Designation: ~~F1904 – 14~~ F1904 – 23

Standard ~~Practice~~Guide for Testing the Biological Responses to ~~Particles~~ Medical Device Particulate Debris and Degradation Products *in vivo*[1]

This standard is issued under the fixed designation F1904; 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 the production of wear particles and degradation products from implanted materials that may lead to a cascade of biological responses resulting in damage to adjacent and remote tissues. In order to ascertain the role of particles in stimulating such responses, the nature of the responses, and the consequences of the responses, established protocols are needed. This is an emerging, rapidly developing area and the information gained from standard protocols is necessary to interpret responses. Some of the procedures listed here may, on further testing, not prove to be predictive of clinical responses to particulate debris. However, only the use of standard protocols will establish which are useful techniques. Since there are many possible and established ways of determining responses, a single standard protocol is not stated. However, this recommended practice indicates which necessary information should be supplied with test results. For laboratories without established protocols, recommendations are given and indicated with an asterisk (*).~~

~~1.2 This standard is not designed to provide a comprehensive assessment of the systemic toxicity, carcinogenicity, teratogenicity, or mutagenicity of the material.~~

~~1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*~~

~~2. Referenced Documents~~

~~2.1 *ASTM Standards:*~~[2]

~~F561 Practice for Retrieval and Analysis of Medical Devices, and Associated Tissues and Fluids~~
~~F619 Practice for Extraction of Materials Used in Medical Devices~~
~~F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices~~
~~F1877 Practice for Characterization of Particles~~

~~3. Summary of Practice~~

~~3.1 Biological responses to particles testing may be done using specimens from animals being tested in accordance with the Practice F748 matrix for irritation and sensitivity, or for implantation. If particles were implanted during the testing procedures or generated during the experimental time period, the response to those particles may form a part of the overall investigation of response to particles. Blood, organs, or tissues from the animals may be used.~~

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

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

3.2 Biological responses to particles may be tested using the actual particulate materials or extracts in accordance with Practice F619. The increased surface area of small particles may enhance the amount of extracted substances but, since the response to particles may be related to the physical size, shape and composition, the use of only extracts will not completely address the question of the impact of particle formation on the tissue response and actual implantation or other testing of particles should be included as a part of the characterization of tissue response when particle generation is likely during actual usage. These materials or extracts may be used in *in vivo* tests or for the *in vitro* tests. Particles generated by other methods may also be used. The method of generation shall be described.

4. Significance and Use

4.1 This practice is to be used to help assess the biocompatibility of materials used in medical devices. It is designed to test the effect of particles from the materials on the host tissues.

4.2 The appropriateness of the methods should be carefully considered by the user since not all materials or applications need to be tested by this practice. The validity of these studies in predicting the human response is not known at this time and studies such as those described here are needed.

4.3 *Abbreviations Used:*

4.3.1 *CD*—Cluster differentiation.

4.3.2 *DNA*—Deoxyribonucleic acid.

4.3.3 *EDS*—Energy dispersive X-ray spectroscopy.

4.3.4 *EU*—Endotoxin unit.

4.3.5 *HLA*—Human leukocyte antigens.

4.3.6 *LAL*—Limulus amebocyte lysate.

4.3.7 *LPS*—Lipopolysaccharide (endotoxin).

4.3.8 *RNA*—Ribonucleic acid.

5. Responses from In Vivo Systems

5.1 *Particles*—Define the nature of the particles used:

5.1.1 Source,

5.1.2 Chemistry,

5.1.3 Size (mean and range),

5.1.4 Shape,

5.1.5 Surface charge (if known),

5.1.6 Method of sterilization,

5.1.7 If the presence of bacterial lipopolysaccharide (LPS) was determined, specify how this was done and the sensitivity of the method. (LAL testing with a sensitivity of at least 0.06 EU is recommended),

5.1.8 Concentration of particles used as weight, or number, or surface area/implant, and

5.1.9 Polystyrene particles, spherical, 1 to 5 μm in size may be used as reference particles.

5.1.10 Practice F1877 may be useful in defining the nature of the particles.

5.2 *Biological System*—One or more of these sites should be used:

5.2.1 *Air Pouch Model*— This is a model to simulate synovial tissue. The volume of air and the time allowed before introduction of the particles should be specified. This model needs to be validated for length of time of implantation and relevance to other *in vivo* systems.

5.2.2 *Cages*—Cages made of porous materials such as stainless steel mesh or porous teflon can be implanted with a test material inside the cage. These may be implanted subcutaneously or intraperitoneally. The material and the implant location chosen should be specified. The fluid accumulating in the cage can be sampled at various time intervals. The time intervals shall be specified. The cage and contained material is removed at the termination of the experiment (specify the time chosen) and evaluated for cell adhesion, cell type, and products. Fluid containing a large number of red blood cells should be discarded since it represents blood, not cage fluid.

