Designation: F981 – 04 (Reapproved 2016)

Standard Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Insertion into Bone[1]

This standard is issued under the fixed designation F981; 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 provides a series of experimental protocols for biological assays of tissue reaction to nonabsorbable biomaterials for surgical implants. It assesses the effects of the material on animal tissue in which it is implanted. The experimental protocol is not designed to provide a comprehensive assessment of the systemic toxicity, immune response, carcinogenicity, teratogenicity, or mutagenicity of the material since other standards deal with these issues. It applies only to materials with projected applications in humans where the materials will reside in bone or soft tissue in excess of 30 days and will remain unabsorbed. It is recommended that short-term assays, according to Practice F763, first be performed. Applications in other organ systems or tissues may be inappropriate and are therefore excluded. Control materials will consist of any one of the metal alloys in Specifications F67, F75, F90, F136, F138, or F562, high purity dense aluminum oxide as described in Specification F603, ultra high molecular weight polyethylene as stated in Specification F648 or USP polyethylene negative control.

1.2 This practice is a combination of Practice F361 and Practice F469. The purpose, basic procedure, and method of evaluation of each type of material are similar; therefore, they have been combined.

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

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 limitations prior to use.*

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

F90 Specification for Wrought Cobalt-20Chromium-15Tungsten-10Nickel Alloy for Surgical Implant Applications (UNS R30605)

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)

F361 Practice for Assessment of Compatibility of Metallic Materials for Surgical Implants with Respect to Effect of Materials on Tissue (Withdrawn 1987)[3]

F469 Practice for Assessment of Compatibility of Nonporous Polymeric Materials for Surgical Implants with Regard to Effect of Materials on Tissue (Withdrawn 1986)[3]

F562 Specification for Wrought 35Cobalt-35Nickel-20Chromium-10Molybdenum Alloy for Surgical Implant Applications (UNS R30035)

F603 Specification for High-Purity Dense Aluminum Oxide for Medical Application

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

[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.

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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] The last approved version of this historical standard is referenced on www.astm.org.

F763 Practice for Short-Term Screening of Implant Materials

3. Summary of Practice

3.1 This practice describes the preparation of implants, the number of implants and test hosts, test sites, exposure schedule, implant sterilization techniques, and methods of implant retrieval and tissue examination of each test site. Histological criteria for evaluating tissue reaction are provided.

4. Significance and Use

4.1 This practice covers a test protocol for comparing the local tissue response evoked by biomaterials, from which medical implantable devices might ultimately be fabricated, with the local tissue response elicited by control materials currently accepted for the fabrication of surgical devices. The materials may include metals (and metal alloys), dense aluminum oxide, and polyethylene that are standardized on the basis of acceptable, well recognized, long-term response. The controls consistently produce cellular reaction and wound healing to a degree that has been found to be acceptable to the host.

5. Test Hosts and Sites

5.1 Rats (acceptable strains such as Fischer 344), New Zealand White rabbits, and other small laboratory animals may be used as test hosts for soft tissue implant response. It is suggested that the rats be age and sex matched. Rabbits or larger animals may be used as test hosts for bone implants. When larger animals such as dogs, goats, or sheep are used, the decision should be based upon special considerations of the particular implant material or study.

5.2 The sacro-spinalis, paralumbar, gluteal muscles, and the femur or tibia can serve as the test site for implants. However, the same site must be used for test and material implants in all the animal species.

5.3 There shall be a minimum of four animals at each sacrifice interval for a total of twelve animals per study. If larger animals are used, in which a greater number of implants may be placed, at least two animals shall be sacrificed at each time period.

6. Implant Specimens

6.1 *Fabrication*—Each implant shall be made in a cylindrical shape with hemispherical ends (see 6.3 and 6.4 for sizes). If the ends are not hemispherical, this shall be reported. Each implant shall be fabricated, finished, and its surface cleaned in a manner appropriate for its projected application in human subjects in accordance with Practice F86. If the specimens are porous, the method of preparation of the porous specimens shall be representative of the contemplated human implant application and shall yield a specimen with characteristic pore size, pore volume, and pore interconnection diameter. The choice between using solid core specimens with porous coatings and specimens that are porous throughout shall be a decision of the investigator and shall be reported.

