Designation: F561 – 19

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

This standard is issued under the fixed designation F561; 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 implanted medical devices and associated specimens that are removed from human and animal subjects during revision surgery and at postmortem. This practice may be used for the analysis of any implant including inert, bioactive, resorbable, and tissue engineered products. This practice can also be used for analysis of specimens and fluids from *in vitro* tests, including those from wear tests and joint simulators. The aim is to provide guidance to minimize iatrogenic damage during the recovery and handling of the associated specimens which could obscure the investigational results. This practice is also intended to provide guidance as to gathering data at the proper time and circumstance.

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. Note that regulations for handling of patient information, tissues, and retrieved devices will vary by geography.

1.3 This practice should be applied in accordance with pertinent regulations or legal requirements regarding the handling of patient data as well as the handling and analysis of retrieved implants and excised tissues, especially with regard to handling devices which may become involved in litigation, as in accordance with Practice E860. Note that regulations for handling of patient information, tissues, and retrieved devices will vary by geography

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

1.6 *This standard may involve hazardous materials, operations, and equipment. As a precautionary measure, explanted devices 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. A detailed discussion of precautions to be used in handling of human tissues can be found in ISO 12891-1. This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

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

2. Referenced Documents

2.1 *ASTM Standards:*[2]

A262 Practices for Detecting Susceptibility to Intergranular Attack in Austenitic Stainless Steels

A751 Test Methods, Practices, and Terminology for Chemical Analysis of Steel Products

C20 Test Methods for Apparent Porosity, Water Absorption,

[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 F04.15 on Material Test Methods.

Current edition approved Jan. 1, 2019. Published February 2019. Originally approved 1978. Last previous edition approved in 2013 as F561 – 13. DOI: 10.1520/F0561-19.

[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.