5.2.3 *Bone Implant Chamber*—This is a modification of the cage system and allows determination of the effect of particles and the resulting biological response on bone remodeling

5.2.4 *Direct Injection*— Intraperitoneal, intravenous, intramuscular, and subcutaneous are the favored routes. The end use application should govern the route of injection and the organ or tissue utilized in this test. Inhalation may be suitable for some end use applications.

5.2.5 *Other Methods*—The use of other biological systems, animal models,or methods of implantation may be appropriate, depending upon the intended use of the material.

5.2.6 Examination of tissue at implant retrieval from animal models or clinical conditions is dealt with in Practice F561, and Practice F1877 may be used to describe the morphology of the particles that may be present in or extracted from those tissues. Some of the procedures defined here are also applicable to these tissues.

5.2.7 All sites used in these studies should be carefully evaluated for infection and inflammation at the termination of the study. The presence of infection or inflammation will have a major impact on the outcome since it stimulates many responses.

5.2.8 *Control Animals*—In the conduct of testing with any of the above described models, appropriate control animals who receive any vehicles, carriers, other treatments received by the experimental models, to control for the effects of factors other than the presence of the particles, should be included as well.

5.3 *Biological Response*—One or more of the following should be performed:

5.3.1 Cell accumulation at the site of the particles should be evaluated for the relative number and type of cells. Standard paraffin or plastic embedded sections are usually sufficient to identify acute inflammatory cells, lymphocytes, macrophages, foreign body giant cells, osteoclasts, osteoblasts, osteocytes, eosinophils, etc. But in some cases special histological procedures, or immunohistochemical stains such as those described in Practice F561, or flow cytometry may be needed to confirm the identity of lymphocytes and macrophages. An evaluation scale of 0 to 5 with 0 being no cell response, 1 being accumulation of a few cells, 2 being a mild response with some cell accumulation, 3 being a moderate response, 4 being a large response, and 5 being a severe response is recommended. It should also be noted whether the response is focal or diffuse.

5.3.1.1 Transport of particles to relevant draining organs and histologic responses in these organs should be determined, especially when direct injection is used. The relevant organs would be spleen, liver, and kidney. In some cases, the lung may also be an appropriate draining organ when it is reasonable to suspect that particles could enter the venous return portion of the vascular system. The draining nodes should be harvested if identifiable. Some types of particles are distinctive (for example, carbon fibers), but lymph nodes and lung commonly contain particles and bits of birefringent material that may be confused with particles used in the experiment. Light microscopy with and without polarized light can be suggestive of particle migration, but other methods (for example, EDS) may be necessary to confirm the composition of the migrating particles. Organs from control animals should also be evaluated.

5.3.2 *Soluble Cell Products Elaborated*—This is a rapidly emerging area of technology. Histochemical and immunohistochemical techniques can be used to great advantage in these studies. Reliable reagents, kits, or hybridization protocols are available to detect

cellular products such as cytokines, prostaglandins, immunoglobulins, as well as the lymphocyte CD markers and some HLA markers. It is not necessary to measure all possible cellular products and the selection should be based on whether there is emphasis on the response of macrophages or other cells involved in the non-specific immune response, or on the specific immune response.

NOTE 1—The identification and study of reactive cellular products is a rapidly expanding field and any listing of specific products from which to choose would necessarily become obsolete quickly. An immunologist should be consulted to assist in the selection of substances for which testing should be performed.

5.3.3 When other products from the cellular response are being detected, they should be specified and the method used specified.

5.4 Effects of the particles on other systems such as bone remodeling, chondrocyte function, cartilage repair, and synovial tissue function and repair are also important studies. The methods used should be fully described.

6. Report Section and Data Analysis

6.1 The histologic response should be compared to that of normal tissues with no particles and to that of tissues receiving the polystyrene reference particles, if used as reference particles. This may be done by counting, by digitization, by cell analyzer, or by estimation in the field of view. In some circumstances, the presence or absence of marker or response will suffice. In some circumstances, the quantitation of the response may be obtained with data on responses such as Ca^{++} released, enzyme levels, DNA or RNA levels, etc.

6.2 The report should include a description of the methods used, route of administration and source of the particles, and other details of the experimental protocol sufficient to allow the results to be interpreted in the context of the testing methods used.

7. Keywords

7.1 biocompatibility; biological response; *in vivo*; interleukins; particles

APPENDIX

(Nonmandatory Information)

X1. RATIONALE

X1.1 The primary purpose of this practice is to describe methodologies to determine the biological response to particles using *in vivo* responses.

X1.2 It is well recognized that the biological responses to particles could be different from those to solid materials. The interaction of the particles with cells in the tissues, notably macrophages and other phagocytic cells, is a key to the final biological response.

X1.3 The interaction of particles with host tissues has been an active research area for many years. Many investigators have developed procedures for doing these studies. This practice is intended to delineate the information necessary for interpretation of the results from these various studies and to describe methodology appropriate for investigators developing such studies.

X1.4 The interaction of the biological system with particles will lead to the accumulation of various cells that may produce soluble mediators that influence the progression of the immune response. Studies such as the ones described here are needed to determine the importance of this response in the biocompatibility and biocompatibility testing of materials.

