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Standard Guide for Design and Maintenance of Low-Temperature Storage Facilities for Maintaining Cryopreserved Biological Materials[1]

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INTRODUCTION

Storage facilities for maintaining cryopreserved biological materials are generally comprised of two types of low-temperature systems: (*1*) freezers that are cooled by mechanical refrigeration and (*2*) freezers that are cooled passively with liquid nitrogen. Either system can be used for the storage of frozen biological materials as long as it meets the criteria specific to the material being stored for ensuring stability. Each system has its own unique handling requirements.

1. Scope

1.1 This guide covers recommended procedures for developing and maintaining low-temperature storage facilities for freezers with mechanical refrigeration.

1.2 This guide covers recommended procedures for developing and maintaining low-temperature storage facilities for freezers cooled with liquid nitrogen.

1.3 This guide does not cover practices for preservation by freezing which are covered in Practice~~s~~ E 1342.

1.4 The values stated in SI units are to be regarded as the standard.

1.5 *This standard does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

E 1342 Practice for Preservation by Freezing, Freeze-Drying, and Low Temperature Maintenance of Bacteria, Fungi, Protista,

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[1] This guide is under the jurisdiction of ASTM Committee E-48 on Biotechnology and is the direct responsibility of Subcommittee E48.02 on Characterization and Identification of Biological Systems.

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Viruses, Genetic Elements, and Animal and Plant Tissues[2]
E 1565 Guide for Inventory Control and Handling of Biological Material Maintained at Low Temperatures[2]
E 1566 Guide for Handling Hazardous Biological Materials in Liquid Nitrogen[2]

3. Terminology

3.1 *Definitions:*

3.1.1 *cryogenic temperatures*—temperatures below or equal to −100°C.

3.1.2 *mechanical refrigeration*—a refrigeration system in which cooling is provided by mechanical means such as a compressor.

3.1.3 *passive refrigeration*—a refrigeration system in which cooling is provided by a refrigerant such as liquid nitrogen.

4. Significance and Use

4.1 The proper design of low-temperature storage facilities ensures that sensitive biological materials are maintained under conditions providing maximum storage stability.

4.2 Properly designed and operated low-temperature storage facilities ensure that the handling of sensitive biological materials at low temperatures does not compromise stability (see Guide E 1565).

4.3 Properly designed low-temperature storage facilities ensure that adequate safeguards are provided to prevent untoward events from compromising the stability of sensitive biological materials.

5. Procedure

5.1 *Low-Temperature Mechanical Freezers*:

5.1.1 Ensure that adequate dedicated electrical power is available to support the number of mechanical freezers required in the facility.

5.1.2 Provide for emergency electrical power to maintain the freezers when commercial power disruptions occur.

5.1.3 Place the freezers in a temperature- and humidity-controlled area with adequate ventilation.

5.1.4 Obtain a source of service support for the refrigeration systems that is reliable and can respond to mechanical problems rapidly. An alternative source should also be available.

5.1.5 When selecting mechanical freezers, be sure that the lowest attainable temperature of the unit is cold enough to ensure the stability of the biological material to be stored in them.

5.1.6 The temperature of mechanical freezers ~~must~~ shall be monitored daily to ensure that consistently low temperatures are maintained. Most units have temperature-sensing devices built in that allow both control and monitoring of the freezer temperature. These internal units can malfunction and fool alarm systems. A temperature detecting probe should therefore be inserted into each mechanical freezer, and the temperature should be read manually, or recorded automatically, on a remote sensing device.

5.1.7 The temperature monitoring device ~~must~~ shall be calibrated annually against a standard traceable to the National Institute for Standards and Technology (NIST).[3]

5.2 *Liquid Nitrogen Freezers*:

5.2.1 Liquid nitrogen freezers are passive cooling systems that use liquid nitrogen as the refrigerant. A reliable source of liquid nitrogen ~~must~~ shall be available to maintain liquid nitrogen freezers.

5.2.2 Liquid nitrogen freezers provide the greatest assurance of long-term stability because of the low temperatures than~~t~~ can be attained (−150 to −196°C).

5.2.3 Choose a liquid nitrogen freezer that is compatible with inventory needs. Small units with large openings for easy access are less expensive to purchase and maintain, but they require more careful monitoring and attention. Larger units may be more efficient. Most of these units have a working opening that allows adequate access but does not compromise the working temperature of the unit.

