Designation: F 561 – 97

Practice for Retrieval and Analysis of Implanted Medical Devices, and Associated Tissues[1]

This standard is issued under the fixed designation F 561; 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 recommendations for the retrieval, handling, and analysis of implantable medical devices and associated specimens which are removed from patients, during revision surgery, at postmortem, or as part of animal studies. The aim is to provide guidance in preventing damage to the associated specimens which could obscure the investigational results, and in gathering data at the proper time and circumstance to validate the study.

1.2 This practice offers guidelines for the analysis of retrieved implants to limit damage to them, and to allow comparisons between investigational results from different studies. The protocols are divided into three stages, where Stage I is the minimum non-destructive analysis, Stage II is more complete non-destructive analysis, and Stage III is destructive analysis. Standard protocols for the examination and collection of data are provided for specific types of materials in relation to their typical applications. For particular investigational programs, additional, more specific, protocols may be required. If special analytical techniques are employed, the appropriate handling procedures must be specified.

1.3 This practice recommendation should be applied in accordance with national regulations or legal requirements regarding the handling and analysis of retrieved implants and excised tissues, especially with regard to handling devices which may become involved in litigation, as per Practice E 860.

1.4 A significant portion of the information associated with a retrieved implant device is often at the device-tissue interface or in the tissues associated with the implant and related organ systems. Attention should be given to the handling of adjacent tissues, so as not to interfere with study of the particles in the adjacent tissue, a chemical analysis for the byproducts of degradation of the implant, or a study of the cellular response to the implant.

1.5 *This standard may involve hazardous materials, operations, and equipment. As a precautionary measure, removed implants should be sterilized or minimally disinfected by an appropriate means that does not adversely affect the implant or the associated tissue that may be subject to subsequent analysis. 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:*

A 262 Practices for Detecting Susceptibility to Intergranular Attack in Austenitic Stainless Steels[2]

A 751 Test Methods, Practices and Terminology for Chemical Analysis of Steel Products[2]

C 20 Test Methods for Apparent Porosity, Water Absorption, Apparent Specific Gravity and Bulk Density of Burned Refractory Brick and Shapes by Boiling Water[3]

C 158 Test Methods for Strength of Glass (Determination of Modulus of Rupture)[4]

C 169 Test Methods for Chemical Analysis of Soda-Lime and Borosilicate Glass[4]

C 573 Test Methods for Chemical Analysis of Fireclay and High-Alumina Refractories[5]

C 623 Test Method for Young's Modulus, Shear Modulus, and Poisson's ratio for Glass and Glass-Ceramics by Resonance[4]

C 633 Test Method for Adhesion or Cohesive Strength of Flame-Sprayed Coatings[6]

C 674 Test Methods for Flexural Properties of Ceramic White Ware Materials[4]

C 730 Test Method for Knoop Indentation Hardness of Glass[4]

C 849 Test Method for Knoop Indentation Hardness of Ceramic Whitewares[4]

C 1069 Test Method for Specific Surface Area of Alumina or Quartz by Nitrogen Adsorption[4]

C 1161 Test Method for Flexural Strength—Advanced Ceramics at Ambient Temperatures[3]

C 1198 Test Method for Dynamic Young's Modulus, Shear

[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 F04.18 on Device Retrieval Analysis.

Current edition approved Dec. 10, 1997. Published June 1998. Originally published as F 561 – 78. Last previous edition F 561 – 87 (1994).

[2] *Annual Book of ASTM Standards*, Vol 01.03.
[3] *Annual Book of ASTM Standards*, Vol 15.01.
[4] *Annual Book of ASTM Standards*, Vol 15.02.
[5] Discontinued—See 1994 *Annual Book of ASTM Standards*, Vol 03.05.
[6] *Annual Book of ASTM Standards*, Vol 02.05.

Modulus, and Poisson's Ratio for Advanced Ceramics by Sonic Resonance[3]
D 256 Test Methods for Determining the Izod Pendulum Impact Resistance of Plastics[7]
D 412 Test Methods for Vulcanized Rubber and Thermoplastic Elastomers-Tension[7]
D 570 Test Method for Water Absorption of Plastics[7]
D 621 Test Methods for Deformation of Plastics Under Load[7]
D 624 Test methods for Tear Strength of Conventional Vulcanized Rubber and Thermoplastic Elastomers[8]
D 638 Test Method for Tensile Properties of Plastics[7]
D 671 Test Method for Flexural Fatigue of Plastics by Constant Amplitude of Force[7]
D 695 Test Method for Compressive Properties of Rigid Plastics[7]
D 732 Test Method for Shear Strength of Plastics by Punch Tool[7]
D 747 Test Method for Apparent Bending Modulus of Plastics by Means of a Cantilever Beam[7]
D 785 Test Method for Rockwell Hardness of Plastics and Electrical Insulating Materials[7]
D 790 Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials[7]
D 792 Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement[7]
D 1004 Test Method for Initial Tear Resistance of Plastic Film and Sheeting[7]
D 1042 Test Method for Linear Dimensional Changes of Plastics Under Accelerated Service Conditions[7]
D 1238 Test Method for Flow Rates of Thermoplastics by Extrusion Plastometer[7]
D 1239 Test Method for Resistance of Plastic Films to Extraction by Chemicals[7]
D 1242 Test Methods for Resistance of Plastic Materials to Abrasion[7]
D 1505 Test Method for Density of Plastics by the Density—Gradient Technique[7]
D 1621 Test Method for Compressive Properties of Rigid Cellular Plastics[7]
D 1622 Test Method for Apparent Density of Rigid Cellular Plastics[7]
D 1623 Test Method for Tensile and Tensile Adhesion Properties of Rigid Cellular Plastics[7]
D 1708 Test Method for Tensile Properties of Plastics by Use of Micro Tensile Specimens[7]
D 2240 Test Method for Rubber Property—Durometer Hardness[7]
D 2842 Test Method for Water Absorbtion of Rigid Cellular Plastics[9]
D 2857 Test Method for Dilute Solution Viscosity of Polymers[9]
D 2873 Test Method for Interior Porosity of Poly(Vinyl Chloride) (PVC) Resins by Mercury Intrusion Porosimetry[9]
D 2990 Test Methods for Tensile, Compressive, and Flexural Creep and Creep-Rupture of Plastics[9]
D 3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships[9]
D 3417 Test Methods for Heats of Fusion and Crystallization of Polymers by Thermal Analysis[9]
D 3418 Test Method for Transition Temperatures of Polymers by Thermal Analysis[9]
D 3835 Test Method for Determination of Properties of Polymeric Materials by Means of a Capillary Rheometer[9]
D 3919 Practice for Measuring Trace Elements in Water by Graphite Furnace Atomic Absorption Spectrophotometry[10]
D 4000 Classification System for Specifying Plastic Materials[9]
D 4001 Test Method for Determination of Weight-Average Molecular Weight by Light Scattering[11]
D 4065 Practice for Determining and Reporting Dynamic Mechanical Properties of Plastics[9]
D 4754 Test Method for Two-Sided Liquid Extraction of Plastic Materials Using FDA Migration Cell[11]
D 5152 Practice for Water Extraction of Residual Solids from Degraded Plastics for Toxicity Testing[11]
D 5227 Test Method for the Measurement of Hexane Extractable Content of Polyolefins[11]
D 5296 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrenbe b Hugh Performance Size-Exclusion Chromatography[9]
E 3 Methods of Preparation of Metallographic Specimens[12]
E 7 Terminology Relating to Metallography[12]
E 8 Test Methods for Tension Testing of Metallic Materials[12]
E 10 Test Method for Brinell Hardness of Metallic Materials[12]
E 18 Test Methods for Rockwell Hardness and Rockwell Superficial Hardness of Metallic Materials[12]
E 45 Test Methods for Determining the Inclusion Content of Steel[12]
E 92 Test Method for Vickers Hardness of Metallic Materials[12]
E 112 Test Methods for Determining the Average Grain Size[12]
E 120 Test Methods for Chemical Analysis of Titanium and Titanium Alloys[13]
E 135 Terminology Relating to Analytical Chemistry for Metals, Ores, and Related Materials[13]
E 168 Practices for General Techniques of Infrared Quantitative Analysis[14]
E 204 Practices for Identification of Materials by Infrared Absorption Spectroscopy Using the ASTM Coded Band and Chemical Classification Index[12]

[7] *Annual Book of ASTM Standards*, Vol 08.01.
[8] *Annual Book of ASTM Standards*, Vol 09.01.
[9] *Annual Book of ASTM Standards*, Vol 08.02.
[10] *Annual Book of ASTM Standards*, Vol 11.01.
[11] *Annual Book of ASTM Standards*, Vol 08.03.
[12] *Annual Book of ASTM Standards*, Vol 03.01.
[13] *Annual Book of ASTM Standards*, Vol 03.05.
[14] *Annual Book of ASTM Standards*, Vol 03.06.

