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Standard Guide for Preservation of Tissue Engineered Medical Products (TEMPs)¹

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INTRODUCTION

The ability to preserve an engineered tissue or cell-based therapeutic device is important for its clinical application. Specifically, preservation permits the ability to store and transport the product, the time for the completion of safety and quality control testing prior to use in patients, and the ability to uncouple the timing of production of the engineered product from its use, ensuring adequate inventory control and the ability to avoid both shortages and product expiration. The aim of this guide is to identify key parameters relevant to the preservation of engineered tissues and cell-based therapies.

1. Scope

1.1 This guide covers the development of standards related to the preservation of cells, tissues, and tissue engineered medical products (TEMPs). Preservation techniques include freezing, vitrification, and hypothermic preservation. This guide discusses preservation, including issues of pre-preservation processing, the process of preservation, storage, transport, recovery, post-preservation processing, quality assurance, and process control.

1.2 This guide contains general guidelines for the preservation of cells, tissues, and tissue engineered medical products (TEMPs) and will identify more specific parameters relevant to the preservation of TEMPs.

1.3 This guide does not apply to any medical products of human origin regulated by the U.S. Food and Drug Administration (FDA) under 21 CFR Parts 16 and 1270 and 21 CFR Parts 207, 807, and 1271.

1.4 *This standard does not purport to address all of the safety concerns, 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 requirements prior to use.*

2. Referenced Documents

2.1 ASTM Standards:²

E1564 Guide for Design and Maintenance of Low-Temperature Storage Facilities for Maintaining Cryopreserved Biological Materials

E1565 Guide for Inventory Control and Handling of Biological Material Maintained at Low Temperatures

2.2 Code of Federal Regulations:³

21 CFR 610.18 Standards for Establishment of Master Cell Banks

21 CFR Parts 16 and 1270 Human Tissue Intended for Transplantation

21 CFR Parts 207, 807, and 1271 Human Cells, Tissues, and Cellular and Tissue-Based Products: Establishment Registration and Listing

2.3 American Association of Blood Banks, Standards:⁴
Preparation of Blood Components
Conditions for Storage, Transportation and Expiration
Tissue Storage and Issue

2.4 American Association of Blood Banks, AABB Technical Manual, Methods:⁴

Method 6.18 Cryopreservation of Hematopoietic Progenitor Cells Using a Controlled Rate Freeze

² 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.

³ Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401.

⁴ Available from American Association of Blood Banks (AABB), 8101 Glenbrook Rd., Bethesda, MD 20814-2749.

¹ This guide is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.45 on Adventitious Agents Safety.

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Method 7.2 Testing Freezer Alarms

Method 7.3 Monitoring Temperature During Shipment

3. Terminology

3.1 Definitions:

3.1.1 *cooling rate*—absolute value of the instantaneous rate of change of temperature during cooling.

3.1.2 *cryopreservation solution*—a preservation medium to which has been added one or more cryoprotectants.

3.1.3 *cryoprotectant*—a chemical or biological substance or mixture of substances used to protect cells or matrix, or both, during cryopreservation and rewarming. In general usage, a cryoprotectant is added to a preservation medium to form a cryopreservation solution.

3.1.4 *cytocrit*—the ratio of cell volume to the total volume of solution and cells for a cell suspension.

3.1.5 *dry shipper*—a storage and transportation device for frozen products that contains a liquid nitrogen (LN) absorbent material in the walls of the container. This device is designed to maintain cryogenic temperature for several days.

3.1.6 *equilibrium freezing temperature*—temperature at which an aqueous solution of a given composition is in equilibrium with ice.

3.1.7 *eutectic point*—a temperature(s) at which the solute(s) in a solution become saturated due to freeze-concentration and precipitate from the solution, causing the unfrozen liquid water to simultaneously freeze.

3.1.8 *glass transition temperature*—the temperature at which the heat capacity associated with translational molecular motions vanishes during cooling or appears during warming. The glass transition temperature is the formal transition point between the glassy state and the liquid state.

3.1.9 *nucleation*—the formation of ice from a supercooled aqueous solution by random aggregation of water molecules into clusters with ice-like properties. Nucleation may be homogenous (spontaneous) or heterogenous (catalyzed by a substrate that reduces the thermodynamic barrier to cluster formation).

3.1.10 *pre-preservation processing*—the manipulation of cells or tissue prior to the initiation of preservation. These steps include, but are not limited to, selection of specific cell populations for freezing, centrifugation to modify cell density, introduction of specific chemical or biological agents, and cooling.

3.1.11 *preservation medium*—a specific formulation of an aqueous or nonaqueous solution in which a population of cells or a tissue will be preserved.

3.1.12 preservation technologies:

3.1.12.1 *anhydrobiotic preservation*—preservation by vaporization and removal of water.

(1) *desiccation*—a process for anhydrobiotic preservation in which water is removed by evaporation.

(2) *freeze-drying*—a process for anhydrobiotic preservation in which ice crystals are formed by freezing, and water is removed by sublimation and evaporation.

(3) *lyophilization*—in common usage, a synonym for freeze-drying. Whereas etymologically, this term also comprises other methods for anhydrobiotic preservation, care should be taken to avoid ambiguity.

3.1.12.2 *cryopreservation*—preservation by cooling to a temperature below the equilibrium freezing temperature of the preservation solution, such that there is solidification. The resulting solid may be either crystalline or amorphous, or a combination of crystalline and amorphous phases.

(1) *freezing*—cryopreservation by formation of crystals. Frozen cell suspensions or TEMPs typically contain both crystalline and amorphous water.

(2) *vitrification*—cryopreservation by formation of glass. Vitified cell suspensions or TEMPs may contain small amounts of crystalline water.

3.1.12.3 *hypothermic preservation*—preservation by cooling to any temperature below normothermic culture temperatures, such that biological components are suspended in a liquid phase. Hypothermic storage temperatures may be either above or below the equilibrium freezing temperature.

3.1.13 *preserve*—to stabilize for the purposes of maintaining the specific mechanical, structural, metabolic, or biological characteristics.

3.1.14 *post-preservation processing*—manipulation of cells or tissue after completion of the preservation process. These steps include, but are not limited to, washing of the cells or tissue to remove the preservation medium/solution and/or specific chemical or biological agents; dilution or concentration of the cells or tissue; and warming to temperatures appropriate for the normal or desired physiological or biochemical functions, or both, of the cells or tissue.

3.1.15 *reconstitute*—to add a solvent or diluent to an anhydrobiotically preserved sample in order to dissolve or suspend its components.

3.1.16 *rewarm*—to warm from preservation temperature to a temperature required for use (for example, additional culture or clinical use).

3.1.17 *seeding*—the deliberate initiation of ice crystal formation in a supercooled aqueous solution under controlled conditions. When ice forms either spontaneously or as a result of the inclusion of nucleating agents in the cryopreservation solution, this is referred to as nucleation.

3.1.18 *storage temperature*—temperature at which the cells, tissue, or TEMP is held after completion of the cooling process.

3.1.19 *supercool*—to cool to a temperature below the equilibrium melting point of the solution without initiating ice formation.

3.1.20 *temperature profile*—the time-temperature history of a sample during cooling or warming.

3.1.21 *thaw*—to warm from a cryopreserved state to a temperature above the melting point of the preservation medium.

3.1.22 *warming rate*—instantaneous rate of change of temperature during warming.