6.2 Reference metallic specimens shall be fabricated in accordance with 6.1 from materials such as the metal alloys in Specifications F67, F75, F90, F138, or F562, ceramic in Specification F603, or polymers such as in Specification F648 polyethylene or USP Negative Control Plastic. If the test materials are porous, consideration should be given to using porous specimens for reference specimens. Alternatively, nonporous reference specimens may be used.

6.3 *Suggested Sizes and Shapes of Implants for Insertion in Muscle:*

6.3.1 The implants shall be cylindrical in shape and may range from 1 mm to 6 mm in diameter and from 10 mm to 20 mm in length depending upon the relative size of the species under study.

6.3.2 The dimensions used shall be reported in accordance with 8.1.

6.3.3 Depending upon the particular device application, other sample shapes may be used. For instance, an investigator might wish to test the biocompatibility of a new material for screws in the form of a screw. If an alternative specimen shape is used, this should be reported in accordance with 8.1.

6.4 *Sizes and Shapes of Implants for Insertion in Bone:*

6.4.1 Implant diameters for use in bone shall be approximately equal to the cortex thickness. Implant lengths shall allow them to reside in one cortex and the medulla without excessive protrusion beyond the periosteum.

6.4.2 The dimensions used shall be reported in accordance with 8.1.

6.5 *Number of Test and Control Implants:*

6.5.1 In each rat, due to size, there may be two implants; one test and one control material implant.

6.5.2 In each rabbit, due to size, there may be six implants; four test and two control material implants.

6.5.3 In larger animals, there may be twelve implants; eight test material and four control material implants.

6.5.4 In rabbits or larger animals, at least sixteen test materials and eight materials shall be tested at each time period.

6.6 *Conditioning:*

6.6.1 Remove all surface contaminants with appropriate solvents and rinse all test and control implants in distilled water prior to sterilization. It is recommended that the implant materials be processed and cleaned in the same way the final product will be.

6.6.2 Clean, package, and sterilize all implants in the same way as used for human implantation.

6.6.3 After final preparation and sterilization, handle the test and control implants with great care to ensure that they are not scratched, damaged, or contaminated in any way prior to insertion.

6.6.4 Report all details of conditioning in accordance with 8.1.

6.7 *Implantation Period*—Insert all implants into each animal at the same surgical session for implantation periods of 12, 26, and 52 weeks.

7. Procedure

7.1 *Implantation (Muscle):*

7.1.1 Place material implants in the paravertebral muscles in such a manner that they are directly in contact with muscle tissue.

7.1.2 Introduce material implants in larger animals by the technique of making an implantation site in the muscle by using a hemostat to separate the muscle fibers. Then insert the implant using plastic-tipped forceps or any tool that is non-abrasive to avoid damage to the implant.

7.1.3 Introduce material implants using sterile technique. Sterile disposable needles or hypodermic tubing and trochar may be used to implant the material implants into the paravertebral muscles along the spine. In rats, insert a negative control implant on one side of the spine and a test material implant on the other side. In rabbits, implant one negative control material on each side of the spine and implant two test materials on each side of the spine. If larger diameter specimens are used, an alternative implantation technique is that described in 7.1.2.

7.2 *Implantation (Femur)*—Expose the lateral cortex of each rabbit femur and drill undersized pilot holes through the lateral cortex using the technique and instrument appropriate for the procedure. Final reaming of the holes should be performed by hand to yield holes which are smaller than the implant specimens by 0.1 mm or less. Into each one of these holes, insert one of the implants by finger pressure. Then close the wound.

NOTE 1—Caution should be taken to minimize the motion of the implant in the tissue to prevent the effects of motion on the desired result.

7.3 *Postoperative Care:*

7.3.1 All animal studies shall be done in a facility approved by a nationally recognized organization and in accordance with all appropriate regulations.

7.3.2 Carefully observe each animal during the period of assay and report any abnormal findings.

7.3.3 Infection or injury of the test implant site may invalidate the results. The decision to replace the animal so that the total number of retrieved implants will be as represented in the schedule shall be dependent upon the design of the study.

7.3.4 If an animal dies prior to the expected date of sacrifice, perform a necropsy in accordance with the procedure in 7.4 to determine the cause of death. Replacement of the animal to the study shall be dependent upon the design of the study. Include the animal in the assay of data if the cause of death is related to the procedure or test material.

7.4 *Sacrifice and Implant Retrieval:*

7.4.1 Euthanize animals by a humane method at the intervals specified in 6.7.

NOTE 2—The necropsy periods start at 12 weeks because it is assumed that acceptable implant data has been received for earlier periods from short term implant testing according to Practice F763. If the 90-day sacrifice period has been utilized under Practice F763, that group need not be repeated under this protocol, and thus, the 12-week group may be eliminated.