E 290 Test Method for Bend Testing of Material for Ductility[12]
E 353 Test Methods for Chemical Analysis of Stainless, Heat-Resisting, Maraging, and Other Similar Chromium-Nickel-Iron Alloys[13]
E 354 Test Methods for Chemical Analysis of High-Temperature, Electrical, Magnetic, and Other Similar Iron, Nickel, and Cobalt Alloys[13]
E 386 Practice for Data Presentation Relating to High Resolution Nuclear Magnetic Resonance (NMR) Spectroscopy[15]
E 407 Practice for Microetching of Metals and Alloys[12]
E 562 Practice for Determining Volume Fraction by Systematic Manual Point Count[12]
E 663 Practice for Flame Atomic Absorption Spectroscopy[14]
E 860 Practice for Examining and Testing Items that are or May Become Involved in Products Liability Litigation[14]
E 883 Guide for Reflected-Light Photomicrography[12]
E 986 Practice for Scanning Electron Microscope Beam Size Characterization[12]
E 1188 Practice for Collection and Preservation of Information and Physical Items by a Technical Investigator[16]
E 1452 Practice for Preparation of Calibration Solutions for Spectrophotometric and for Spectroscopic Atomic Analyses[14]
E 1479 Practice for Describing and Specifying Inductively-Coupled Plasma Optical Emission Spectrophotometers[14]
F 316 Test Method for Pore Size Characteristics of Membrane Filters for Use with Aerospace Fluids[10]
F 619 Practice for Extraction of Medical Plastics[17]
F 981 Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Bone[17]
F 1044 Test Method for Shear Testing of Porous Metal Coatings[17]
F 1147 Test Method for Tension Testing of Porous Metal Coatings[17]
F 1501 Test Method for Tension Testing of Calcium Phosphate Coatings[17]
F 1658 Test Method for Shear Testing of Calcium Phosphate Coatings[17]

2.2 *Other Document:*

ISO/DIS 12891-1, Retrieval and Analysis of Implantable Medical Devices, Part 1: Standard Practice for Retrieval and Handling

3. Terminology

3.1 *Definition of Terms Specific to Issues of Microbial Contamination:*

3.1.1 *antiseptic*—a germicide that is used on skin or living tissue for the purposes of inhibiting or destroying microorganisms.

3.1.2 *decontamination*—a process or treatment that renders a medical device, instrument, or environmental surface safe to handle. Ranges from sterilization to cleaning with soap and water.

3.1.3 *disinfectant*—a germicide that is used solely for destroying microorganisms on inanimate objects.

3.1.4 *disinfection*—generally less lethal than sterilization. It eliminates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms (e.g., bacterial endospores) on inanimate objects. It does not insure overkill.

3.1.5 *sterilization*—use of a physical or chemical procedure to destroy all microbial life; including large numbers of highly resistant bacterial endospores.

4. Summary of Practice

4.1 This practice provides recommendations for collection of clinical data, analysis of adjacent tissues and the material characterizations to be performed when an implant is retrieved as part of a clinical or an animal study.

4.2 The clinical data to be recorded include a case history review, roentgenogram reviews, tissue culture, and observations of the implant site.

4.3 Protocols are provided for the handling of the implant tissue interface, and adjacent tissues for subsequent analysis. These protocols are intended to facilitate (a) histologic and immunohistochemical examination of the tissues, (b) chemical analysis of the tissues for identification and quantification of implant corrosion or degradation products, and (c) digestion of tissues for subsequent harvesting and analysis of particulate debris.

4.4 The material characterizations include observation and description of the explanted device and adjacent tissues, determination of chemical composition, macroscopic and microscopic examinations and mechanical property determinations. The guidelines are separated in three stages. Stage I is considered to comprise an essential minimum analysis for routine examination of all types of materials. Stage II is nondestructive but provides more detail and is intended for special studies of devices with or without impaired function, made of all types of materials. Stage III includes destructive methods for and material-specific protocols for detailed failure, microstructural, and chemical analysis as well as determination of physical and mechanical properties.

5. Significance and Use

5.1 The investigation of retrieved implantable medical devices and adjacent tissues can be of value in the assessment of clinical complications associated with the use of a specific prosthetic device design; can expand the knowledge of clinical implant performance and interactions between implants and the body; provide information on implant performance and safety; and thus further the development of biocompatible implant materials and devices with improved performance.

5.2 A significant portion of the information associated with a retrieved implant is obtained with detailed studies of the device-tissue interface. Appropriate methods are provided to facilitate a study of the particles in the tissues, and chemical analysis for the byproducts of degradation of the implant, and histologic evaluation of the cellular response to the implant.

5.3 For the analysis to be accurate, it is essential that the

[15] *Annual Book of ASTM Standards*, Vol 14.02.
[16] *Annual Book of ASTM Standards*, Vol 13.01.
[17] Available from American National Standards Institute, 1430 Broadway, New York, NY 10018.

device and associated tissues be removed without alteration of their form and structure. It is also essential that the tissues be handled in such a way as to avoid microbial contamination of the work place or the investigator. Standard protocols for the examination and collection of data are provided for retrieval and handling of implantable medical devices, as well as for specific types of materials in relation to their typical applications. For particular investigational programs, additional, more specific, protocols may be required. If special analytical techniques are employed, the appropriate procedures must be specified.

5.4 In order to interpret the analysis of materials and tissues, it is also essential to capture a minimum data set regarding the clinical findings and laboratory studies documenting device performance and reasons for removal.

5.5 Any destructive analysis of implants must be done so as to not destroy any features that may become the subject of litigation, as per Practice E 860. This standard recommendation should be applied in accordance with state or national regulations or legal requirements regarding the handling and analysis of retrieved implants and tissues.

6. Interferences

6.1 Some critical features of the retrieved implant, tissue and the interface can only be accurately described by observation at the time of removal, and prior to sterilization or disinfection. Such observation must be made using appropriate aseptic precautions.

6.2 Due to the destructive nature of some of the analysis protocols provided in this practice, their use precludes any other type of analysis. It is therefore essential that handling of the device and tissues be done in concert with the requirements of all of the analyses to be performed, including analyses that may be done in the future.

7. Hazards

7.1 The handling of retrieved implants and tissues may involve handling of infectious material.

7.2 It is suggested that individuals handling the devices be vaccinated against Hepatitis B. As a precautionary measure, removed implants should be sterilized by an appropriate means that does not adversely affect the implant.

7.3 There are situations where tissues or implants can not be sterilized or disinfected prior to analysis, for example, requirements of specialized protocols in which sterilization will adversely effect tissue or material properties. In such cases, extreme care should be taken to use aseptic technique and disinfection. Where institutional guidelines for the handling of septic material do not exist, details for handling and sterilizing retrievals, and laboratory practice recommendations can be found in ISO 12891-1.

8. Clinical Information Gathered at the Time of Implant Explantation

8.1 The extent of clinical information to be obtained will depend in part on the type of implant and reasons for removal. Similarly, the amount of information provided about the implant site will depend on the circumstances regarding the removal. A detailed listing and format for documentation of the clinical information associated with removal are provided in Appendix X1.

