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Standard Guide for Handling Hazardous Biological Materials in Liquid Nitrogen¹

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INTRODUCTION

Hazardous biological materials maintained at liquid nitrogen temperatures require special handling and storage procedures. The penetration of vials by liquid nitrogen during storage can result in contamination of the nitrogen itself² or contamination of personnel resulting from improperly sealed vials exploding.^{3,4} Ensuring safe storage conditions and taking precautions during the retrieval of cultures from liquid nitrogen can help reduce the potential hazards. To avoid the penetration of vials by liquid nitrogen and resulting hazards, they should be filled to a point that minimizes the air space in the vial, and they shall be sealed completely. Vials may be examined for leaks by immersing them in an aqueous methylene blue (0.05 %) solution at 4°C.

1. Scope

1.1 This guide covers recommended procedures for maintaining and handling hazardous biological materials at liquid nitrogen temperatures.

1.2 This guide covers the safety precautions recommended when handling material stored in liquid nitrogen.

1.3 This guide does not cover the maintenance and handling of hazardous biological materials maintained at cryogenic temperatures in systems other than liquid nitrogen.

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:*⁵

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

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² Shafer, T. W., Everett, J., Silver, G. H., and Came, P. E., "Biohazard: Virus-Contaminated Liquid Nitrogen," *Science*, Vol 191, 1976, pp. 24-26.

³ Simione, F. P., Jr., Daggett, P.-M., McGrath, M. S., and Alexander, M. T., "The Use of Plastic Ampoules for Freeze Preservation of Microorganisms," *Cryobiology*, Vol 14, 1977, pp. 500-502.

⁴ Grieff, D., Melton, H., and Rowe, T. W., "On the Sealing of Gas-Filled Glass Ampoules," *Cryobiology*, Vol 12, 1975, pp. 1-14.

⁵ For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

E 1342 Practice for Preservation by Freezing, Freeze-Drying, and Low Temperature Maintenance of Bacteria, Fungi, Protista, Viruses, Genetic Elements, and Animal and Plant Tissues

3. Terminology

3.1 *Definitions:*

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

3.1.2 *hazardous biological materials*—living biological materials, and products derived therefrom, that pose a potential threat to human health.

3.1.3 *liquid nitrogen storage*—storage directly in liquid nitrogen or in the vapor phase above liquid nitrogen.

4. Significance and Use

4.1 This guide is intended for use by individuals maintaining and handling hazardous biological material in liquid nitrogen freezers.

4.2 This guide does not cover all aspects of every situation that may be encountered in maintaining hazardous biological material in liquid nitrogen; each situation must therefore be assessed individually using these guidelines.

4.3 This guide is not intended for use with systems other than liquid nitrogen storage.

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

5. Procedures

5.1 *All Liquid Nitrogen Freezers:*

5.1.1 Liquid nitrogen freezers vary from all-liquid storage, to liquid and vapor storage, to all-vapor storage. Freezers with all-liquid storage present the greatest hazard, and freezers with