Designation: F1408 – 20

Standard Practice for Subcutaneous Screening Test for Implant Materials[1]

This standard is issued under the fixed designation F1408; 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 practice covers a short-term testing method to screen the subcutaneous tissue reaction to implant candidate materials in small laboratory animals. The material may be dense or porous. This method may not work for absorbable materials, depending on the absorption kinetics. The tissue reactions will be evaluated in comparison to those evoked by control materials that are accepted as clinical implant materials.

1.2 This practice, along with other appropriate biological tests (including other ASTM test methods), may be used to assess the biocompatibility of candidate materials for use in the fabrication of devices for clinical application. It may be also applied to evaluate the effect of special surface textures and preparations of known materials.

1.3 This practice does not provide a comprehensive assessment of the systemic toxicity, carcinogenicity, teratogenicity, or mutagenicity of the material. Additional information may be needed on the material in its final finished form, such as implantation assessment at the clinically relevant location.

1.4 The values stated in SI units, including units officially accepted for use with SI, are to be regarded as standard. No other systems of measurement are included in this standard.

1.5 *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, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *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]

F67 Specification for Unalloyed Titanium, for Surgical Implant Applications (UNS R50250, UNS R50400, UNS R50550, UNS R50700)

F75 Specification for Cobalt-28 Chromium-6 Molybdenum Alloy Castings and Casting Alloy for Surgical Implants (UNS R30075)

F86 Practice for Surface Preparation and Marking of Metallic Surgical Implants

F136 Specification for Wrought Titanium-6Aluminum-4Vanadium ELI (Extra Low Interstitial) Alloy for Surgical Implant Applications (UNS R56401)

F138 Specification for Wrought 18Chromium-14Nickel-2.5Molybdenum Stainless Steel Bar and Wire for Surgical Implants (UNS S31673)

F648 Specification for Ultra-High-Molecular-Weight Polyethylene Powder and Fabricated Form for Surgical Implants

F763 Practice for Short-Term Screening of Implant Materials

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

2.2 *ISO Standard:*[3]

ISO 10993-6:2016 Biological Evaluation of Medical Devices—Part 6: Tests for Local Effects After Implantation

3. Summary of Practice

3.1 Under strict aseptic conditions, test or control samples are implanted subcutaneously along the dorsal midline at the level of the cervical or thoracic vertebra of an anesthetized animal. The size of the implant should not impede the normal movement of the animal. After one, four, and nine to twelve weeks the animals are euthanized, and a comprehensive

[1] This practice is under the jurisdiction of ASTM Committee F04 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

Current edition approved June 1, 2020. Published August 2020. Originally approved in 1992. Last previous edition approved in 2013 as F1408 – 97 (2013). DOI: 10.1520/F1408-20.

[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 International Organization for Standardization (ISO), ISO Central Secretariat, BIBC II, Chemin de Blandonnet 8, CP 401, 1214 Vernier, Geneva, Switzerland, http://www.iso.org.

necropsy is performed. The samples are excised with an intact tissue envelope for histological evaluation. The tissue response to the test sample is compared to the response of the control material.

4. Significance and Use

4.1 This practice is a guideline for a short-term screening test for the evaluation of the tissue response to materials that may be selected for implantation in the human body and should be done in accordance with good laboratory practices. This test may be performed prior to long-term testing (for example, Practice F981) to eliminate unsuitable candidate materials early and to avoid unnecessary animal testing.

4.2 This practice may be used to detect toxic effects of materials in general (see Appendix X1). However, it is particularly suitable for the testing of materials that are intended to have contact with subcutaneous tissues or soft tissues in general. For materials intended to be inserted specifically into muscle tissues, Practice F763 should be considered as a short-term test method.

4.3 The suggested implant specimens are cylindrical. A special grooved type of cylinder may be used (see Fig. X2.1 of Appendix X2) to allow tissue interlocking that could keep the implant in place and minimize tissue irritation through motion at the interface that otherwise could contribute to increased variance of the results. In case ungrooved cylinders are used (see Fig. X1.2 of Appendix X2), probable motion at the implant/tissue interface must be taken into account. Control cylinders should be shaped like the test cylinders.

4.4 The type of surface preparation of the specimens can affect the tissue reaction; therefore the preparation procedure should be noted in the report. The test may be used to compare the effect of different surface structures or conditions of the same material or to assess the effect of various treatments of modifications of a material.

NOTE 1—If this method is used for material research, testing for endotoxin prior to implantation should be considered.

5. Test Animals and Sites

5.1 Laboratory mice (for example, C57BL/6, BALB/c) are used. The test may be adapted to other suitable test animals (for example, rats). Institutional and government animal use and care policies and regulations shall be followed.

5.2 The implant specimens are inserted subcutaneously in the neck of the host.

5.3 One implant (either test or control) is inserted per mouse. Therefore, the number of animals is identical with the number of implants.

5.4 If rats or other larger suitable animals are used, more than one implant may be inserted per animal, but the implants should never be allowed to come in contact with each other. If this is the case, cylinders of the test and control material may be implanted separately on the right and the left side of the neck in a single animal. However, insertion of test and control implants in the same animal may prevent analysis of systemic toxicity.

6. Implant Specimens

6.1 *Specimen Design*—Cylinders of 7 mm length and 4 mm diameter are prepared for implantation in mice. Special specimens with two grooves are designed corresponding to the figures in Appendix X2. If larger animal hosts are used, the implant dimensions may be increased proportionally. If it is impossible to prepare specimens of this kind, the specimen configuration used must be described fully in the report. Implant specimens from the candidate and control material should always have the same dimensions. Control articles with a clinically established acceptable biocompatibility response should be matched as closely as reasonably possible for significant physical characteristics such as surface features (for example, smooth solid sheet versus highly porous mesh, presence of surface finish, etc.).