8.2 As a minimum, the clinical information for device tracking should include the following information:

8.2.1 Date of implantation, and date of explantation.

8.2.2 Identification of hospitals, or physicians' offices, where device implantation and removal was performed.

8.2.3 Confidential, unique, patient ID Code to link to hospitals implantation and removal records.

8.2.4 Device identification (manufacturer's name and device catalogue number).

8.2.5 Device lot and serial number.

8.2.6 Indication for use and reason for explantation (clinical diagnosis).

8.3 For purposes of implant retrieval studies, the following information is considered essential:

8.3.1 Patient or animal age and sex.

8.3.2 A generic statement as to level of patient activity relative to the device.

8.3.3 A statement as to any gross evidence of inflammation, implant site infection, or tissue damage such as osteolysis.

8.3.4 Orientation of the implant relative to the patient. It is suggested that the proximal end of the device be identified with a nondestructive marking scheme.

8.4 More detailed clinical information should be gathered, where feasible, as indicated in Appendix X1. Obtaining an in situ, intraoperative photograph of the implant is highly desirable.

8.5 To facilitate subsequent analysis, it is recommended that the device be removed with the tissue interface intact.

8.5.1 In cases of animal studies of tissue responses to implants, the implant should be removed with at least a 4 mm thick layer of adjacent tissue, as per F 981.

9. Analysis of the Tissues and the Tissue-Implant Interface

9.1 *Macroscopic Examination of Tissue*:

9.1.1 Record a gross pathologic description of the tissue immediately adjacent to the implant, as to consistency and color, as seen by the naked eye, or with a hand lens or dissecting microscope. Record any differences between the implant-tissue interface and the tissues not in direct contact with the implant. Describe the specimen size either by dimensions or weight.

9.1.2 Since the color of tissue is altered by sterilization and fixation methods, it is recommended that gross observations be made prior to sterilization. Such observations should be made utilizing aseptic techniques.

9.1.3 Where appropriate and feasible, obtain photographic documentation of the explant and adjacent tissue, as well as a photographic record of subsequent dissections.

9.2 *Histopathological Analysis of Tissue*:

9.2.1 Process the excised tissue using standard laboratory procedures for the histological dehydration, embedding and sectioning. These procedures may be for paraffin embedding, methacrylate embedding or other special procedures. Routine staining with hematoxylin and eosin (H & E), or toluidine blue are recommended for light microscopy of soft tissues and bone.

Special stains, for example, von Kassa, Masson, Movat pentachrome, may be utilized as indicated and should be fully described.

9.2.2 Provide a detailed histopathologic description of the tissue-implant interface as well as all adjacent tissue specimens, for example, extracellular matrix, necrotic changes, thickness of fibrous capsule, cell types, particulates, hyperplasia, dysplasia, type of inflammatory reaction.

9.2.3 If the implant material is porous, then tissue analysis must include evaluation of the reaction within the pores as well as in the adjacent tissues. This should include the degree and nature of tissue ingrowth, and biological fixation.

9.2.4 For detailed studies of tissue reactions, the use of a quantitative scoring scheme, such as that in Practice F 981 is recommended.

9.2.5 Since some polymeric materials, for example, PMMA bone cement, are altered or dissolved by the solutions used for routine histology, special techniques may be indicated, or special note made of voids formerly occupied by the material.

9.3 *Immunohistochemical and Other Special Histopathology Protocols*:

9.3.1 These procedures can be used for identifying specific cell types and extracellular matrix tissue responses to implantable materials and prosthetic devices. This field is constantly changing, and therefore only one such approach is provided as an example.

9.3.1.1 Typical markers chosen are for the presence of immunoglobulins on lymphocytes to indicate B cells or on monocytes/macrophages to indicate activation, the presence of CD2 markers to indicate immature T cells, the presence of CD3 markers to indicate mature T cells, and markers to indicate activated macrophages.

9.3.1.2 The protocols consist of a series of steps or reactions which have been developed to amplify the reactions, and to be cost effective. First, an antibody specific for the CD marker is used (typically mouse anti-human). Then, a biotinilated antibody to the first antibody is applied (typically goat anti-mouse); biotin serves as a marker in this amplification phase of the reactions. Strept-avidine peroxidase is then added to bind to the biotin and immobilize the peroxidase. Finally, a substrate is added which will react with the peroxidase, change color and precipitate. Diaminobenzidine (DAB) is often used, although several substrates are available for different kits or automatic systems. The end result is the peroxidase oxidation of DAB to give a yellow-brown precipitate at the site of the reaction. The sections can be stained with hematoxylin to enhance the visibility of cells.

9.3.1.3 An example of a method to be used is briefly summarized below and is based on standard techniques. Although it was originally described for use on frozen tissues, the use of embedded tissues allows for examination of the same tissue blocks used for routine pathology. This is only one of many approaches.

9.3.2 *Reagents*:

9.3.2.1 The reagents used come from a variety of companies including DAKO, Becton Dickinson, Kirkegaard & Perry, and Oncogene.

9.3.2.2 Antibody for specific markers, for example, CD2, CD3.

9.3.2.3 Biotinilated goat anti-mouse or anti-rabbit IgG.

9.3.2.4 Strept-avidine peroxidase.

9.3.2.5 Diaminobenzidine (DAB), or other suitable substrate.

9.3.3 Sections are deparaffinated in xylene for 5 min twice, and then rehydrated with absolute ethanol for 3 min, 95 % ethanol for 3 min, and then in 70 % ethanol for 3 min.

9.3.4 The sections are then placed in a methanol-hydrogen peroxide solution for 30 min to diminish the background level of peroxidase in the tissue. The sections are rinsed in water, next placed in buffered saline, and then the slide around the section is dried.

9.3.5 The slide is then placed in a humidity chamber, covered with buffer, and the first antibody is added. This will be the antibody specific for the marker (for example, CD2) and will be either of mouse or rabbit origin. This is incubated overnight, then rinsed with buffer, drained, and the slide around the tissue dried.

9.3.6 The second antibody, which is biotinolated, is added. This is usually goat anti-mouse or anti-rabbit IgG. This is incubated for 30 min, rinsed, the slide dried, and then strept-avidin peroxidase is added.

9.3.7 The strept-avidin peroxidase is incubated for 30 min, rinsed, and then a substrate such as DAB is added. The development of the color is watched under the microscope, the action stopped with water, then the slides are dipped into osmium tetroxide for final fixation. The slides may be counterstained with hematoxylin for visualization of all cells. The slides are processed for mounting with eukitt and can be evaluated for presence of label.

9.3.8 This method can be used to detect the production of cytokines in the cells in the tissues. However, caution should be used in the interpretation of findings, since these are soluble mediators and rapidly leave the site of origin.

9.4 *Chemical Analysis of Tissues By Flame Atomic Absorption Spectroscopy (AAS), Graphite Furnace Atomic Absorption Spectroscopy (GFAAS), by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) or Mass Spectroscopy (ICPMS)*:

9.4.1 *Reagents and Materials*:

9.4.1.1 Standard AAS grade solutions (MCB reagents, Fisher, and VWR) are used to make calibration curves. Calibration solutions should be prepared according to Practices E 1452 and D 3919, using the same matrix solution as the test specimen. Solutions of low concentration should be made fresh daily. The sensitivity and possible interferences depend on the particular element.

9.4.1.2 Any fixing agents, chemicals and solvents must be of analytic purity. The use of 70 % ethanol is recommended as a transport and storage solution. The use of double distilled, deionized water is necessary.

9.4.1.3 Handling of tissues for subsequent chemical analysis requires special precautions to be taken to insure that the specimens are not contaminated with the elements to be analyzed. Surgical knives or instruments used for tissue excision shall be free of any contamination or loose particulates.

The use of ceramic or glass knives is recommended for preparation of specimens associated with metallic implants. Glass knives are not recommended for subsequent silicone analysis.

9.4.1.4 Tissues should be transferred to plastic or glass containers of high quality which have been thoroughly acid cleaned or unused from a lot tested to be free of contamination. Acid cleaning which may etch the glass surfaces is not recommended for subsequent silicone analysis. Tissue transfer should be done in a dust free environment.

