Designation: F 1408 – 97 (Reapproved 2002)ε1

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

This standard is issued under the fixed designation F 1408; 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.

ε[1] NOTE—Footnote 3 was editorially corrected in November 2002.

1. Scope

1.1 This practice covers a short-term testing method to screen the subcutaneous tissue reaction to metallic or other implant candidate materials in small laboratory animals. The material may be dense or porous. 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 experimental protocol is not designed to provide a comprehensive assessment of the systemic toxicity, carcinogenicity, teratogenicity, or mutagenicity of the material.

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 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 limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*

F 67 Specification for Unalloyed Titanium for Surgical Implant Applications (UNS R50250, UNS R50400, UNS R50550, UNS R50700)[2]

F 75 Specification for Cobalt-28Chromium-6Molybdenum Alloy Castings and Casting Alloy for Surgical Implants (UNS R30075)[2]

F 86 Practice for Surface Preparation and Marking of Metallic Surgical Implants[2]

F 136 Specification for Wrought Titanium-6Aluminum-4Vanadium ELI (Extra Low Interstitial) Alloy for Surgical Implant Applications (UNS R56401)[2]

F 138 Specification for Wrought 18Chromium-14Nickel-2.5Molybdenum Stainless Steel Bar and Wire for Surgical Implants (UNS S31673)[2]

F 648 Specification for Ultra-High-Molecular-Weight Polyethylene Powder and Fabricated Form for Surgical Implants[2]

F 763 Practice for Short-Term Screening of Implant Materials[2]

F 981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants With Respect to Effect of Materials on Muscle and Bone[2]

3. Summary of Practice

3.1 Under strict aseptic conditions, specimens of the candidate and control materials are implanted subcutaneously in the neck of mice (or other suitable animals). After one, three, and nine weeks the animals are anesthetized and the test samples are excised with an intact tissue envelope. On histologic sections the tissue reactions to the candidate materials are compared with the tissue response to clinically accepted control materials.

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. This test may be performed prior to longterm testing (for example, Practice F 981) to eliminate unsuitable candidate materials early and to save further 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 F 763 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

[1] This practice is under the jurisdiction of ASTM Committee F-4 on Medical and Surgical Materials and Devices and is the direct responsibility of Subcommittee F04.16 on Biocompatibility Test Methods.

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[2] *Annual Book of ASTM Standards*, Vol 13.01.

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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.

5. Test Animals and Sites

5.1 Mice of an established strain, (preferably females), are used as test hosts. The test may be adapted to other suitable test animals (for example, rats).

5.2 The implant specimens of control and candidate materials are inserted subcutaneously in the neck of the host.

5.3 One implant is inserted per mouse. Therefore, the number of animals is identical with the number of test specimens. If rats or other larger suitable animals are used, more than one test specimen may be implanted, but the implants should never be allowed to come in contact with each other. If animals other than mice are large enough, cylinders of the candidate and control material may be implanted separately at the right and the left side of the neck in one animal.

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.

6.2 *Selection of Control Materials*—Recommended metals for use as control materials include those given in Specifications F 67, F 75, F 136, and F 138. However, for specific applications any metal of known compatibility and standardized as implant material may be employed as 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, RS, or UHMWPE (see Specification F 648) 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 particular issue, 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 tested candidate material. For preparation of metallic materials Practice F 86 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 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 plastified 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 above the sacrum and prepare a subcutaneous tunnel toward the neck.

7.1.2 Push the implant through the tunnel and position at the neck. In some distance of the implant close the tunnel with three stitches with a thread of a non-metallic suture material to prevent moving of the implant. Then close the incision. (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. Keep mice up to three or four weeks in individual 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*.[3]

7.2.1 Carefully observe each animal during the period of assay, and report any abnormal clinical findings.

7.2.2 If infection or injury of the test implant site invalidates the results, replace the animal so that the number of retrieved implants will be at least that of the schedule.

