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Standard Guide for *in vivo* Evaluation of Osteoinductive Potential for Materials Containing Demineralized Bone (DBM)[1]

This standard is issued under the fixed designation F2529; 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.

1. Scope

1.1 This guide covers general guidelines to evaluate the effectiveness of DBM-containing products intended to cause and/or promote bone formation when implanted or injected *in vivo*. This guide is applicable to products that may be composed of one or more of the following components: natural biomaterials (such as demineralized bone), and synthetic biomaterials (such as calcium sulfate, glycerol, and reverse phase polymeric compounds) that act as additives, fillers, and/or excipients (radioprotective agents, preservatives, and/or handling agents) to make the demineralized bone easier to manipulate. It should not be assumed that products evaluated favorably using this guidance will form bone when used in a clinical setting. The primary purpose of this guide is to facilitate the equitable comparison of unique bone-forming products in *in vivo* heterotopic models of osteoinductivity. The purpose of this guide is not to exclude other established methods.

1.2 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with the use of DBM-containing bone-forming/promoting products. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices involved in the development of said products in accordance with applicable regulatory guidance documents and in implementing this guide to evaluate the bone-forming/promoting capabilities of the product.*

1.4 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D1193 Specification for Reagent Water

D5056 Test Method for Trace Metals in Petroleum Coke by Atomic Absorption

E508 Test Method for Determination of Calcium and Magnesium in Iron Ores by Flame Atomic Absorption Spectrometry

F565 Practice for Care and Handling of Orthopedic Implants and Instruments

F895 Test Method for Agar Diffusion Cell Culture Screening for Cytotoxicity

F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Insertion into Bone

F1854 Test Method for Stereological Evaluation of Porous Coatings on Medical Implants

F2131 Test Method for ***In Vitro*** Biological Activity of Recombinant Human Bone Morphogenetic Protein-2 (rhBMP-2) Using the W-20 Mouse Stromal Cell Line

F2721 Guide for Pre-clinical ***in vivo*** Evaluation in Critical Size Segmental Bone Defects

2.2 *Federal Documents:*[3]

21 CFR 58 Good Laboratory Practice for Nonclinical Laboratory Studies

21 CFR 820 Quality System Regulation

21 CFR 1270 Human Tissue Intended for Transplantation

21 CFR 1271 Human Cells, Tissues, and Cellular and Tissue-Based Products

21 CFR 610.12 General Biological Products Standards—General Provisions—Sterility

Q1E Evaluation of Stability Data FDA Guidance for Industry

[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 F04.44 on Assessment for TEMPs.

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[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 Food and Drug Administration (FDA), 10903 New Hampshire Ave., Silver Spring, MD 20993-0002, http://www.fda.gov.

Container and Closure Integrity Testing in Lieu of Sterility Testing as a Component of the Stability Protocol for Sterile Products FDA Guidance Document
Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products Guidance for Industry
Guidance for the Preparation of a Premarket Notification Application for a Surgical Mesh Guidance for Industry and/or for FDA Reviewers/Staff and/or Compliance

2.3 *AAMI/ISO Documents:*[4]

AAMI TIR 17 Compatibility of Materials Subject to Sterilization
AAMI/ISO 22442-01 Medical Devices Utilizing Animal Tissues and Their Derivatives—Part 1: Application of Risk Management
AAMI/ISO 22442-03 Medical Devices Utilizing Animal Tissues and Their Derivatives—Part 3: Validation of the Elimination and/or Inactivation of Viruses and Transmissible Spongiform Encephalopathy (TSE) Agents

2.4 *ANSI/AAMI/ISO Documents:*[5]

ANSI/AAMI/ISO 11137 Parts 1–3 Sterilization of Health Care Products—Radiation
ANSI/AAMI/ISO 10993 Biological Evaluation of Medical Products

2.5 *ICH Documents:*[6]

ICH Harmonised Tripartite Guideline Q5C Quality of Biotechnological Products—Stability Testing of Biotechnological/Biological Products
ICH Harmonised Tripartite Guideline Q5A (R1) Viral Safety Evaluation of Biotechnology Products Derived from Cell Lines of Human or Animal Origin

2.6 *USP Document:*[7]

United States Pharmacopeia Chapter <71>

3. Terminology

3.1 *Definitions:*

3.1.1 *additive*—ingredients that may be used to preserve the product, provide radioprotection, and/or act as bulk filler and/or binding agent.