9.4.2 Test solutions should be analyzed in triplicate, either as is or after dilution with 1 % nitric acid to a concentration which falls within the standards, and the results averaged. Concentrations are determined in µ/l (ppb), or µ/g of tissue (ppm). Results from solutions of known volumes from *in vitro* studies can be converted to total micrograms in solution.

9.4.2.1 The concentration of metallic species in tissue may vary according to the location of the specimen relative to the implant. It is therefore important to carefully record the location of the specimen.

9.4.3 These methods of analysis require chemical digestion of the tissue samples prior to analysis, and therefore the samples can not be used for any other analysis. The ability to digest tissue is influenced by the method of tissue fixation. It is recommended that tissues be fixed in analytical grade 70 % ethanol in analytical grade water. The methods of digestion depend on the type of tissue to be analyzed.

9.4.3.1 *Blood* samples drawn from patients or animals should be done using polypropylene syringes. The blood can be allowed to clot at room temperature and centrifuged at 1850 g for 30 min to separate serum and clot fractions. Blood may also be drawn in heparinized vacutainer tubes. The blood may be allowed to settle so as to isolate red and white cells, or be centrifuged at 400 g and the plasma supernatant drawn off. Plasma is diluted at least 2× in 1 % nitric acid.

9.4.3.2 *Cells*, either red blood cells or cells from cell culture experiments may receive special treatment, such as separation of cell contents and cell membranes. The cells are washed and centrifuged 3 times with physiologic saline to remove trapped serum or growth media. The cell pellet is then lysed with 1 % Triton X100 to release intracellular contents, centrifuged and the supernatant harvested. This solution is pipetted off, diluted 2× in 1 % nitric acid, and referred to as "cell contents." The pellet of cell membranes is then washed and centrifuged 3 times in saline to remove the Triton and remaining contents. The membranes are then digested in 50 % nitric acid, diluted in 0.5 % nitric acid for analysis as "cell membranes."

9.4.3.3 *Tissue* from implant sites or joint capsules should be weighed and placed in plastic bags for the mechanical tissue digestion machine. These are then frozen until use. For preparation, 5 ml of 50 % nitric acid will be added to each bag. The tissue is then homogenized in a mechanical blender. The samples are then pipetted into the GFAAS for analysis. Dilution as necessary is done with nitric acid.

9.4.3.4 *Bone* specimens can be subjected to a two-stage digestion procedure to separate them into two phases, mineralized and demineralized (or organic). The bones are placed in 0.5 N HCl for 48 hrs at 4°C to demineralize them. The rubbery demineralized samples are then placed in 50 % nitric acid to digest the organic portion. Both the HCl and HNO_3 samples are diluted as necessary and analyzed by GFAAS.

9.4.4 Chemical analysis by flame AAS should be done according to manufacturer's instructions, in accordance with E 663 and E 135.

9.4.5 Analysis with graphite furnace (GFAAS) should be done according to D 3919 using the manufacturer's specifications for analyte wavelength and slit width. Calibration standards, as per E 1452, should be made up in the same matrix as the test specimens.

9.4.5.1 A multi-cycle protocol is recommended to insure complete drying of the specimen prior to charring and atomization; additional steps may be required for post analysis clearing of the tubes.

9.4.5.2 The use of ultra pure argon for clearing is essential.

9.4.5.3 There are two types of graphite tubes used in the furnace: pyrolytic carbon coated graphite tubes, and tubes with L'vov platforms. The analysis for Ni, Mo, Pt, Ti, and V are done from the wall of the tubes while Co, Cr and Al are done on platforms.

9.4.6 Chemical analysis by ICP should be done in accordance with Practice E 1479.

9.5 *Analysis of Tissues for Particulate Debris*:

9.5.1 Analysis for particulates can be done histopathologically as in 9.2, or by tissue or fluid digestion and particle separation. Tissues subjected to digestion become dedicated to this type of analysis.

9.5.1.1 Preparation of tissue for drying and digestion will depend on the state of the tissue. If the tissue is embedded in paraffin, the tissue block should be deparaffinized with xylene overnight at room temperature, and then washed with 100 % ethanol. If tissue is fixed in formalin, it should be dehydrated through a series of alcohols using standard histological protocol, and infiltrated with 100 % ethanol. If tissue is fresh, it should be frozen and free of embedding media.

9.5.1.2 The tissue will be weighed, or the fluid volume determined before digestion. If a wet weight is desired (only applicable for the fresh tissue), 0.3–0.5 g are typically weighed out. For a dry tissue weight, the tissue should be freeze dried, sliced with a ceramic knife, and 0.02–0.03 g weighed out with a microbalance. Weighed tissue should then be placed in acid washed polystyrene vials. Once weighed, the tissue is suitable for digestion.

9.5.1.3 Several digestion protocols are described in the literature. The choice of protocol depends in part on the type of particle of interest, for example, metal versus polymer, and on the type of tissue. Four methods of digestion are described in this recommended practice.

9.5.2 *Reagents for Digestion*:

9.5.2.1 *Ultrapure water*—Distilled H_2O filtered with 0.2 µm filter.

9.5.2.2 *Phosphate Buffer*—3.55 g Na_2HPO_4, 3.45 g NaH_2PO_4, 0.744 g EDTA, in 100 ml ultrapure water.

9.5.2.3 *Papain solution*—1 ml phosphate buffer, 100 µL pure papain, 3.26 mg N-acetylcysteine, 9 ml ultrapure water.

9.5.2.4 *Strong bases*—Tissues have been digested in solutions of sodium hydroxide ranging from 1–10 N NaOH (5–50

ml/g of tissue), or in potassium hydroxide 2 N KOH (10 ml/g of tissue), or 4 M KOH (2 ml/gram of tissue).

9.5.2.5 *Pronase*—2000 U/ml, 50 mM Tris, 75 mM NaCl.

9.5.2.5.1 Concentrated nitric acid (HNO_3) has been used when tissues are difficult to digest, especially after fixation and embedding.

9.5.3 *Procedure for Digestion in Papain*:

9.5.3.1 Place 1 gram of tissue sample in a clean 50 ml conical tube.

9.5.3.2 Add 5 ml of papain solution and vortex.

9.5.3.3 Incubate sample at 65°C for 24 h. If sample does not dissolve add an additional 100 µl papain and vortex.

9.5.3.4 Centrifuge in an ultracentrifuge for 1 h at 100 000 g. A variable gradient may be used comprising of 2.0 ml each of 5, 10, 20, and 50 % sucrose.

9.5.3.5 Wash particles in 10 cc of hot filtered water.

9.5.4 *Procedure for Digestion in Strong Base and Pronase*

9.5.4.1 Rinse tissue samples in phosphate buffered saline, and place 1 g in a 15 ml glass tube.

9.5.4.2 Add 2 ml of 4 M KOH.

9.5.4.3 Incubate sample at 56°C for 48 h. Mix samples occasionally.

9.5.4.4 Centrifuge at 1000 g for 1 h. To facilitate sedimentation of debris, add 6 ml of 95 % ethanol to the 2 ml aliquots. Discard clear supernatant, and repeat digestion and centrifugation steps.

9.5.4.5 Wash debris in distilled water, mix and place in 37°C for 8 h. Mix with excess amounts of ethanol, and centrifuge at 1000 g, 1 h. Repeat washing procedure 3 times.

9.5.4.6 Digest the organic material with debris in pronase at 37°C for 24 h. During the first 5 min in pronase, ultrasonicate the debris to disaggregate the particles.

9.5.4.7 Wash the debris 3 times in distilled water.

9.5.4.8 Resuspend debris (0.5 ml debris/3 ml of ethanol) and add an equal volume of hexane and vortex.

9.5.4.9 Transfer the cream colored fraction containing the polymeric particles, which stabilized at the hexane-ethanol interface into a clean, sterile tube. Metal debris and bone particles will be in the sediment at the bottom.