Apparent Specific Gravity, and Bulk Density of Burned Refractory Brick and Shapes by Boiling Water
C158 Test Methods for Strength of Glass by Flexure (Determination of Modulus of Rupture)
C169 Test Methods for Chemical Analysis of Soda-Lime and Borosilicate Glass
C623 Test Method for Young's Modulus, Shear Modulus, and Poisson's Ratio for Glass and Glass-Ceramics by Resonance
C633 Test Method for Adhesion or Cohesion Strength of Thermal Spray Coatings
C674 Test Methods for Flexural Properties of Ceramic Whiteware Materials
C730 Test Method for Knoop Indentation Hardness of Glass
C1069 Test Method for Specific Surface Area of Alumina or Quartz by Nitrogen Adsorption
C1161 Test Method for Flexural Strength of Advanced Ceramics at Ambient Temperature
C1198 Test Method for Dynamic Young's Modulus, Shear Modulus, and Poisson's Ratio for Advanced Ceramics by Sonic Resonance
C1322 Practice for Fractography and Characterization of Fracture Origins in Advanced Ceramics
C1326 Test Method for Knoop Indentation Hardness of Advanced Ceramics
C1327 Test Method for Vickers Indentation Hardness of Advanced Ceramics
D256 Test Methods for Determining the Izod Pendulum Impact Resistance of Plastics
D412 Test Methods for Vulcanized Rubber and Thermoplastic Elastomers—Tension
D624 Test Method for Tear Strength of Conventional Vulcanized Rubber and Thermoplastic Elastomers
D638 Test Method for Tensile Properties of Plastics
D695 Test Method for Compressive Properties of Rigid Plastics
D732 Test Method for Shear Strength of Plastics by Punch Tool
D747 Test Method for Apparent Bending Modulus of Plastics by Means of a Cantilever Beam (Withdrawn 2019)[3]
D785 Test Method for Rockwell Hardness of Plastics and Electrical Insulating Materials
D790 Test Methods for Flexural Properties of Unreinforced and Reinforced Plastics and Electrical Insulating Materials
D792 Test Methods for Density and Specific Gravity (Relative Density) of Plastics by Displacement
D1004 Test Method for Tear Resistance (Graves Tear) of Plastic Film and Sheeting
D1238 Test Method for Melt Flow Rates of Thermoplastics by Extrusion Plastometer
D1239 Test Method for Resistance of Plastic Films to Extraction by Chemicals
D1505 Test Method for Density of Plastics by the Density-Gradient Technique
D1621 Test Method for Compressive Properties of Rigid Cellular Plastics
D1622 Test Method for Apparent Density of Rigid Cellular Plastics
D1623 Test Method for Tensile and Tensile Adhesion Properties of Rigid Cellular Plastics
D1708 Test Method for Tensile Properties of Plastics by Use of Microtensile Specimens
D2240 Test Method for Rubber Property—Durometer Hardness
D2842 Test Method for Water Absorption of Rigid Cellular Plastics
D2857 Practice for Dilute Solution Viscosity of Polymers
D2990 Test Methods for Tensile, Compressive, and Flexural Creep and Creep-Rupture of Plastics
D3016 Practice for Use of Liquid Exclusion Chromatography Terms and Relationships
D3418 Test Method for Transition Temperatures and Enthalpies of Fusion and Crystallization of Polymers by Differential Scanning Calorimetry
D3835 Test Method for Determination of Properties of Polymeric Materials by Means of a Capillary Rheometer
D3919 Practice for Measuring Trace Elements in Water by Graphite Furnace Atomic Absorption Spectrophotometry
D4000 Classification System for Specifying Plastic Materials
D4001 Test Method for Determination of Weight-Average Molecular Weight of Polymers By Light Scattering
D4065 Practice for Plastics: Dynamic Mechanical Properties: Determination and Report of Procedures
D4754 Test Method for Two-Sided Liquid Extraction of Plastic Materials Using FDA Migration Cell
D5227 Test Method for Measurement of Hexane Extractable Content of Polyolefins
D5296 Test Method for Molecular Weight Averages and Molecular Weight Distribution of Polystyrene by High Performance Size-Exclusion Chromatography
E3 Guide for Preparation of Metallographic Specimens
E7 Terminology Relating to Metallography
E8 Test Methods for Tension Testing of Metallic Materials [Metric] E0008_E0008M
E10 Test Method for Brinell Hardness of Metallic Materials
E18 Test Methods for Rockwell Hardness of Metallic Materials
E45 Test Methods for Determining the Inclusion Content of Steel
E92 Test Methods for Vickers Hardness and Knoop Hardness of Metallic Materials
E112 Test Methods for Determining Average Grain Size
E135 Terminology Relating to Analytical Chemistry for Metals, Ores, and Related Materials
E353 Test Methods for Chemical Analysis of Stainless, Heat-Resisting, Maraging, and Other Similar Chromium-Nickel-Iron Alloys
E354 Test Methods for Chemical Analysis of High-Temperature, Electrical, Magnetic, and Other Similar Iron, Nickel, and Cobalt Alloys
E407 Practice for Microetching Metals and Alloys

