Designation: E 1566 – ~~93~~00

Standard Guide for Handling Hazardous Biological Materials in Liquid Nitrogen[1]

This standard is issued under the fixed designation E 1566; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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 itself[2] 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, ~~vials must~~ they 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.

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:*

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.

Current edition approved ~~Aug. 15, 1993.~~ Sept. 10, 2000. Published ~~O~~ Dec~~to~~ember 2000.

Originally published as E1566–93. Last previous edition E1566–93.

[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.

Viruses, Genetic Elements, and Animal and Plant Tissues[5]

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 all-vapor storage present the least hazard. The type of system in use should be assessed before attempting to apply these guidelines.

5.1.2 When working in freezers with all liquid storage, the operator ~~must~~ shall work in or near the liquid nitrogen when inserting and retrieving material.

5.1.3 Freezers with both liquid and vapor storage reduce the proximity of the operator to the liquid nitrogen, but the risk of liquid splashing remains.

5.1.4 In all vapor freezers, the operator is protected from the liquid by a barrier.

5.2 *Liquid Storage Freezers*:

5.2.1 Hazardous biological material should never be stored directly in liquid nitrogen. However, if a situation occurs in which hazardous biological materials are maintained in liquid nitrogen, the following guidelines should be followed:

5.2.2 Wear a laboratory coat over a layer of protective clothing, such as a heavy jacket, that provides some protection from the splashing of liquid nitrogen.

5.2.3 Wear insulated gloves over rubber surgical gloves.

5.2.4 Wear a full face shield extended to cover the neck.

5.2.5 Immerse the material slowly into liquid nitrogen in order to avoid splashing.

5.2.6 Remove the material from liquid nitrogen slowly.

5.2.7 Immediately place the retrieved material into dry ice for transport, or into a metal canister containing a liquid disinfectant for immediate thawing.

5.3 *Liquid/Vapor Storage Freezers*:

5.3.1 Protective clothing should include a laboratory coat and surgical gloves. A face shield should be worn when retrieving from the liquid, and eye protection should be used when retrieving from the vapor.

5.3.2 Immerse the material slowly into liquid nitrogen in order to avoid splashing.

5.3.3 Remove the material from the liquid slowly, and allow it to warm to the temperature of the vapor phase prior to removing it from the freezer.

5.3.4 Material stored in the vapor phase may be removed from the freezer immediately for transport in dry ice or thawing.

5.3.5 Precautions ~~must~~ shall be taken to ensure that the liquid nitrogen does not come into contact with material stored in the vapor phase.

5.4 *Vapor Storage Units*:

5.4.1 Protective clothing should include a laboratory coat, surgical gloves, and eye protection.

5.4.2 Material may be removed from the freezer immediately for transport in dry ice or for thawing.

5.5 *Decontamination*:

5.5.1 Whenever a liquid nitrogen freezer, used to store hazardous biological material, is emptied and warmed it should be decontaminated. Decontamination procedures should be based on the nature of the hazardous material maintained in the unit.

5.5.2 When vial breakage occurs, involving hazardous biological material, an assessment of the nature of the hazard ~~must~~ shall be made. If decontamination is required, the remaining material should be surface decontaminated rapidly in order to avoid warming and moved to a different freezer. Before decontaminating the empty liquid nitrogen freezer, it should be warmed to room temperature.

[5] *Annual Book of ASTM Standards*, Vol 11.05.

6. Keywords

6.1 cryogenic; hazardous biologicals; liquid nitrogen