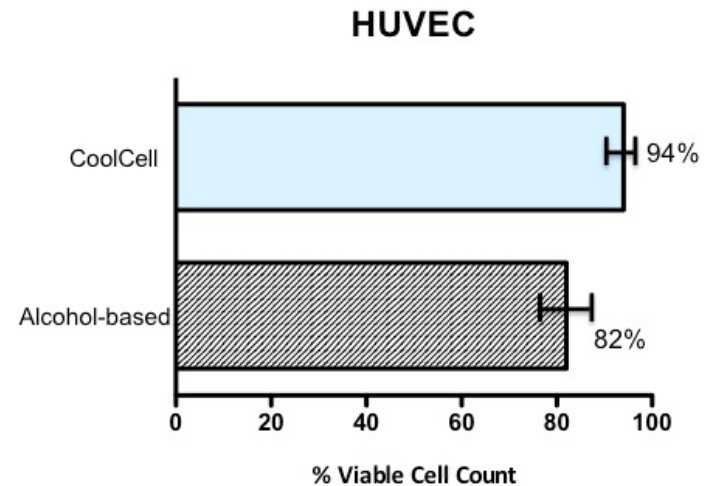
CoolCell® (ATCC® ACS-6000™)

- Reliable $-1^{\circ}\text{C}/\text{min}$ cooling rate
- 4 Hours in -70°C Freezer
- Comfortable to touch
- No alcohol use or maintenance

Freezing cells



- Can be used with most cell types
 - Verified use with organoids
- Performs as well or better than comparable products



Vial selection

Several types of vials exist for storage at ultra low and cryogenic temperatures

- Plastic vials
 - Internal thread
 - External thread
- Straws
- Glass ampoules (heat sealed)

Considerations for vial type selection

- Storage temperature
- Liquid submersion
- Head space
- Effect on warming
- Material stresses

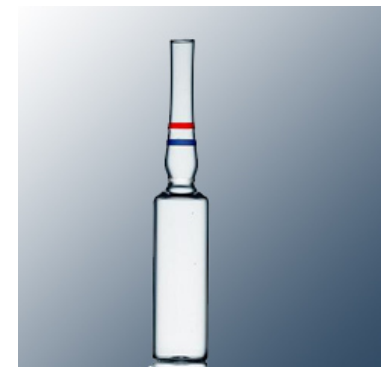
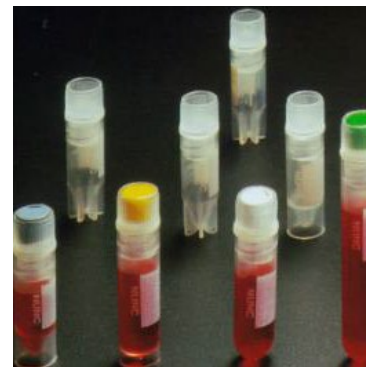


External

Ideal



Internal



Post thawing

Thaw as quickly as possible

- Thaw in 37°C water bath for 2 minutes
- Transfer to 10 mL centrifuge tube
- Add 9 mL of growth media (10% FBS)
 - Dropwise to avoid osmotic shock
- Centrifuge, resuspend in 2 mL of growth media