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

E539 Test Method for Analysis of Titanium Alloys by X-Ray Fluorescence Spectrometry
E562 Test Method for Determining Volume Fraction by Systematic Manual Point Count
E860 Practice for Examining And Preparing Items That Are Or May Become Involved In Criminal or Civil Litigation
E883 Guide for Reflected–Light Photomicrography
E986 Practice for Scanning Electron Microscope Beam Size Characterization
E1188 Practice for Collection and Preservation of Information and Physical Items by a Technical Investigator
E1479 Practice for Describing and Specifying Inductively Coupled Plasma Atomic Emission Spectrometers
F316 Test Methods for Pore Size Characteristics of Membrane Filters by Bubble Point and Mean Flow Pore Test
F619 Practice for Extraction of Medical Plastics
F981 Practice for Assessment of Compatibility of Biomaterials for Surgical Implants with Respect to Effect of Materials on Muscle and Insertion into Bone
F1044 Test Method for Shear Testing of Calcium Phosphate Coatings and Metallic Coatings
F1147 Test Method for Tension Testing of Calcium Phosphate and Metallic Coatings
F1635 Test Method for ***in vitro*** Degradation Testing of Hydrolytically Degradable Polymer Resins and Fabricated Forms for Surgical Implants
F1854 Test Method for Stereological Evaluation of Porous Coatings on Medical Implants
F1877 Practice for Characterization of Particles
F2102 Guide for Evaluating the Extent of Oxidation in Polyethylene Fabricated Forms Intended for Surgical Implants
F2182 Test Method for Measurement of Radio Frequency Induced Heating On or Near Passive Implants During Magnetic Resonance Imaging
F2214 Test Method for***In Situ*** Determination of Network Parameters of Crosslinked Ultra High Molecular Weight Polyethylene (UHMWPE)
E2451 Practice for Preserving Ignitable Liquids and Ignitable Liquid Residue Extracts from Fire Debris Samples
F2502 Specification and Test Methods for Absorbable Plates and Screws for Internal Fixation Implants
F2739 Guide for Quantifying Cell Viability within Biomaterial Scaffolds
F2977 Test Method for Small Punch Testing of Polymeric Biomaterials Used in Surgical Implants
F2995 Guide for Shipping Possibly Infectious Materials, Tissues, and Fluids
F2979 Guide for Characterization of Wear from the Articulating Surfaces in Retrieved Metal-on-Metal and other Hard-on-Hard Hip Prostheses
F3036 Guide for Testing Absorbable Stents
F3129 Guide for Characterization of Material Loss from Conical Taper Junctions in Total Joint Prostheses

2.2 *Other Document:*[4]
ISO 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*—a process or treatment using a disinfectant. Disinfection is generally less lethal than sterilization. It eliminates virtually all recognized pathogenic microorganisms but not necessarily all microbial forms (for example, bacterial endospores) on inanimate objects. It does not ensure overkill.

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

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. It also provides for analysis of specimens and lubrication fluids from *in vitro* wear tests.

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 and fluids 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 and fluids for subsequent harvesting and analysis of particulate debris.

4.4 The material characterizations include observation and description of the retrieved 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

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

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 various 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. The flowchart below can be used to guide the type of analysis that can be completed (Fig. 1).

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. Comparison of wear patterns and wear particle morphology observed with retrievals and those observed with *in vitro* joint simulator tests can provide valuable insight into the validity of the *in vitro* simulation.

5.2 A significant portion of the information associated with a retrieved implant is obtained with detailed studies of the device-tissue interface healing response. 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 device and associated tissues be removed minimizing as best as possible alteration of their form and structure. It is also essential that the tissues be handled in such a way as to avoid microbial or viral contamination of the work place or the investigator. The tissue-device interface may need to be stabilized with chemical fixation prior to separation of the device from it's in-situ position. It is also highly recommended to document detailed information about the tissue specimens, including location of extraction. 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 reason for device removal, method of removal, method and timing preservation and clinical findings and laboratory studies documenting device performance.

5.5 Planning of the overall retrieval analyses prior to execution of any of the protocols or methods within this practice is essential to maximize the overall effectiveness of the analyses. The plan shall be based on initial observations from the available clinical information, tissues, and implants. Subsequently, the plan may need to be revised based on results obtained throughout the analyses. Due to the potential interferences described in Section 6, protocols and methods should be executed in a sequence such as to minimize the impact of interferences

5.6 Any destructive analysis of implants must be done so as to not destroy any features that may become the subject of litigation, in accordance with Practice E860. 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 fixation, sterilization, or disinfection. Such observation must be made using appropriate aseptic precautions. Photomicrographs are recommend at this stage of device retrieval.

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.

6.2.1 For example, when harvesting tissues for subsequent chemical analysis, it is important to use tools that do not contain the materials or elements of interest in the tissues.

6.2.2 If possible, retrieved implants should be placed in bags or containers, unless the bone-implant interface will be evaluated. There is possibility of material degradation following exposure to formalin (or other fixatives such as glutaraldehyde) such as in a Morse taper of a ceramic femoral head.