7.4.2 At necropsy, record any gross abnormalities of color or consistency observed in the tissue surrounding the implant. Remove each implant with an intact envelope of surrounding tissue. Include in the tissue sample a minimum of a 4-mm thick layer of tissue surrounding the implant. If less than a 4-mm thick layer of tissue is removed, report in accordance with 8.1.

7.5 *Postmortem Observations*—In accordance with standard laboratory practice, perform a necropsy on all animals that are sacrificed for the purposes of the assay or die during the assay period. Establish the status of the health of the experimental animal during the period of the assay. Report as described in Section 8.

7.6 *Histological Procedure:*

7.6.1 *Tissue Sample Preparation*—Prepare appropriate blocks from each implantation site and indicate the orientation of the axis of the femur relative to the axis of the implant for bone implants. Also indicate the orientation of the implant relative to the axis of rotation of the femoral condyles.

7.6.1.1 Process the excised tissue block containing either a test implant or control implant for histopathological examination and such other studies as are appropriate. Cut the sample midway from end to end into appropriate size and in the appropriate orientation for each study. Transfer, or record, or both, the orientational details noted in 7.6.1 to each part of the sample. Record the gross appearance of the implant and the tissue. If the sample is porous, it is imperative that sectioning procedures be used that maintain the implant within its tissue envelope to allow the evaluation of tissue within the pores. Such procedures may include ground section preparation.

7.6.1.2 If special stains are deemed necessary, prepare additional sections and make appropriate observations.

7.7 *Histopathological Observations*—Compare the amount of tissue reaction adjacent to the test implant to that adjacent to a similar location and orientation on the control implant with respect to thickness of scar, presence of inflammatory or other cell types, presence of particles, and such other indications of interaction of tissue and material as might occur with the actual material under test. A suggested method for the evaluation of tissue response after implantation is Turner, et al. **(1)**[4]. If a porous sample is being tested, the evaluation of the tissue reaction shall include areas within the pores of the test and control samples at similar locations.

7.7.1 *Suggested Method for Tissue Response Evaluation:*

7.7.1.1 A suggested format with tissue response and cell accumulation to be evaluated and a scoring range of 0 to 3 is shown in Table 1.

7.7.1.2 The scoring system of 0 to 3 is based upon the observation of high power fields (400-500X) and an average of five fields.

Tissue Response/Cell Accumulation	Score
0	0
1–5	0.5
6–15	1
16–25	2
26 or more	3

7.7.1.3 The necrosis and/or degeneration score is determined using the same range of 0 to 3, as follows:

[4] The boldface numbers in parentheses refer to the list of references at the end of this standard.

TABLE 1 Suggested Evaluation Format and Scoring Range

Animal Number						
Duration of Implant (weeks)						
Sample Description						
Gross Response						
Histopath-Number						
Score		0	0 .5	1	2	3
Necrosis						
Degeneration						
Inflammation						
	Polymorphonuclear Leukocytes					
	Lymphocytes					
	Eosinophils					
	Plasma Cells					
	Macrophages					
Fibrosis						
Giant Cells						
Foreign Body Debris						
Fatty Infiltration						
Relative Size of Involved Area in mm						
Histopathologic Toxicity Rating						

Degree	Score
No reaction	0
Very slight reaction	0.5
Mild reaction	1
Moderate reaction	2
Marked reaction	3

7.7.1.4 An overall rating of test samples may be given using a rating range of 0 to 4, as follows:

Rating	Score
No reaction	0
Very slight reaction	1
Mild reaction	2
Moderate reaction	3
Marked reaction	4

(1) Pathologists may choose to use the scoring system of comparing the negative control to the test material as an aid in their evaluation. The overall reaction to the test material as compared to the negative control is to be evaluated independently for all time periods.

7.7.2 *Suggested Criteria for Comparing Responses to Test and Control Specimens*—In discussing and reporting the results of this testing, a test article may be reported as having satisfied the requirements of this test if the response of tissues surrounding the test article is not significantly greater than that for the control specimen.

8. Report

8.1 Report the following information:

8.1.1 All details of implant characterization, fabrication, and conditioning (including cleaning, handling, and sterilization techniques employed). For porous implants, a measure of the porosity and pore interconnection diameters shall have been measured and reported.