9.5.4.10 Repeatedly add hexane or ethanol to the original tube and vortex. Harvest and pool the polyethylene particles.

9.5.4.11 To the pooled particles, add 2 ml of ethanol and evaporate any remaining hexane.

9.5.4.12 Add excess ethanol to the tube and centrifuge at 1000 g for 30 min.

9.5.4.13 Discard the supernatant and resuspend debris in KOH at 56°C for 8 h.

9.5.4.14 Add excess ethanol to the tube and centrifuge at 1000 g for 30 min.

9.5.4.15 Discard the clear supernatant and resuspend particles in 1 ml of distilled water, incubate for 8 h followed by centrifugation in ethanol. Repeat wash procedure three times.

9.5.4.16 Resuspend approximately 0.5 ml of debris in 2 ml of ethanol. Ultrasonicate for 5 min before transfer to a stub for SEM and particle analysis.

9.5.5 *Procedure for Digestion in Nitric Acid*

9.5.5.1 With this protocol, the digest solution is never filtered, therefore, the submicron particles are not lost and can be evaluated (with SEM and Coulter), without complications that could arise from hemosiderin particles which remain with non-acid digestion protocols.

9.5.5.2 If paraffin embedded sections are deparaffinated in xylene or toluene, replace the solution with 2 changes of absolute ethanol.

9.5.5.3 Critical point dry the tissue specimen, slice it and place 0.02 to 0.03 g pieces in plastic tubes.

9.5.5.4 Add 1.0 ml of concentrated nitric acid.

9.5.5.5 Digest the tissue for 24 h at room temperature. Shake the tube and sonicate for 2 min. Continue digestion for an additional 24 h.

9.5.5.6 Centrifuge digestion solution at 9500 g for 5 min. Depending on the amount of debris, there will be a floating band of polymeric debris and a sedimented pellet of metallic debris. These can either be isolated separately, or together.

9.5.5.7 Separate the fluid from debris by aspirating the clear liquid between the floating band and the pellet.

9.5.5.8 Add 1 ml of concentrated nitric acid to the debris, sonicate for 2 min and centrifuge at 11 600 rpm for 5 min.

9.5.5.9 Aspirate the clear liquid and add 1 ml of acetone, sonicate 2 min, and centrifuge at 11 600 rpm for 20 min. Both metallic and polymeric debris will sediment in the acetone.

9.5.5.10 Aspirate the supernatant and resuspend the debris in 10 µl of dispersant (Coulter I B) and slowly add 1 ml of ultrapure water, with intermittent sonication.

9.5.6 *Procedure for Tissue or Dried Lubricant or Synovial Fluid Digestion in Sodium Hydroxide*:

9.5.6.1 For tissue only: mince 2.0–5.0 g of tissue and place in a glass container. To extract lipid, add 2:1 chloroform-:methanol solution and place on an orbital shaker overnight, or until the tissues sink to the bottom of the container. Rinse tissues with 3× filtered (0.2 µm) deionized H_2O.

9.5.6.2 For extracted tissues and dried (lyophilized) fluid, add 12 ml 5*N* NaOH and incubate at 65°C for 1 to 3 hours on a water bath-shaker.

9.5.6.3 Allow the digested solution to cool to room temperature, then ultrasonicate for 10 minutes.

9.5.6.4 Into two clean, particle-free polyallomer centrifuge tubes, place 7 ml each of the digested solution and top off with 5 ml of 5 % sucrose. Ultracentrifuge for 3 hours at 40 000 rpm, 5–15°C.

9.5.6.5 UHMWPE will rise to the top of each tube to form an opaque layer. Carefully pipette this band into another clean, particle-free vial. Other bands that sometimes appear can be collected separately or pooled with the UHMWPE.

9.5.6.6 To wash off the sucrose, add 3× filtered deionized H_2O to the collected band until the total volume is 21 ml. Ultrasonicate for 5 min, then heat sample for 1 h at 80°C.

9.5.6.7 Into three clean, particle-free polyallomer centrifuge tubes, place 7 ml each of the solution and top off with an isopropanol gradient consisting of 3 ml of 0.96 g/cm^3 isopropanol/deionized H_2O and 2 ml of 0.90 g/cm^3 solution. Ultracentrifuge for 1 h at 40 000 rpm, 20–25°C.

9.5.6.8 Collect the UHMWPE band at the 0.90 and 0.96 g/cm^3 interface into a clean, particle-free vial. Other bands that may appear can be collected separately.

9.5.7 *Isolation of Debris*:

9.5.7.1 The debris obtained by the above procedures may contain particles of a wide range of sizes and morphology. There may also be clumping or agglomeration of particles. As a result, several stages of filtration may be necessary to effectively isolate the different particles of interest.

9.5.7.2 The final common pathway for the above procedures is filtration with submicron filters. These are then subjected to optical or scanning electron microscopy, or particle counting. Particle morphology may be characterized.

9.5.7.3 To characterize retrieved particles with light microscopy, the particle solution should be sonicated for 10 min. Some particles may melt and agglomerate further by sonication for 10 min. For these, short bursts with time to cool are recommended. From this, 10 µl of the solution can be removed and filtered using a 0.1 µm filter (nitrocellulose, Millipore, Nucleopore). The filter should then be mounted and coverslipped on a glass slide. Since the filters become optically transparent when in contact with standard mounting media, the particles can be analyzed with a transmitted light microscope.

9.5.7.4 To characterize the morphology of retrieved particles with Scanning Electron Microscopy, 10–200 µl of particle solution should be filtered using a 0.2 µm polycarbonate filter, or 10 and 0.4 µm filters (nitrocellulose, Millipore, Nucleopore). The filtering vacuum should be stopped before all the liquid is removed, and the remaining liquid allowed to evaporate. The filter should then be fixed with double sided tape and copper conductive strip, on an SEM mount. Specimens may be coated with palladium or gold to make the polymeric particles conductive. Coating is not necessary when operating the SEM in the low vacuum Nature mode.

9.5.7.5 The large pore filters can be cut in half, dried at 50°C and coverslipped for viewing with transmitted polarized light microscopy.

9.5.7.6 Chemical analysis methods such as EDXA and FTIR may also be employed to determine the chemical nature of the particles.

10. Stage I Analysis of the Implant Components

10.1 Stage I examination and documentation should be conducted routinely on all retrieved devices. The procedures are intended to take the investigator a minimal amount of time and expense to complete, while providing sufficient information to permit device tracking.

10.1.1 Perform a separate analysis for each component of a device, if possible and necessary, as outlined below. A coating shall be considered as a separate component. A sample form is provided as Appendix X2.

10.1.2 Document chain of device custody as per Practice E 1188.

10.2 *Device Description*:

10.2.1 Identify the device type and describe all of the components retrieved.

10.2.2 Record the manufacturer and catalogue and serial numbers where possible.

10.2.3 Record any other information which describes the device.

10.3 *Macroscopic Examination*:

10.3.1 Perform this examination with the unaided eye or with the aid of a stereo-microscope.

10.3.2 Examine all surfaces of the components for evidence of in service or iatrogenic damage.

10.3.3 Record an estimate as to the degree of findings as per Appendix X2.

10.4 *Visual Examination for Evidence of Mechanical Failure*:

10.4.1 Observe the implant surface by suitable techniques to ascertain any mode of failure, as applicable. In no event should any surface of a failed implant be destructively evaluated at this time.

10.4.2 Sketch any significant features. These may also be recorded photographically, although this adds significant cost to routine, Stage I analysis.

11. Stage II Analysis

11.1 Stage II analysis should be carried out after Stage I, if deemed necessary, to further evaluate the characteristics, or failure mode, of the implant. This level of testing primarily relates to an assessment of the modes of failure and deterioration of an implant in the most non-destructive manner possible. Record the results on form Appendix X3.

11.1.1 Perform Stage II analysis for severely damaged components, or where optical fractography is sufficient to determine mechanisms involved in mechanical failure of the device.

11.1.2 Fabrication of surface replications may be indicated in some Stage II investigations. These could then be used for examination without damaging the specimen.

11.2 *Photography*:

11.2.1 A photographic documentation of findings should be made.

11.3 *Optical Fractographic Examination*:

11.3.1 If the implant is fractured, analyze the fracture surface by suitable techniques to ascertain the mode of fracture.