Post thawing considerations

Cell recovery – measuring viability of cells

Microbial cells

- Serial dilutions

Animal/human cells

- Stain

Animal embryos

- Morphology

Vessel selection

- Cell culture dishes
- Flasks
- Multiwell plates
- Roller bottles

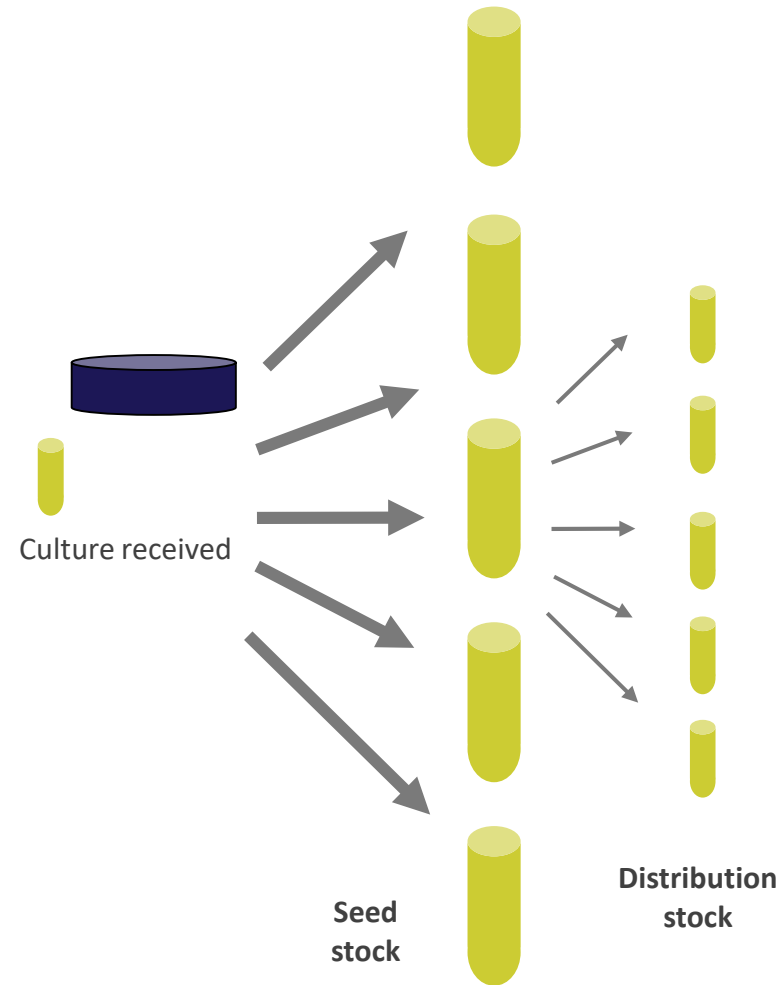


Inventory management

- Seed lot system
- Low temperature storage
- Biological materials management
- Inventory control
- Safety considerations



Seed lot system

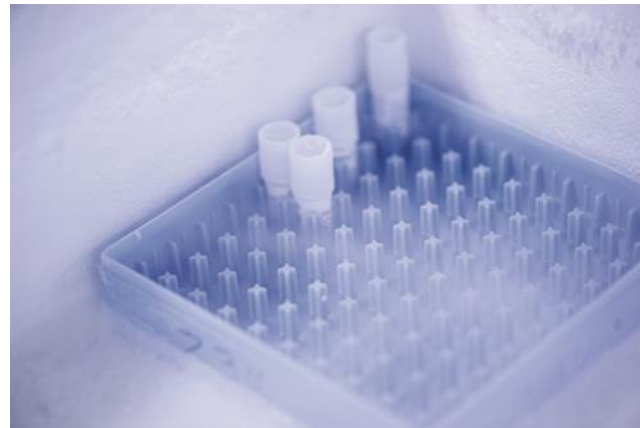


- Preserved cultures remain as close as possible to the original culture
- Seed stock is archived for future replenishment
- Distribution stock are used for distribution
- Authentication compares:
 - Seed, Distribution, Initial culture