5.2.4 Install liquid level sensing probes and controlling devices on the liquid nitrogen freezer, if possible, to monitor liquid levels and to allow automatic and manual filling. Manual dipstick measurements should also be performed to ensure that the monitoring equipment is working properly.

5.2.5 If possible, do not store vials directly in the liquid phase since improperly sealed vials can leak, leading to potential contamination and exploding vials upon retrieval. Maintain the vials in the vapor phase above the liquid. When vials are stored directly in the liquid, they ~~must~~ should be filled ~~only~~ to ~~two-thirds capacity,~~ a point that minimizes the air space in the vial, and they ~~must~~ shall be sealed completely. Vials may be examined for leaks by immersing them in an aqueous methylene blue (0.05 %) solution at 4°C~~)~~.

5.2.6 Storing material in the vapor phase of the liquid nitrogen freezer requires validating the unit to ensure that sufficient liquid nitrogen is kept in the bottom of the unit to maintain a working temperature at the top of the unit of −130°C or below. To validate the freezer, measure the temperature at the top with the freezer open, and raise the level of the liquid nitrogen until the temperature

[2] *Annual Book of ASTM Standards*, Vol 11.05.
[3] National Institute of Standards and Technology, (NIST), Gaithersburg, MD 20899.

remains at or below −130°C for at least 1 h.[4] The requirements for storing material in the vapor phase must shall be determined prior to purchasing a liquid nitrogen freezer since not all freezer designs are amenable to all vapor storage.

5.2.7 Avoid using small liquid nitrogen units for permanent storage since they require constant surveillance and increase the probability of problems occurring.

5.2.8 To ensure a constantly available source of liquid nitrogen, a bulk storage tank should be dedicated to liquid nitrogen storage only. This can be a portable unit located within the facility, or an externally stand tank with insulated piping for transporting the liquid nitrogen to the freezers. Solenoids and pressure safety valves should be located in the piping to allow the control of nitrogen flow and ventilation of excess pressure. Care must shall be taken to avoid moisture accumulation on the valves that could freeze and cause a malfunction.

5.3 *Safety*:

5.3.1 Precautions must shall be taken to provide personnel protection from the extreme cold encountered at cryogenic temperatures. Extremities must shall be protected at all times using gloves. Thin nylon gloves provide some protection when handling ampules; however, insulated gloves designed for working at cryogenic temperatures should be used when possible.

5.3.2 When vials are stored directly in the liquid, they must should be filled only to two-thirds capacity a point that minimizes the air space in the vial, and they must shall be sealed completely. Vials may be examined for leaks by immersing them in an aqueous methylene blue (0.05 %) solution at 4°C.

5.3.2.1 Because of the potential for exploding vials when retrieving from liquid nitrogen, precautions must shall be taken to protect the operator from glass shards.[5,6] Heavy gloves, laboratory coat, and face shield should be mandatory whenever retrieving vials from liquid nitrogen. In addition, glass vials should be placed inside a metal canister before removing the vial to warmer temperatures.

5.3.3 NHazardous biological materials should not be maintained directly in liquid nitrogen, because nitrogen will displace oxygen in confined areas with poor ventilation, especially during fill operations. Care must shall be taken to ensure proper ventilation of all areas housing liquid nitrogen freezers, and an oxygen monitor with alarm should be installed in the area if possible.

5.3.4 Hazardous materials should not be maintained directly in liquid nitrogen because of the potential for leaking and exploding vials (see Guide E 1566).

5.3.5 When freezers are no longer used, they should be decontaminated after warming before removal or reuse.

6. Keywords

6.1 biological materials; cryopreserved; facilities

[4] Simione, F.P. and Karpinsky, J.Z., "Points to Consider Before Validating a Liquid Nitrogen Freezer", In: *Validation Practices for Biotechnology Products*, ASTM STP 1260, Shillenn, J.K., ed., ASTM, 1996, pp. 24–30.

[5] Simione, F.P. Jr., Daggett, P.M., McGrath, M.S., and Alexander, M.T., "The Use of Plastic Ampules for Freeze Preservation of Microorganisms", *Cryobiology*, Vol 14, 1977, pp. 500–502.

[6] Grieff, D., Melton, H., and Rowe, T.W., "On the Sealing of Gas-Filled Glass Ampoules", *Cryobiology*, Vol 12, 1975, pp. 1–14.