7.2.3 If an animal dies prior to the expected date of sacrifice, autopsy it and determine the cause of death. Replace the animal if the cause of death is unrelated to the test procedure or the test material. Include the test animal in the assay of data if the cause of death is related to the procedure or test material.

7.3 *Sacrifice and Implant Retrieval*:

7.3.1 Sacrifice the animals after one, three, and nine weeks. If longer time intervals are of interest, mice may be kept up to 24 weeks. Examine and report the status of the health of the animals prior to euthanasia.

7.3.2 At sacrifice, record any gross abnormalities of color or consistency observed on the tissues surrounding the implant. Remove each implant with an intact tissue envelope. If the tissue envelope was damaged during the excision, such should be reported. Transfer the tissue specimen as soon as possible in a fixing agent that does not interfere with the implant material and its probable degradation products.

8. Histologic Evaluation

8.1 *Histological Preparation*:

[3] *The Guide for Care and Use of Laboratory Animals*, Institute of Laboratory Animal Research Publication. Available from National Academy Press, 500 Fifth St., NW, Lockbox 285, Washington, DC 20055.

8.1.1 In general, standard laboratory practices for histological preparation of the implant/tissue specimens and staining are used.

8.1.2 If the implant/tissue interface is to be studied, embedding of the intact tissue envelope with the implant in situ using hard plastics is preferred. Appropriate microtomes or cutting and grinding techniques must be employed for the preparation of histologic slides. Before sectioning, hard metals may be removed by an electrochemical technique. In this case, after embedding, one cuts the sample longitudinally through the implant and dissolves the implant parts electrochemically, providing that the electrochemical procedure does not markedly alter the contacting tissues embedded in the plastics. The empty space may be filled with plastic material to protect the original contacting surface during sectioning.

If the implant material is a ceramic or calcified material, other procedures may need to be considered. Where possible, the material may be dissolved after embedding, thus preserving the interface, and allowing standard histologic procedures. If the material cannot be dissolved after embedding, the use of thick sections and grinding to desired thickness may be preferable.

8.1.3 For quantitative evaluations the cutting geometry in relation to the cylinder must be considered. The implant orientation and cutting geometry shall be reported.

8.1.4 If techniques described under 8.1.2 are not available, conventional (for example, paraffin) embedding and standard microtomy may be employed. However, with this technique the tissue layers closest to the implant are usually destroyed.

8.1.5 If such conventional technique is used, the tissue envelope should be opened before or after exposure to a fixative and the condition of the implant surface and the tissue bed shall be reported.

8.1.6 The stained histologic sections of the surrounding tissues from the candidate- and control-material implants are compared, and their characteristics are reported. 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.

8.1.7 The counted cell populations at defined distances from the implant interface, and the thickness of the tissue capsula 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.

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

9.1.3 *Hosts and Implantation*—Report type of test host and number of implants inserted, if other animals than mice are used. Comment on age, sex, and strain of animals, insertion techniques, and special diet. Any pathologic signs shall be diagnosed and reported. If test animals are lost the cause of death should be noted.

9.2 Include a description of retrieval technique, observations made on 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. The techniques employed for the preparation of the histologic sections shall be described.

10. Keywords

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

APPENDIXES

(Nonmandatory Information)

X1. RATIONALE

X1.1 This practice complements existing ASTM standards on in vivo biocompatibility testing of prospective implant materials. The two particular related standards, Practices F 981 and F 763, provide only procedures for long term testing in muscle and bone and short term testing in muscles, respectively. Thus, a short term subcutaneous screening test is desirable for the assessment of the tissue response to materials intended to be used either for typical subcutaneous implants (for example, in plastic or tumor surgery) or for implants for fracture treatment that are usually in contact with subcutaneous tissues (for example, midface, hand, tibia), muscles, fasciae, tendons, etc. This practice is also of interest for the testing of materials for short term implants such as drains or leads.

X1.2 The test procedure in this practice is used during many years of in vivo implant material testing and is designed to give reproducible results.

X1.3 The use of mice as test hosts has the advantage that the tests are not expensive, and that the animals can be kept separate during the period of wound healing. However, the test method in this practice can be adapted when larger test animals are requested. Then, more than one implant of the same kind can be tested in one animal.