6.2 *Selection of Control Materials*—Recommended metals for use as control materials include those given in Specifications F67, F75, F136, and F138. However, for specific applications, any metal of known compatibility and standardized as implant material may be employed as a control material for comparison. To study adverse tissue reactions, a non-compatible material like copper may be used as a positive control material. A suitable polymeric control material like the polyethylene USP negative control plastic, Referenced Standard (RS), or ultrahigh molecular weight polyethylene (see Specification F648) may be used.

6.3 *Specimen Surface*—The surface of specimens from prospective implant materials should be treated in the same manner as the implant intended for clinical application in the human patient. Depending on the objective of the test, the control specimens should have either a surface condition as it is normally used for clinical applications or a surface condition most similar to that of the test material. For preparation of metallic materials, Practice F86 should be considered.

6.4 *Numbers of Test and Control Implants*—Per each time period, at least six implant specimens of each candidate and control material should be evaluated in mice (one per mouse). If more than one specimen is implanted in larger (non-mouse) test hosts, at least four animals should be used per material and time period.

6.5 *Conditioning*—The cleaning, sterilization, and packaging should be the same as used for implantation in the human patient. After surface preparation and sterilization, the implant specimens should be protected from surface alterations and contamination and should be handled with non-metallic forceps when appropriate. When plastic forceps are used, be sure that no plastic material is transferred to the implant surface.

7. Procedure

7.1 *Implantation:*

7.1.1 Implant the specimens under sterile conditions in anesthetized animals. The incision site is remote from the implantation site to prevent infection around the implant. In mice, make a 1 cm long incision on the dorsal midline at the level of the sacrum and prepare a subcutaneous tunnel along the dorsum towards the cervical area.

7.1.2 Push the implant through the tunnel and position it in the subcutaneous pocket created in the dorsal cervical area. Then, close the incision with sterile sutures. (Do not place the implant directly underneath the incision to avoid infection.)

7.1.3 Keep the individually marked animals in standard cages that comply with current animal protection requirements. A strong scientific justification and enrichment provisions should be provided if the animals are kept singly in cages.

7.2 *Post-Operative Care*—Care of the animals should be in accordance with accepted standards as outlined in the *Guide for the Care and Use of Laboratory Animals*.[4]

7.2.1 Carefully observe each animal at least once daily, and report any abnormal clinical findings.

7.2.2 If infection or injury occurs, it should be investigated for root cause, and reported.

7.2.3 If an animal dies or needs to be euthanized prior to the scheduled termination study time point, perform a comprehensive necropsy and, if necessary, collect tissues with lesions for histological evaluation to determine the cause of death or morbidity, and relationship to the test or control implant and/or procedure.

7.3 *Euthanasia and Implant Retrieval:*

7.3.1 Euthanize the animals after one, four, and nine to twelve weeks. Examine and report the status of the health of the animals prior to euthanasia.

NOTE 2—If tissue resolution hasn't occurred by the nine to twelve-week assessment, longer term assessments with additional animals may be needed.

7.3.2 Perform a comprehensive necropsy, and record any gross abnormalities. Remove each implant with an intact tissue capsule. If the tissue capsule was damaged during the excision, this should be reported. As soon as possible, transfer the tissue specimen into a fixative that does not alter the implant material.

8. Histologic Evaluation

8.1 *Histological Preparation:*

8.1.1 Use standard laboratory practices for histological preparation and staining (for example, Hematoxylin and Eosin) of the implant/tissue specimens.

8.1.2 If the implant/tissue interface is to be studied, embed the intact tissue envelope with the implant in situ using hard plastics. Appropriate cutting and grinding techniques must be employed for the preparation of histologic slides.[5,6,7,8]

8.1.3 The cutting orientation in relation to the cylinder must be considered and shall be reported.

8.1.4 For non-metallic materials, conventional (for example, paraffin) embedding and standard microtomy may be employed. The stained histological sections of the surrounding tissues from the test and control implants are compared, and their characteristics are reported. A predefined grading scale (for example, ISO 10993-6, Annex E) should be used. The comparison should be made between the same cylinder sections. With grooved implants the center portions between the grooves and the flat top surfaces of the implant are usually used for evaluation.

8.1.5 The counted cell populations at defined distances from the implant interface, and the thickness of the tissue capsule, may be parameters for quantitative evaluation.

9. Report

9.1 Report the following information:

9.1.1 *Implants*—Describe implant material, material condition, fabrication, surface condition, and modifications of the recommended shape and size of implants. If possible, document by taking photographs of the implant after retrieval.

9.1.2 *Conditioning*—Describe cleaning, handling, and sterilization techniques employed.

9.1.3 *Animal Model and Implantation*—Report the species, strain, sex, age, and weight of animal used for testing and number of implants inserted. Describe insertion techniques, special diet, and any medication used during the study. All clinical signs shall be reported. Any animal death should be investigated and reported.

9.2 Include a description of retrieval technique, observations made regarding control and test implants, as well as the gross appearance of the tissues surrounding the implants. The number of implants tested per time interval should be stated.

9.3 Report the observation of each histological examination, including representative photomicrographs. The techniques employed for the preparation of the histological sections shall be described.

9.4 Provide the credentials of the person conducting the gross and histopathological evaluations.

10. Keywords

10.1 biocompatibility; medical devices; mice; short-term tissue screening; subcutaneous tissue screening; tissue compatibility; toxicity/toxicology

[4] National Research Council, *Guide for the Care and Use of Laboratory Animals*, 8th ed., Institute of Laboratory Animal Research Division on Earth and Life Sciences, Washington, DC, National Academies of Science Press, 2011 (http://www.nap.edu/catalog.php?record_id=12910)

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