Low temperature storage



For the best security, always store your cells in liquid nitrogen freezers



Low temperature storage

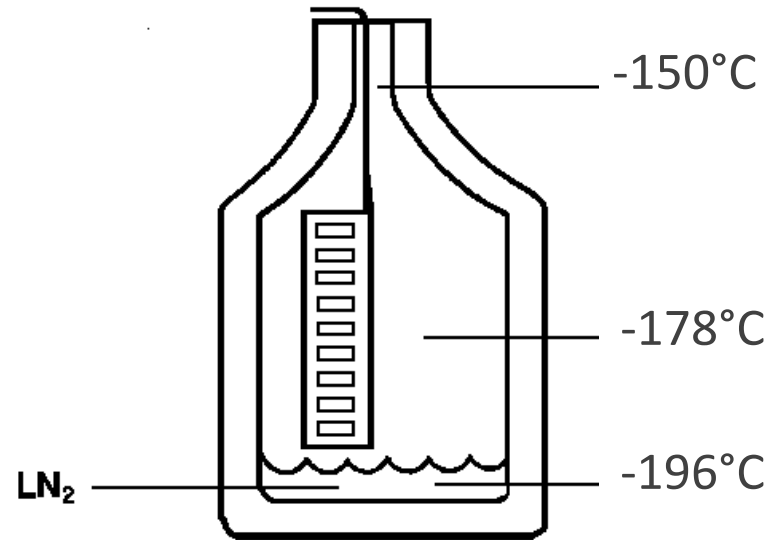
Mammalian cells

Long-term storage should be below -140°C

- -140°C for an indefinite length of time
- -80°C for less than 1 year

Vials should be stored in a liquid nitrogen unit **above** the volume of liquid at the bottom of the tank

This temperature should be between **-140°C** and **-180°C**



Biological materials management

Ensuring preserved material remains unchanged

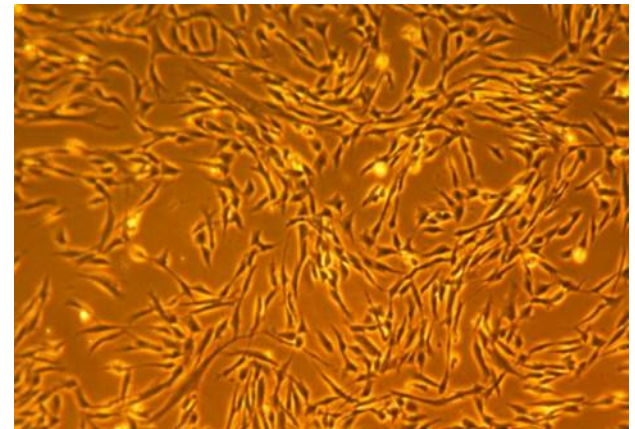
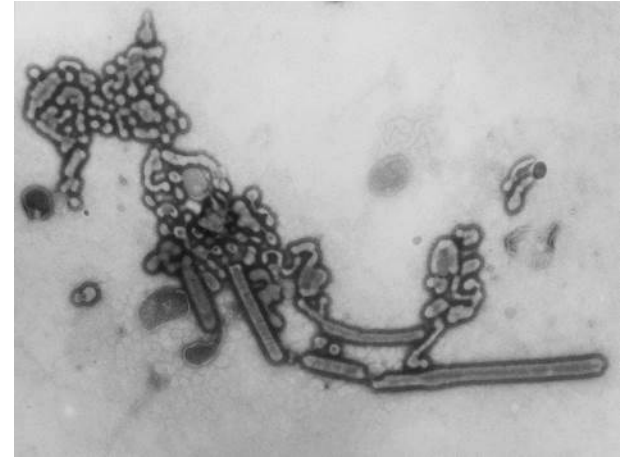
Manageable levels of biological material

Keeping material that is needed

- Continuing monitoring for contamination
- Removing unwanted, contaminated, misidentified items

Create a system of identification

- Complete characterization of new material
- Cataloging and data recording



Inventory control

Record keeping of vital information

- Preservation methodology used
- Location/identification of stored material
- Preservation date
- Number of passages



Inventory control

Locator codes

For rapid and easy retrieval

- Freezer unit number
- Code for freezer section or rack
- Box/canister number
- Grid spot within each box



Good inventory control practices minimizes the time needed to find material, reducing the risk that the freezer unit and biological materials will warm

Safety considerations

U.S. Public Health Service Biosafety Guidelines

- Most mammalian cells – biosafety level 1
- Human/primate cells – biosafety level 2
 - *If not thoroughly characterized*
- Bacteria / Viruses – biosafety level 3

Personal protective equipment

- Insulated gloves when using liquid nitrogen tanks
- Long sleeve laboratory coats
- Full face mask
 - Possible ampoule explosion