6.2.3 Soft tissue implants especially those with a lumen or cavity that may be compressed during transport should be placed in a hard wall container with a fluid tight lid.

6.2.4 Ensure that air-fluid interfaces are minimized to avoid focal desiccation of explanted tissue surfaces.

7. Hazards

7.1 The handling of retrieved implants and tissues may involve handling of infectious material (bacterial, viral, fungal, or protozoal).

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

7.3 There are situations where tissues or implants cannot be sterilized or disinfected prior to analysis, for example, requirements of specialized protocols in which sterilization will adversely affect cell/tissue morphology and staining quality 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. For

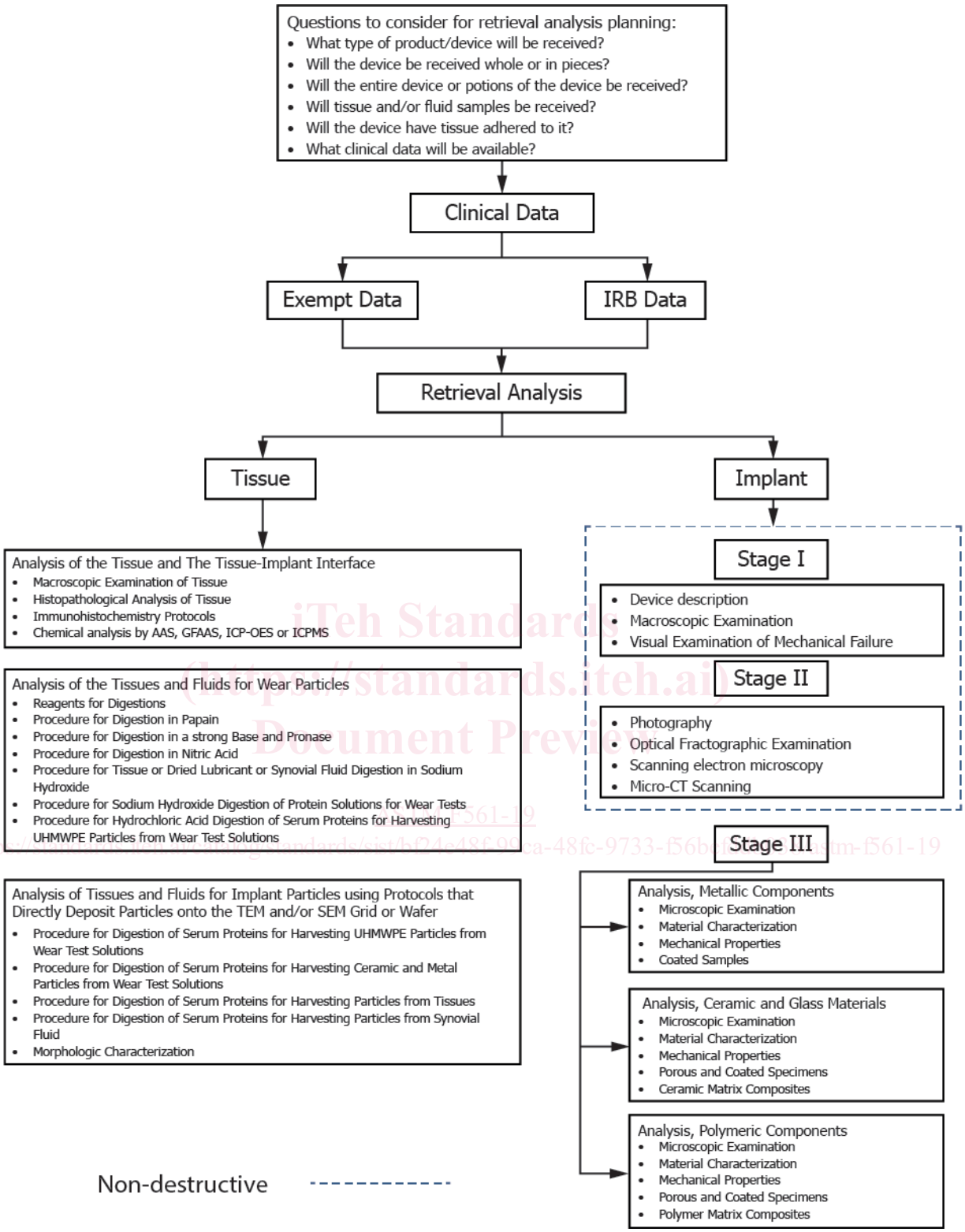

FIG. 1 Retrieval Analysis Flowchart

decontamination of metallic implants, the use of bleach should be considered and possibly avoided to prevent corrosion damage from the decontamination process.

7.4 Handling of explanted devices with an electrical energy source such as implantable cardioverter defibrillators may be hazardous. It is recommended that manufacturer's inactivation procedures be followed. When all required equipment, such as a device-specific wireless programmer, are not available it is recommended that the device be disabled using a magnet or other alternative method that is appropriate for the device. Handling with double gloves will reduce the risk of an unintended shock.

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. The investigator is responsible for ensuring patient privacy and protecting patient data. The investigator shall follow all relevant regulations in the geography where the retrieval and analysis is performed. Steps to enhance patient protection may include obtaining patient consent and engaging an Institutional Review Board (IRB) to review and approve study details prior to initiation. Clinical data should be de-identified at the earliest practical point. A detailed listing and format for documentation of the clinical information associated with removal are provided in Appendix X1. Standard patient evaluation scoring schemes such as those developed by clinical societies may also be utilized.

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 healthcare institution's 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 and original placement. It is suggested that the proximal end of the device or other critical landmark 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. However, interface preservation should not jeopardize the practice of medicine and patient safety. The investigator shall follow all rules and guidelines for implant and tissue handling established by the clinical center where the retrieval is performed.