8.1.2 Procedures for implantation and implant retrieval.

8.1.3 Details of any special procedure (such as an unusual or unique diet fed to test animals).

8.1.4 The observations of each control and test implant as well as the gross appearance of the surrounding tissue in which the implants were implanted.

8.1.5 The observation of each histopathological examination, including a descriptive pathology narration and the pathologist's evaluation as to the reaction to the test material provided.

9. Keywords

9.1 biocompatibility; bone implant materials; cellular reaction; histology/histopathology; implants muscle; New Zealand rabbits; orthopaedic medical devices—bone; plastic surgical devices/applications; polyethylene (PE)—surgical implant applications; rabbits; rats; scar; test animals; tissue compatibility; tissue response evaluation

APPENDIX

(Nonmandatory Information)

X1. RATIONALE FOR PRACTICE F981

X1.1 This practice is based on the research techniques utilized by Cohen (**2**), and by Laing, Ferguson, and Hodge (**3, 4**) in the early 1960s. These studies involved the implantation of metal cylinders in paravertebral muscle of rabbits. The biological reaction to the cylinders was described as the thickness of the fibrous membrane or capsule formed adjacent to the implant. The thickness of the capsule and the presence of inflammatory cells was used as a measure of the degree of adverse reaction to the test material.

X1.2 As first published in 1972, Practice F361 was a test for the biological response to metallic materials. The scope was expanded beyond that of the published reports to include bone as well as muscle as an implant test site. To avoid species-specific reactions, the method called for the use of rats and dogs as well as rabbits. Cylindrical test specimens with rounded ends were used to avoid biological reactions associated with sharp corners or other variations in specimen shape.

X1.3 In 1978, Practice F469 was published as a parallel document for the testing of polymeric materials. Since the methods were essentially the same, the scope of Practice F361 was expanded to include the testing of specimens made of metallic, polymeric or ceramic materials, thereby including and superseding Practice F469.

X1.4 Stainless steel, cobalt chromium, and titanium alloys are used as reference materials since the biological response to these materials has been well characterized by their extensive use in research. The response to these materials is not defined as compatible, but rather the response is used as a reference against which reactions to other materials are compared.

X1.5 This practice is a modification of the original Practice F361 in that it only involves long-term test periods. The short term response to materials is to be evaluated using Practice F763.

X1.6 This practice was revised in 1987 to allow for alternative specimen dimensions for rats and rabbits for muscle implantation. The original specimen dimensions were intended to be implanted through a needle, which was a change from Practice F361 and Practice F469. The alternate dimensions restore those specified since 1972, which some members felt were more appropriate for some material types.

X1.7 This practice was revised in 1990 to add a ceramic material (Specification F603) as a reference material when testing ceramics.

X1.8 In 1991, this practice was revised to add the testing of porous materials to the Scope. Previously, the committee had been unable to achieve consensus on the appropriate modifications to the technique to allow the testing of porous materials.

X1.9 This practice is based upon over 30 years of published experience in the use of these techniques for the evaluation of the response of tissue to implant materials. In revision, there was discussion of the appropriate length of a long-term study. Comments received suggested that one- or two-year studies were excessive. It was the decision of the task force that the one-year sacrifice interval would be maintained but that the two-year interval for larger animals could be removed.

X1.10 The revision of this document in 1992 removed all reference to the use of the canine for these studies to encourage the use of rats and rabbits when practical. Larger animals such as dogs, goats, and sheep may be utilized when found to be appropriate by the investigator.

REFERENCES

(1) Turner, J. E., Lawrence, W. H., and Autian, J., "Subacute Toxicity Testing of Biomaterials using Histopathologic Evaluation of Rabbit Muscle Tissue," *Journal Biomedical Material Research*, Vol. 7, No. 39, 1973.

(2) Cohen, J., "Assay of Foreign-Body Reaction," *Journal of Bone and Joint Surgery*, No. 41A, 1959, pp. 152–166.

(3) Ferguson, A. B., Jr., Laing, P. G., and Hodge, E. S.," The Ionization of Metal Implants in Living Tissues," *Journal of Bone and Joint Surgery*, No. 42A , 1960, pp. 77–90.

(4) Laing, P. G., Ferguson, A. B., Jr., and Hodge, E. S.," Tissue Reaction in Rabbit Muscle Exposed to Metallic Implants," *Journal Biomedical Materials Research*, No. 1, 1967, pp. 135–149.