Hazardous biological materials

- Thaw and open vials of hazardous material inside biological safety cabinet
- Decontaminate liquid nitrogen freezer



Summary

Freezing cells

- $-1^{\circ}\text{C}/\text{min}$ is ideal for most cells
- 10% DMSO, 20% FBS, or 20% BSA – mammalian cells
- 10% glycerol - bacteria
- Use a controlled rate freezing container, i.e. CoolCell[®]

Cell recovery

- Thaw quickly in a 37°C water bath
- Bring cells out of DMSO slowly
- Measure the viability of cells

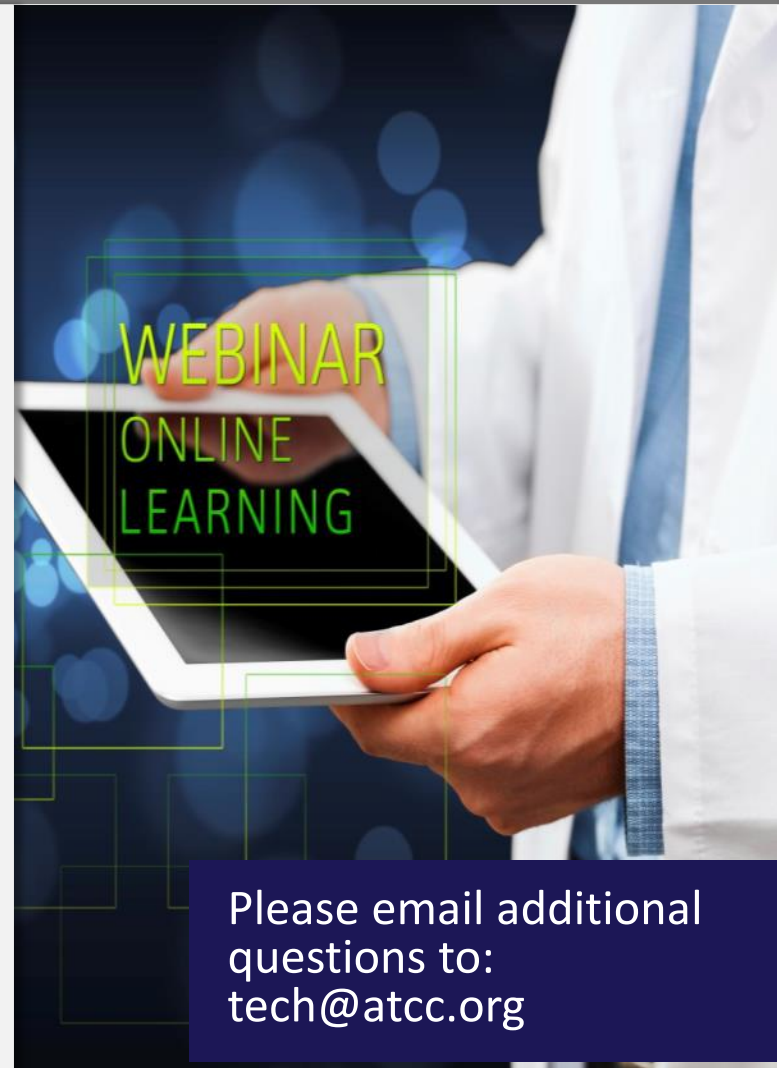
Inventory management

- Store at -140°C in Liquid Nitrogen
- Maintain biological inventory to keep needed material; discard unwanted material
- Record, document, and track all material
- Follow safety guidelines!

Thank you for joining today!

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- **April 28, 2016**
10:00 AM, 3:00 PM EST
Frank Simione, M.S., *Director, Standards Standards Resource Organization, ATCC*
The ATCC Story: A Ninety Year Celebration
- **May 5, 2016**
10:00 AM, 3:00 PM EST
Cara Wilder, Ph.D., *Technical Writer, ATCC*
Carbapenem-resistant Enterobacteriaceae (CRE) –
A Growing Superbug Population



Please email additional questions to:
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