3.1.1.1 *Discussion*—It has no intended mode of action, such as causing cells to transform lineage, once implanted.

3.1.2 *biologically active carrier*—a component added to a DBM-containing bone-forming product that results in a physiological and/or biochemical transformation in the implant site independent of other constituents in the bone-forming product.

3.1.2.1 *Discussion*—The transformation may be desirable or untoward. A biologically active carrier may also contribute to the physical or chemical properties/characteristics of that bone-forming product.

3.1.3 *bone*—hard connective tissue of the skeletal system in vertebrates comprised of collagen, growth factors, and an inorganic rigid matrix containing calcium, phosphate and other minerals, and various cellular elements, including osteoblasts, osteocytes, osteoclasts, and hematopoietic cells.

3.1.3.1 *Discussion*—Bone is made up of cortical and cancellous bone tissue. It serves as the point of attachment for muscles and tendons and is load-bearing.

3.1.4 *bone-forming product (containing DBM)*—as used in this guide, a DBM-containing bone-forming product may be comprised of multiple components including, but not restricted to, demineralized bone, growth factors, differentiation factors, osteoprogenitor cells, mesenchymal stem cells, biologically active carrier(s), and/or non-biologically active carrier(s).

3.1.5 *bone marrow*—tissue located in the cancellous portion and cavities (medullary canal) of most bones.

3.1.5.1 *Discussion*—Bone marrow is highly vascular and occurs in two forms: white/yellow marrow, comprised mostly of adipose cells located primarily in the long bones, and red marrow, which primarily produces and contains pluripotent stems cells and red blood cells, platelets, and white blood cells derived from them. In adults, red marrow is located primarily in the flat bones.

3.1.6 *bone tissue*—the tissue component of a bone comprised of a mineralized collagenous matrix formed and maintained through the action of osteoblastic cells and osteocytes remodeled through the action of osteoclasts.

3.1.7 *cartilage*—connective tissue that is a major constituent of the embryonic and young vertebrate skeleton, largely replaced with bone and bone marrow bone with maturation.

3.1.7.1 *Discussion*—It is comprised mostly of Type II collagen and proteoglycans and found in joints, the outer ear, bronchi, and larynx. There are three major types of cartilage: hyaline cartilage, which is adapted for joint surfaces by virtue of its smoothness and ability to withstand compression; fibrocartilage, found in the outer ear, nose, and meniscus; and elastic cartilage, found in the outer ear and epiglottis. Cartilage is also formed by the action of bone morphogenetic protein(s) (BMPs) in concert with other peptide factors on mesenchymal stem cells.

3.1.8 *cortical bone*—thin superficial layer of compact bone tissue that constitutes the primary load-bearing component of a bone.

3.1.9 *demineralized bone*—bone tissue wherein the average mineral content, typically measured as calcium, is less than or equal to 8 % by dry weight.

3.1.9.1 *Discussion*—As used in this guide, dry weight means lacking significant or measurable residual moisture (<5 %).

3.1.10 *differentiation factors*—proteins with the ability to induce or alter cell differentiation and/or proliferation with subsequent tissue morphogenesis.

3.1.10.1 *Discussion*—For example, BMP-2, BMP-4, and BMP-7(OP-1) are differentiation factors with the ability to

[4] Available from Association for the Advancement of Medical Instrumentation (AAMI), 4301 N. Fairfax Dr., Suite 301, Arlington, VA 22203-1633, http://www.aami.org.

[5] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.

[6] Available from International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), ICH Secretariat, c/o IFPMA, 15 ch. Louis-Dunant, P.O. Box 195, 1211 Geneva 20, Switzerland, http://www.ich.org.

[7] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.