11.3.2 In no event should the fracture surface be destructively evaluated. If the device has mechanically failed, it is important to remember that it may be classified as legal evidence.

12. Stage III Analysis, Metallic Components

12.1 If further testing is necessary to assess the properties of the implant, the tests listed under Stage III Analysis, Metallic Components, in Appendix X4 shall be carried out as deemed necessary to further characterize the implant and its history.

12.1.1 Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated. Care should be taken to insure that potential legal evidence is not destroyed, as per Practice E 860.

12.2 *Microscopic Examination*:

12.2.1 Prepare appropriate metallographic sections in accordance with Methods E 3 and Terminology E 7.

12.2.2 Specimens should be etched as per Practice E 407, and examined microscopically using light microscopy as per Guide E 883.

12.2.3 Determine the inclusion content using Test Methods E 45 as a guide. Compare the observed content with that of the applicable material standard, if appropriate.

12.2.4 Determine the grain size as per Test Methods E 112 and compare results with the applicable material standard.

12.2.5 Characterize the grain boundaries in terms of inclusion content. Microscopic evidence of corrosion should be noted and recorded. Evidence of sensitization of stainless steels can be determined according to Practices A 262.

12.2.6 Any evidence of microporosity can be documented using Test Methods E 45 as a guideline.

12.2.7 The use of scanning electron microscopy as per Practice E 986 may be utilized for fractographic analysis.

12.3 *Material Characterization*:

12.3.1 Determine the physical and chemical composition and identity of the metallic alloy as per Test Methods A 751, E 353, E 354, and E 120. In the event that the composition does not meet a recognized material standard, the appropriate referee analysis procedure shall be used.

12.3.2 Chemical analysis can also be conducted with electron microprobe analysis.

12.4 *Mechanical Properties*:

12.4.1 The type of measurements to be carried out at this Stage of characterization will be dependent upon the implant and its use. Suggested property tests are shown in Appendix X4 under Mechanical Properties.

12.4.2 Determine the hardness as per Test Methods E 10, E 18, or E 92.

12.4.3 Determine the tensile, flexural, compressive, and the like properties in accordance with applicable material specification if indicated, and such other tests as are appropriate to the specimen which may be fabricated from the implant. Deviation from the specimen dimensions as described in the standard methods may be necessary to accommodate the shape and size of the device under investigation.

12.5 *Coated Samples*:

12.5.1 Examine coated samples microscopically for missing sections of coating. Estimate the fraction of coating missing.

12.5.2 Where possible determine the shear strength of the adherent coating as per Test Method F 1044.

12.5.3 Where possible determine the tensile strength of the adherent coating as per Test Method F 1147.

13. Stage III Analysis: Polymeric Components

13.1 If further testing is necessary to assess the properties of the implant, the tests listed under "Stage III Analysis, Polymeric Components," in Appendix X5 shall be carried out as deemed necessary to further characterize the implant and its history.

13.1.1 Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated. Care should be taken to insure that potential legal evidence is not destroyed, as per E 860.

13.1.2 A wide variety of polymeric materials are used in implantable devices. Actual identification of the material type may not be possible without Stage III chemical analysis. Where possible, classify the material according to Classification D 4000.

13.1.3 Each type or class of materials has an associated battery of tests designed for that specific type. As a result, the investigator is directed to the specific material standards to identify the specific tests which are appropriate. The methods and properties described in this section are intended to serve as a guide to the selection of test methods.

13.2 *Microscopic Examination*:

13.2.1 Examine the specimen for evidence of surface damage or degradation.

13.2.2 The use of thin sections (5–10 μm) and optical microscopy using reflected and transmitted, polarized and nonpolarized light has been found useful in identifying evidence of inclusions or porosity and for identification of changes in crystallinity or density.

13.2.3 In the case of mechanical failure, scanning electron microscopy (SEM) may provide insight into the mode of failure.

13.3 *Material Characterization*

13.3.1 Determine the molecular weight or weight distribution of the specimen. Possible methods and ASTM standards to be utilized are gel permeation chromatography (GPC) (Practice D 3016, Test Method D 5296), osmometry, light scattering (Test Method D 4001), viscometry (Test Methods D 3835, D 2857), or melt index (Test Method D 1238).

13.3.2 Determine the density by an appropriate method. Suggested methods are by displacement (Test Methods D 792), the gradient method (Test Method D 1505), or, for rigid cellular plastics, Test Method D 1622 is suggested.

13.3.3 Determine the thermal properties of the material. The glass transition (Tg), and melt temperature (Tm) can be determined by differential scanning calorimetry (DSC) as per Test Method D 3417. Phase identification by differential thermal analysis (DTA) as per Test Method D 3418. Softening point, thermomechanical properties, coefficient of expansion can be determined by thermomechanical analysis (TMA). The thermal stability can be determined by thermogravimetric analysis (TGA).

13.3.4 Determine the chemical composition and determine whether there has been any oxidation or degradation of the material. Analysis by infrared analysis and FTIR is suggested, as per Practices E 168 and E 204. Chemical composition and structure can be determined by nuclear magnetic resonance spectroscopy (NMR) as per Practice E 386. Free radicals can be identified with electronic spin resonance (ESR).

13.3.5 Of particular interest are the presence of any low molecular weight extractables. These may be from degradation of the polymer or from absorption of moieties such as lipids from the *in vivo* environment. There are a number of extraction protocols that could be utilized, for example, Test Methods D 1239, D 2842, D 4754, Practice D 5152, Test Method D 5227, Practice F 619. Appropriate chemical analysis assays can then be applied to identify the composition of the etractables. The results will depend on the method chosen.

13.4 *Mechanical Properties*:

13.4.1 The type of measurements to be carried out at this Stage of characterization will be dependent upon the implant and its use. In making such measurements, it is important to note and record the sample location and orientation.

13.4.2 Determine the hardness according to the applicable material standard. Testing by Rockwell, as per Test Method

D 785, or Durometer according to Test Method D 2240 are suggested.

13.4.3 Determine the tensile, flexural, compressive, etc. properties in accordance with applicable material specification if possible, and such other tests as are appropriate to the specimen which may be fabricated from the implant. Suggested test methods are: tensile (Test Methods D 412, D 638, D 1623, D 1708), flexural (Test Methods D 621, D 671, D 747, D 790), compression (Test Methods D 695, D 1621), shear (Test Method D 732), impact resistance (Test Methods D 256), tear resistance (Test Methods D 624, D 1004), abrasion resistance (Test Method D 1242), and tensile creep (Test Method D 2990). Deviation from the specimen dimensions as described in the standard methods may be necessary to accommodate the shape and size of the device under investigation.

13.4.4 Dynamic mechanical properties may also be of interest. These could be measured by means of a torsional pendulum as per Test Method D 4065 or in flexural fatigue as per Test Method D 671.

13.5 *Coated or Porous Specimens*:

13.5.1 Coatings should be microscopically and chemically characterized utilizing the methods as described in this section.

13.5.2 The porosity of porous materials or porous coatings should be determined according to Test Method D 2873, Practice E 562 or Test Method F 316.

13.6 *Polymer Matrix Composites*:

13.6.1 Analysis and characterization of polymer matrix should be as described in this section.

13.6.2 Analysis of the reinforcing material should be in accordance with the material type.

13.6.3 Of special interest with composites is the analysis of the interfacial relationships between matrix and the second phase. Microscopic methods should be utilized to establish degrees and nature of interfacial bonding, or degradation thereof.

14. Stage III Analysis: Ceramic and Glass Materials

14.1 If further testing is necessary to assess the properties of the implant, the tests listed under “Stage III Analysis: Ceramic and Glass Materials” In Appendix X6 shall be carried out as deemed necessary to further characterize the implant and its history.

14.1.1 Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated. Care should be taken to insure that potential legal evidence is not destroyed, as per Practice E 860.

14.1.2 There is a wide variety of materials and devices that fit this category. Therefore, these protocols shall serve as a guide to ascertaining the important features of retrieved devices.

14.2 *Microscopic Examination*:

14.2.1 Use standard light optical or electron optical microscopic preparation techniques suitable for the material under investigation.