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, in accordance with Practice F981. Care should be taken to avoid unnecessary manipulation of the device-tissue interface before chemical stabilization. Trimming of tissues, orientation of blocks and the creation of histology slides should be sufficiently detailed and tracked to aid in the subsequent interpretation of any histology reactions.

8.5.2 When handling of devices with electrical energy source that have internal data storage such as implantable cardioverter defibrillators, pacemakers or neurostimulators, the investigator is responsible for ensuring patient privacy and protecting patient data

9. Packaging and Shipping of Explanted Devices, Tissues and Fluids

9.1 In the event that an implant is explanted at a facility where the device needs to be transported to a laboratory for analysis, the components, tissues, and fluids must be packaged in a manner that provides adequate protection to the items during the shipping process.

9.2 Care should be taken during any handling of the retrieved components to avoid damage to the components (i.e. rubbing together the articulating surfaces, dropping or knocking the parts, or allowing tissues to dry out).

9.3 Disassembly of components beyond what is necessary for the explanation of the implanted device should be avoided.

9.4 Each component should be packaged individually and device packaging should be dictated by the type of analysis that will be performed (for example, formalin submersion for tissue ongrowth analysis of coated metallic components). All packaging should be labeled with a unique identification code. Double bagging is preferred and each bag should be labeled with the unique identification code. The addition of an absorbent material may also be required.

9.5 Transportation of the devices and tissue for analysis may be time sensitive. Anticipate worst-case scenario timelines to make sure the sample evaluation is not compromised by delays in the transportation process. The potential for extremes of temperature exposure and possible effects of specimen should also be considered.

9.6 Specific details with regard to the packaging and shipping of medical devices, tissues, and fluids is covered in Guidance F2995.

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

10.1 *Macroscopic Examination of Tissue:*

10.1.1 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 such as tissue downstream from an intravascular or intracardiac implant. Describe the specimen size either by dimensions or weight.