X1.4 The testing of a wide variety of metallic and plastic materials in mice has shown that the reaction to compatible and

non-compatible materials is significantly different. The reaction scale ranges from the death of the animals after three weeks, over tissue necrosis, inflammation, indifferent material layers at the interface, and varying tissue capsulae. The thickness of the tissue capsula surrounding the implants, as well as the cell population, can vary significantly in response to different materials.

X1.5 For information the following literature references may be consulted:

Turner, E., Lawrence, W. H., and Autian, J. "Subacute Toxicity Testing of Biomaterials. Using Histopathological Evaluation of Rabbit Muscle Tissue," *Journal of Biomedical Materials Research, 7,* 39, 1973.

Geret, V., Rahn, B. A., Mathys, R., Straumann, F., and Perren, S. M., "In vivo Testing of Tissue Tolerance of Implant Materials: Improved Quantitative Evaluation through Reduction of Relative Motion at the Implant Tissue Interface," from *Current Concepts of Internal Fixation of Fracture*, Uhthoff, H. K., Springer Verlag, 1980.

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Rahn, B. A., Gerber, H. W., Simpson, J., Straumann, F., and Perren, S. M., *Cultured Cells Contacting Implant Material of Different Surface Treatment Biomaterials 1980*, Edited by Winter, G. D., Gibbson, D. F., and Plenk, Jr. H., John Wiley and Sons Ltd., 1982.

Rahn, B. A., Gerber, H. W., Geret, V., and Perren, S. M. *Assessment of Biocompatibility*, World Congress on Medical Physics and Biomedical Engineering, Hamburg, 1982.

Geret, V., Rahn, B. A., Mathys, R., and Perren, S. M., Quantitative Analyse der In vivo Gewebeverträglichkeit von Hydroxylapatit, Ceros 80, Hefte zur Unfallheilkunde, Heft 165, Hrsg: C. Burri/U. Heim/J. Poigenfürst, Springer-Verlag Berlin Heidelberg, 1983.

X2. SHAPE AND DIMENSIONS OF CYLINDERS FOR IMPLANTATION IN MICE (GIVEN IN MILLIMETRES)

X2.1 See Fig. X2.1 and Fig. X2.2

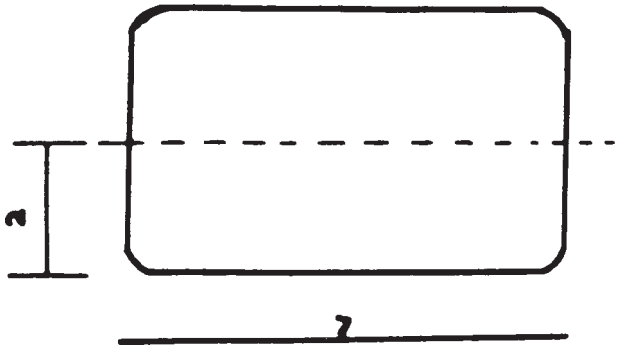

FIG. X2.1 Cylinders Without Grooves

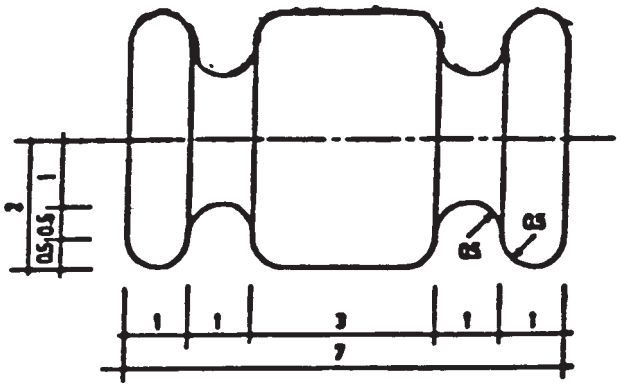

FIG. X2.2 Special Cylinders With Grooves

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