14.2.2 Determine the inclusion content in accordance with the applicable material standard, if appropriate.

14.2.3 Determine the grain size in accordance with the applicable material standard and method.

14.2.4 Evidence of degradation should be noted and recorded as per Appendix X6. Surfaces should be examined for evidence of surface etching or grain excavation.

14.2.5 Fracture surfaces may be examined by scanning electron microscopy (SEM).

14.3 *Material Characterization*:

14.3.1 Determine the chemical composition and identity of the component. Chemical composition of glasses may be conducted according to Test Methods C 169. Composition of ceramics may be conducted according to Test Methods C 573.

14.3.2 Determine the density and apparent porosity, as per Test Methods C 20.

14.3.3 Appropriate x-ray diffraction (XRD) methods should be utilized to determine degree of crystallinity and phase composition. Phase composition may also be determined by FTIR.

14.3.4 Thermal properties may be determined by differential scanning calorimetry (DSC), or thermogravimetric analysis (TGA) as appropriate, and by differential thermal analysis (DTA).

14.4 *Mechanical Properties*:

14.4.1 The type of measurements to be carried out at this Stage of characterization will be dependent upon the implant and its use. Suggested property tests are shown in Appendix X6 under Mechanical Properties.

14.4.2 Determine the hardness as per Test Method C 730 for glasses or Test Method C 849 for ceramics.

14.4.3 Determine the tensile, flexural, compressive, etc. properties in accordance with applicable material specification, if possible. Modulus and Poisson’s ratio may be determined as per Test Method C 623 for glasses, or Test Method C 1198 for ceramics. Flexural properties may be determined as per Test Methods C 158 for glasses, and Test Methods C 674 or C 1161 for ceramics.

14.5 *Porous and Coated Samples*:

14.5.1 Examine coated samples microscopically for missing sections of coating. Estimate the fraction of coating missing.

14.5.2 Determine the shear strength of the coating using the appropriate method, such as Test Method F 1658.

14.5.3 Determine the tensile strength of the coating using the appropriate method, such as Test Method F 1501.

14.5.4 Determine adhesion or cohesive strength of flame-sprayed coatings as per Test Method C 633.

14.5.5 Surface area of porous materials may be determined by Test Method C 1069. Porosity may be determined as per Practice E 562, or Test Method F 316.

14.6 *Ceramic Matrix Composites*:

14.6.1 Analysis and characterization of ceramic matrix should be as described in this section.

14.6.2 Analysis of the reinforcing material should be in accordance with the material type.

14.6.3 Of special interest with composites is the analysis of the interfacial relationships between matrix and the second phase. Microscopic methods should be utilized to establish degrees and nature of interfacial bonding, or degradation thereof.

15. Materials of Biological Origin or Tissue Engineering

15.1 If further testing is necessary to assess the properties of

biologically derived implants, the tests shall be carried out as deemed necessary to further characterize the implant and its history.

15.1.1 Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated. Care should be taken to insure that potential legal evidence is not destroyed, as per Practice E 860.

15.2 The protocols in this document provide for analysis of biological tissues and of synthetic materials. The application of the appropriate methodologies should be utilized in the study of various biologic and synthetic components of retrieved biological or tissue engineered devices.

16. Keywords

16.1 ceramics; chemical analysis; composites; histology; implant retrieval; metals; particles; polymers

APPENDIXES

(Nonmandatory Information)

X1. CLINICAL INFORMATION SUGGESTED TO BE RECORDED DURING RETRIEVAL OF MEDICAL DEVICES

Minimum data set
Date of implantation, and date of removal
Hospital, or physician's office of implantation and of removal
Confidential patient ID Code to link to hospitals of implantation and removal records
Implant anatomical site
Device identification (manufacturer's name and device catalogue number), Device lot and serial number
Indication for use and Reason for explantation
Additional Clinical Information (confidential)
Surgeon (name & address)
Patient (name & address and/or ID number)
female [ ] male [ ] date of birth ____ occupation; weight height
History of substance abuse (smoking, etc.)
Reason for Investigation: routine series [ ], research [ ] documentation [ ], complaint [ ], liability claims [ ], clinical investigation [ ], other _____
Diagnosis at Insertion (or Reason for Insertion)
Additional Diagnoses and Complications
Antibiotics: pre op. [ ] peri op. [ ] post op. [ ] prophylactic [ ]
Relevant pharmaceuticals duration:
Post Operative Treatment:
Complications between insertion and removal: e.g. infection [ ]
Observations prior to removal (functional)
Clinical Reason for Removal:
routine [ ] pain [ ] revision [ ] failure [ ] infection [ ]
allergy [ ] other _____
Patient level of activity at removal
Observations at Removal (indicate yes, no, not applicable,
doubt, etc.)

normal tissue [ ]	bursal fluid [ ]	scar tissue [ ]
granulation tissue [ ]	bone reaction [ ]	infection [ ]
loose implant [ ]	discoloration, implant debris [ ]	other _____

Additional Material Provided for Analysis

radiographs	no [ ]	yes [ ]	how many
tissue	no [ ]	yes [ ]	type origin
bacteriol. specimen	no [ ]	yes [ ]	type origin
immunol. specimen	no [ ]	yes [ ]	type origin
fluid	no [ ]	yes [ ]	type

photographs; pathology reports
surgical reports; additional documentation
Type of disinfection used

X2. STANDARD FORM FOR GUIDING THE STAGE I ANALYSIS OF RETRIEVED IMPLANTABLE MEDICAL DEVICES

Reason for Investigation:
routine clinical series [ ] animal study [ ] research [ ]
complaint [ ] liability claims [ ] other ____
Patient or animal ID code _____
Date Retrieved _____ Implant duration _____
Date of analysis _____
Record for all Components retrieved:
device type & component names _____
manufacturer(s) _____
catalog number(s) _____ Serial number(s) _____
identification marks, e.g. size: _____
material(s) _____
Macroscopic examination (YES, NO, DOUBT, or NOT APPLICABLE)

		Location	Size/Area	Severity/ Degree
_____ a)	wear or burnishing	_____	_____	_____
_____ b)	galling	_____	_____	_____
_____ c)	scratching	_____	_____	_____
_____ d)	change of shape	_____	_____	_____
_____ e)	mechanical damage	_____	_____	_____
_____ f)	macro porosity	_____	_____	_____
_____ g)	pitting or crevice corrosion	_____	_____	_____
_____ h)	fretting	_____	_____	_____
_____ i)	embedded particles	_____	_____	_____
_____ j)	discoloration or staining	_____	_____	_____
_____ k)	calcification	_____	_____	_____
_____ l)	thrombosis	_____	_____	_____
_____ m)	degradation	_____	_____	_____
_____ n)	stress cracking or crazing	_____	_____	_____
_____ o)	loss of coating	_____	_____	_____
_____ p)	mechanical failure	_____	_____	_____
_____ q)	cold flow	_____	_____	_____

Sketch(s):

X3. STANDARD FORM FOR GUIDING THE STAGE II ANALYSIS OF RETRIEVED IMPLANTABLE MEDICAL DEVICES

Photograph orientation and descriptions.
Mechanical failure (if YES, identify mode, indicate location of failure and method of identification)
a) static-overstress, causing plastic deformation
b) shear
c) fatigue
d) torsion
e) impact
f) stress corrosion or environmental cracking
g) fatigue, or corrosion-fatigue
h) combination of above (identify)
i) other (specify)_____
j) unable to identify

X4. STAGE III ANALYSIS: METALLIC MATERIALS

Standard form for guiding the Stage III analysis of retrieved metallic implantable medical devices

1. **Microscopic examination** (indicate location and orientation of sample)
a) Inclusion content
b) Grain size
c) Grain boundary constituents
d) Microporosity
e) Other distinguishing features
2. **Failure analysis** (if appropriate)
a) Presence of fatigue striations
b) Fraction of surface with ductile overload
c) Evidence of significant ductile overload
d) Defects associated with crack initiation
3. **Type of material** (indicate method of determination)
a) Chemical composition
4. **Mechanical properties** (indicate N/A if not available). Samples should be taken from areas representative of the original material.
a) Sample size and orientation
b) Hardness (indicate type and method)
c) Other ASTM tests as applicable (for example, tensile, or transverse bend tests)
5. **Metallic Coating**
a) Coating material
b) Estimated fraction of coating missing
c) Shear strength
d) Tensile strength

X5. STAGE III ANALYSIS: POLYMERIC MATERIALS

Standard form for guiding the Stage III analysis of retrieved polymeric components

Samples should be taken from areas representative of the original material, (D 1898).