10.1.2 Since the color and texture of the tissue is altered by sterilization and fixation methods, it is recommended that gross observations be made prior to fixation or sterilization. Such observations should be made utilizing appropriate blood borne pathogen protective techniques and equipment. The type of fixative and time of fixation should be considered depending on the type of pathologic analysis to be conducted. For example, if simply routine H & E staining then fixation in appropriate volume of 10 % buffered formalin for 24 to 72 hours may be fine but if immunohistochemistry (IHC) is considered then short duration (24 hr or <) in 4 % paraformaldehyde may be required to maintain epitope antigenicity.

10.1.3 Where appropriate and feasible, obtain photographic documentation of the explant and adjacent tissue, as well as a photographic record of subsequent dissections. A scale and unique ID # should be included in each photograph if possible.

10.2 *Histopathological Analysis of Tissue:*

10.2.1 Process the excised tissue using standard laboratory procedures for the histological processing to create stained microscope slides for pathologic interpretation. Processing may require that the device remains in the in-situ position within the tissue. Alternatively, the device may have been completely removed from the tissue. Depending on the study objectives or diagnostic goals, these procedures may be for frozen sections, paraffin embedding, methacrylate embedding or other special procedures. Routine staining with hematoxylin and eosin (H & E), a trichrome or pentachrome stain, or toluidine blue are recommended for light microscopy of most soft tissues and bone. Special stains (for example, von Kossa, Lendrum fibrin, Pearls iron, Picrosirius red, Massons trichrome, Movat pentachrome, or other) may be utilized as indicated and should be fully described.

10.2.2 Provide a detailed histopathologic description of the tissue-implant interface as well as all adjacent tissue specimens (for example, acute or chronic nature of the response, edema, thrombosis, calcification the type and extent of extracellular matrix deposition, necrotic changes, thickness of fibrous capsule, inflammatory cell types, giant cells, particulates, hyperplasia, dysplasia, type of inflammatory reaction).

10.2.3 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, interstitial inflammation, and overall biological fixation.

10.2.4 For detailed studies of tissue reactions, the use of a quantitative scoring scheme, such as that in Practice F981 is recommended.

10.2.5 Since some polymeric materials, for example, Polymethyl methacrylate (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.

10.3 *Immunohistochemistry Protocols:*

10.3.1 IHC can be used to identify specific cell types and ECM protein deposition in response to implantable materials and prosthetic devices. Typically, monoclonal or polyclonal antibodies raised against a particular epitope are used to identify cells, proteins or enzymes of interest that may aid in determining the cause of failure. For example, positive identification of B-cells in a tissue that contains may lymphocytes in addition to only small numbers of plasma cells and neutrophils could aid in determining if a patient is sensitive to a metal present in the retrieved implant. Other markers may be useful to identify activated cells or chemokines. This field is constantly changing as new markers are developed and new or improved methods are developed that increase specificity of current markers. Therefore, it is not possible to provide anything but a basic introduction, and a recommendation to follow published methods and methods provided by the manufacturer of the antibody to be used whenever possible.

10.3.1.1 Methods for typical markers chosen for studies of human tissues such as anti-human B cell, T cell, or monocytes/macrophages markers are readily available from a number of reputable manufacturers. However, specific markers for other species (for example, rats, mice, rabbits, goats), may not be readily available and may require extensive methods development of development of new antibodies specific for the species under investigation. It is essential that appropriate positive and negative controls be used to enable appropriate interpretation of the data. It is recommended that positive controls be processed in the same manner as test tissue.

10.3.1.2 IHC methods consist of a series of steps or reactions that have been developed to amplify the signal on the markers. First, a primary antibody specific for the CD marker (for example, mouse anti-human) is applied. Then, a secondary antibody that binds specifically to the first antibody is applied (for example, goat anti-mouse). This antibody is typically tagged with a reagent such as biotin, which serves as a marker in this amplification phase of the reaction. In this example, strept-avidine peroxidase would then be added to bind to the biotin and immobilize the peroxidase. Finally, a substrate is added that will react with the peroxidase, change color and precipitate at the location of the antigen. Diaminobenzidine (DAB) is often used, although several substrates are available for different kits or automatic systems. The end result in this example 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 or other counterstain to enhance the visibility of cells and contrast with the colored reaction identifying the epitope. Additional amplification steps may be needed to ensure adequate visualization, or addition of blocking steps such as addition of hydrogen peroxide or horse serum may be needed to reduce background labeling. Background labeling can be introduced by binding of the reagents to endogenous proteins or enzymes (for example, peroxidase) or to block non-specific binding of minor or substantive known contaminants in the reagents may be needed.

10.3.1.3 It is best if an antibody is selected that has been previously used successfully on tissues embedded in the same manner as those being tested as not all methods that work on

one type of section (for example, frozen sections) will work on another type of section (for example, paraffin-embedded or MMA-embedded sections). This is because the embedding processes may mask, alter or destroy antigens. While antigen retrieval processes (for example, citrate, heat and enzyme digestion) can be used to unmask the antigen for which the antibody is specific, these methods are not always successful, can result in tissue falling off the slides and can even increase background labeling.

10.3.1.4 Irrespective of the embedding technique or the method used, the desired outcome is one where the is low or no nonspecific coloration of the tissues with the substrate used to localize the signal identifying the antigen of choice (for example, CD-2), one where positive control tissues embedded and labeled in the same manner show an expected pattern of labeling and one where test sections labeled with all the same methods except the primary antibody (no-antibody controls) are negative (for example, show no label). Some additional general considerations are provided below that will apply to all methods used.

10.3.2 *Reagents and Other Considerations:*

10.3.2.1 Tissue should be fixed in reagents that are known to support the IHC methods you intend to use.

10.3.2.2 HC reagents can be purchased from a variety of companies. It is advisable to use those recommended by the manufacturer or shown in the literature to produce a strong label with low or no background.

10.3.2.3 t is important to use a humidified chamber when labeling to ensure that slides do not dry out during the procedure.

10.3.2.4 It is important to completely wash away the prior reagent from the section with buffered saline or other recommended wash before adding the next reagent.

10.4 *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):*

10.4.1 *Reagents and Materials:*

10.4.1.1 Standard Atomic Absorption Spectroscopy (AAS) grade solutions (MCB reagents, Fisher, and VWR) are used to make calibration curves. Calibration solutions should be prepared according to Practice D3919, 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.

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

10.4.1.3 Handling of tissues for subsequent chemical analysis requires special precautions to be taken to ensure 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 particles. 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.

10.4.1.4 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.

10.4.2 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.

10.4.2.1 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.

10.4.3 These methods of analysis require chemical digestion of the tissue samples prior to analysis, and therefore the samples cannot 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.

10.4.3.1 *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.

10.4.3.2 *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."

10.4.3.3 *Tissue* from implant sites or joint capsules should be weighed and placed in plastic bags.

(1) For the mechanical tissue digestion appropriate bags for the machine should be used. These may then be frozen until use. For preparation, 5 mL of 50 % nitric acid shall be added to each bag. The tissue is then homogenized in a mechanical blender.

(2) Alternatively, tissue should be placed in analytical grade 70 % ethanol in analytical grade water in a proper container that will not allow cross-contamination.

(3) The tissue sample may be dried (15 min at 90°C) prior to digestion, to determine the dry weight. Acid digestion of the dry sample can then be accomplished with nitric acid. The dried tissue sample (~100 mg) should be mixed with 5 mL of

low trace nitric acid (minimum 70 % HNO_3) and heated for approximately 2 h (or more if needed) at 90°C. Alternatively, the same solution can be placed in a microwave digestion bomb (that is, Parr Microwave Digestion Bombs Model No. 4781 23 mL or 4782 45 mL),[5] which is a chemically inert vessel designed for high heat where venting may be required. These vessels can be placed in a household microwave for more rapid sample dissolution (that is, 2 min at medium power). There are also commercially available microwave digestion systems available that operate at higher pressures and temperatures which also have vessels and carrousels as part of the system. (See Note 1.)

(4) The samples are then pipetted into the GFAAS or ICPMS for analysis. Dilution as necessary is done with nitric acid. Following the digestion procedure, the solution may need to be diluted to a pre-determined amount (that is, 5:1) using de-ionized water. Dilutions may be necessary if the analytical equipment cannot operate with samples containing high acid concentrations.