1. **Microscopic examination** (indicate location and orientation of sample)

a) Grain size

b) Grain boundary constituents

c) Microporosity

d) Evidence of damage or degradation, or other distinguishing features.

2. **Failure analysis:**

Mode of failure, delamination, oxidation, optical microscopy with polarized light.

3. **Material Characterization**

a) Molecular weight (use most applicable technique)

1) Gel permeation chromatography (GPC)

2) Osmometry

3) Light scattering

4) Viscometry

5) Melt index

b) Density

c) Thermal characterization (most appropriate techniques)

1) Glass transition (Tg), melt temperature (Tm), by differential scanning calorimetry (DSC). Phase identification by differential thermal analysis (DTA).

2) Softening point, thermomechanical properties, coefficient of expansion by thermomechanical analysis (TMA)

3) Thermal stability by thermogravimetric analysis (TGA)

d) Chemical analysis

1) Infrared spectroscopy (IR, FTIR)

2) Chemical composition and structure (NMR)

3) Other chemical composition determinations

e) Low molecular extractables, e.g. absorbed lipid content of implant.

4. **Mechanical properties** (indicate N/A if not available).

a) Record sample size and orientation

b) Hardness

c) Other tests as appropriate, e.g. tensile, flexural, compression, shear, impact resistance, tear resistance, abrasion resistance, tensile creep.

d) Dynamic mechanical measurements, fatigue.

5. **Porous or Coating materials**

a) Coating material

b) Any damage of loss of coating

c) Porosity

6. **Composites**

a) Type of composite materials

b) Matrix phase relationships.

X6. STAGE III ANALYSIS: CERAMIC AND GLASS MATERIALS

STANDARD FORM FOR GUIDING THE STAGE III ANALYSIS OF RETRIEVED CERAMIC AND GLASS IMPLANTABLE MEDICAL DEVICES

1. **Microscopic examination** (indicate location and orientation of sample)

a) Inclusion content

b) Grain size

c) Grain boundary constituents

d) Microporosity

e) Grain excavation, grain orientation, (texturing) of surface vs bulk

f) Surface morphology (SEM)

g) Other distinguishing features

2. **Failure Analysis** (if appropriate)

a) Defects associated with crack initiation

b) Fracture morphology indicative of failure stresses

3. **Material Characterization**

a) Type of material (indicate method of determination)

b) Chemical composition

c) Surface chemistry, etching, new crystals

d) Chemical glass transition (DSC or DTA)

e) Crystallinity (XRD)

f) Phase composition (FTIR)

4. **Mechanical properties** (indicate N/A if not available). Samples should be taken from areas representative of the original material.

a) Sample size and orientation

b) Hardness (indicate type and method)

c) Other ASTM tests as applicable (for example, flexural properties)

5. **Porous or Ceramic Coatings**

a) Coating material

b) Estimated fraction of coating missing

c) Shear strength

d) Tensile strength

e) Adhesive and cohesive strength

6. **Composite Materials**

a) Material types

b) Composite phase relationships

X7. RATIONALE

X7.1 The ultimate test for an implant or device and the materials out of which is fabricated, is its performance as an implant. In many situations, critical information can only be ascertained by examination of retrieved implants and the surrounding tissues. Such analysis could be part of experimental studies in animals, clinical studies, where devices are routinely removed or removed for revision, or in post-mortem autopsy studies. The information suggested for collection could also be of value in device tracking.

X7.2 This standard practice was first published in 1978 for analysis of retrieved metallic orthopaedic implants. Rather than develop a separate standard for each material type or class, the document was revised to cover all material types and analysis of the associated tissues.

X7.3 The intent of this document is to provide standard protocols for analysis of retrieved devices. It specifies standard methods of analysis and data collection for comparing similar sets of data between research centers. For information and data on retrieved devices to be useful on a national and international basis, standard methods of analysis and reporting are necessary. Therefore, collected data should conform to this standard practice.

X7.4 Because of the complexity of analysis of implant devices, and because of the large number of potential tests suggested in this standard, it has appeared desirable to divide the investigation into stages. The analyses of the implant are separated into three groups with the degree of characterization increasing from Stage I through Stage III. The implant characterizations may include macroscopic and microscopic examinations, chemical composition, as well as physical and mechanical property determinations to ascertain mechanisms and degree of degradation.

X7.5 The tests performed may depend upon the reason for removal of the implant. Furthermore, analysis can be an expensive and time consuming process. To facilitate capturing information on a large number of implants, a minimum dataset obtained by inexpensive means is proposed in the first two stages. Perform a Stage I analysis for all removals. Perform a Stage II analysis for severely damaged components, or where optical fractography is sufficient to determine mechanisms involved in mechanical failure of the device. Stage III examinations involve destructive methods for microscopy and chemical analysis. These should only be conducted when complete material characterization and failure analysis is indicated.

X7.6 The American Society for Metals, International, has published Metals Handbook, Failure Analysis and Prevention, which may be used as a reference to supplement the practices in this document. As part of an Implant retrieval meeting held at the National Bureau of Standards in 1980, an extensive collection of retrieval forms was assembled as an appendix **(9)**, which may also be used as a guide for documenting the analyses.

X7.7 This standard practice is intended for clinical and other research purposes. Users interested in product liability litigation may refer to E 860. In any event, care should be taken to insure that potential legal evidence is not destroyed.

REFERENCES

(**1**) L'Vov, B. V., "Electrothermal atomization—The Way Towards Absolute Methods of Atomic Absorption Analysis," *Spectrochimica Acta*, 33B, 153–193, 1978.

(**2**) Long, G. L., and Winefordner, J. D. "Limit of Detection. A closer look at the IUPAC definition. *Analyt. Chem.*, 55, 712A–724A, 1983.

(**3**) Jacobs, J. J., Skipor, A. K., Black, J., Urban, R. M., and Galante, J. O., "Release and Excretion of Metal in Patients Who Have a Total Hip-Replacement Component Made of Titanium-Base Alloy," *J Bone and Joint Surgery*, 73A (10), 1475–1486, 1991.

(**4**) Gill, R. J., "AAS or ICP-OES: Are They Competing Techniques?," *American Laboratory*, November, 24F–24K, 1993.

(**5**) Campbell, P., Ma, S., Schmalzried, T., and Amstutz, H. C., "Tissue Digestion for Wear Debris Particle Isolation," *J Biomedical Materials Research*, 28, 523–526, 1994.

(**6**) Shanbhag, A. S., Jacobs, J. J., Glant, T. T., Gilbert, J. L., Black, J., and Galante, J. O., "Composition and Morphology of Wear Debris in Failed Uncemented Total Hip Replacement," *J. Bone and Joint Surgery*, 76B, 60–67, 1994.

(**7**) Margevicius, K. J., Bauer, T. W., McMahon, J. T., Brown, S. A., and Merritt, K., "Isolation and Characterization of Debris in Membranes around Total Joint Prostheses," *J Bone Joint Surgery*, 76A, 1664–1675, 1994.

(**8**) Favero, M. S., and Bond, W. W., "Sterilization, Disinfection, and Antisepsis in the Hospital," Ch 24 in: *Manual of Clinical Microbiology*, Balows & Hausler, Herrman, Isenberg, Shadomy, ASM Washington, 1991.

(**9**) Weinstein, A., Gibbons, D., Brown, S., and Ruff, W., *Implant Retrieval: Material and Biological Analysis*, National Bureau of Standards Special Publication 601, 1981.

(**10**) Metals Handbook Vol 11, "Failure Analysis and Prevention," American Society of Metals, Metals Park, OH 1986.