NOTE 1—Microwave digestion is a good alternative to traditional acid digestion, since microwave dissolution is faster, safer, and simpler, and provides more controlled reproducible conditions than conventional methods. Depending on the power ratings of the microwave, an appropriate level will need to be chosen in conjunction with the adequate time to achieve full digestion of the tissue.

10.4.3.4 *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.

10.4.4 Analysis with graphite furnace (GFAAS) should be done according to Practice D3919 using the manufacturer's specifications for analyte wavelength and slit width. Calibration standards should be made up in the same matrix as the test specimens.

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

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

10.4.4.3 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 nickel, molybdenum, platinum, titanium, and vanadium are done from the wall of the tubes while cobalt, chromium, and aluminum are done on platforms.

10.4.5 Chemical analysis by ICP should be done in accordance with Practice E1479.

[5] The sole source of supply of the apparatus known to the committee at this time is Parr Instrument Co., 211 Fifty Third St., Moline, IL 61265–9984. If you are aware of alternative suppliers, please provide this information to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee,[1] which you may attend.

11. Analysis of Tissues and Fluids for Wear Particles

11.1 Analysis for implant particles can be done histopathologically as in 10.2, or by tissue or fluid digestion and particle separation. Tissues subjected to digestion become dedicated to this type of analysis.

11.1.1 Preparation of tissue for drying and digestion will depend on the state of the tissue. If the tissue was previously 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 fixed in formalin, it should be dehydrated through a series of alcohols using a standard histological protocol, and infiltrated with 100 % ethanol. If tissue is fresh, it should be frozen and free of embedding media.

11.1.2 The tissue shall 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.

11.1.3 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.

11.2 *Reagents for Digestion:*

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

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

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

11.2.4 *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).

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

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

11.3 *Procedure for Digestion in Papain:*

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

11.3.2 Add 5 ml of papain solution and vortex.

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

11.3.4 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.

11.3.5 Wash particles in 10 cc of hot filtered water.

11.4 *Procedure for Digestion in a Strong Base and Pronase:*

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

11.4.2 Add 2 ml of 4 M KOH.

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

11.4.4 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.

11.4.5 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 for 1 h. Repeat washing procedure 3 times.

11.4.6 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.

11.4.7 Wash the debris 3 times in distilled water.

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

11.4.9 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.

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

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

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

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

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

11.4.15 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.

11.4.16 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.

11.5 *Procedure for Digestion in Nitric Acid:*

11.5.1 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.

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

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

11.5.4 Add 1.0 ml of concentrated nitric acid.

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

11.5.6 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.

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

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

11.5.9 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.

11.5.10 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.

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

11.6.1 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.

11.6.2 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.

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

11.6.4 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 at 5–15°C.

11.6.5 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.

11.6.6 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.

11.6.7 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 at 20–25°C.

11.6.8 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.

11.7 *Procedure for Sodium Hydroxide Digestion of Protein Solutions from Wear Tests:*

11.7.1 Lubrication fluids harvested during wear or joint simulation tests may be stored frozen at −20°C until digestion.

11.7.2 Digest a minimum volume of 80 ml serum or protein containing solutions by adding KOH pellets to a concentration of 12M. Digest at 60°C for 48 h or until solution clears.

11.7.3 The digested fluids are cooled to 4°C. Lipids and proteins are removed by the addition of an equal volume of chloroform:methanol (2:1). The solutions are then incubated at room temperature for 24 h, and then centrifuged at 2000 g for 10 min at RT. The contaminating lipids and proteins form a layer at the interface of the two solutions. The top layer containing the polyethylene wear particles is then decanted by pipetting into a clean tube and the procedure repeated three times or until the supernatant clears completely (that is, all visible lipids and proteins removed).

11.7.4 Any remaining proteins are removed by precipitation with the addition of an equal volume of ice-cold ethanol. The