~~X1.5 This practice was revised in 2014 to incorporate new information and update some of the information originally included in the 1998 version.~~

1. Scope

1.1 The purpose of this standard guide is to describe the principles and approaches to testing of medical device debris and degradation products from device materials (for example, particles from wear) for their potential to activate a cascade of biological responses at local and systemic levels in the body. In order to ascertain the role of device debris and degradation products in stimulating such responses, the nature of the responses and the consequences of the responses should be evaluated. This is an emerging area. The continuously updated information gained from the testing results and related published literature is necessary to improve the study designs, as well as predictive value and interpretation of the test results regarding debris/degradation product related responses. Some of the procedures listed here may, on further testing, not prove to be predictive of clinical responses to device-related debris and degradation products. However, only the continuing use of standard protocols will establish the most useful testing approaches with reliable study endpoints and measurement techniques. Since there are many possible and established ways of determining the debris/degradation product related responses *in vivo*, a single standard protocol is not stated. However, this recommended guide indicates which testing approaches are most applicable per expected biological responses and which necessary information should be supplied with the test results. To address the general role of chronic inflammation in exaggerating device-related foreign body response (FBR), the recommendations in this standard include the assessment of device-related pro-inflammatory responses and subsequent tissue remodeling potential.

1.2 This document is to provide the users with updated scientific knowledge that may help better characterize medical device debris related responses. It is to help the users to optimize their plans for particle characterization and biocompatibility assessment by considering the testing principles and methods available in published literature that are appropriate to their products.

1.3 This standard is not sufficient to address device-related degradation products that result in gas formation or that are exclusively represented by nanoparticles, or soluble species such as dissolved metal ions.

1.4 While devices should be designed and manufactured in such a way as to reduce as far as possible the risks posed by substances or particles (including wear debris, degradation products, and processing residues) that may be released from the device, this standard guide may help users to identify the presence of wear debris and degradation products and subsequent adverse reactions that may occur.

1.5 Although this guide is based on the available device debris-related knowledge that is largely based on orthopedic devices, most of the recommendations are also applicable to other (non-orthopedic) device areas.

1.6 *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]

F561 Practice for Retrieval and Analysis of Medical Devices, and Associated Tissues and Fluids
F619 Practice for Extraction of Materials Used in Medical Devices
F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices
F1877 Practice for Characterization of Particles
F1903 Practice for Testing for Cellular Responses to Particles ***in vitro***

2.2 *ISO Standards:*[3]

ISO 14242-1 Implants for surgery—Wear of total hip-joint prostheses—Part 1: Loading and displacement parameters for wear-testing machines and corresponding environmental conditions for test—Amendment 1
ISO 14242-3 Implants for surgery—Wear of total hip-joint prostheses—Part 3: Loading and displacement parameters for orbital bearing type wear testing machines and corresponding environmental conditions for test
ISO 14243-1 Implants for surgery—Wear of total knee-joint prostheses—Part 1: Loading and displacement parameters for wear-testing machines with load control and corresponding environmental conditions for test
ISO 14243-3 Implants for surgery—Wear of total knee-joint prostheses—Part 3: Loading and displacement parameters for wear-testing machines with displacement control and corresponding environmental conditions for test
ISO 17853 Wear of implant materials—Polymer and metal wear particles—Isolation and characterization
ISO 22622 Implants for surgery—Wear of total ankle-joint prostheses—Loading and displacement parameters for wear-testing machines with load or displacement control and corresponding environmental conditions for test

3. Terminology

3.1 *Definitions of Terms Specific to This Standard:*

3.1.1 *mechanistic, adj*—of or relating to the theory of mechanism which, in the science of biology, is defined as a system of causally interacting parts and processes that produce one or more effects.

3.1.2 *phagocytosable, adj*—capable of being phagocytosed.

4. Summary of Guide

4.1 Evaluation of biological responses to medical device debris and degradation products may be performed using specimens from animals being tested in accordance with Practice F748 which provides recommendations for biocompatibility assessment including local and systemic toxicity. When biocompatibility testing is performed (for example, implantation or injection of the test material), evaluation of the tissues surrounding the application site represent the best opportunity for assessing FBR and other local tissue responses. Bodily fluids such as blood and urine, as well as different organ tissues from the tested animals should be used for the assessment of systemic responses. Procedures according to Practice F561 may be used to assess the cellular and tissue responses *in vivo*.

4.2 Biological responses to device-related wear debris and degradation products may be tested using materials or extracts in accordance with Practice F619. The increased surface area of small particles may enhance the amount of extracted substances but, since the response to particles may be related to the physical size, shape, composition, and dose, the use of only extracts will not completely address the question of the impact of particle formation on the tissue response, and actual implantation or other testing of particles should be included as a part of the characterization of tissue response when particle generation is likely during actual usage. These materials or extracts may be used for the *in vivo* tests described here or *ex vivo* / *in vitro* approaches described in Practice F1903. Particles and other device-related debris/degradation products generated by alternative methods (for example, from animal studies, clinical use, or *in vitro* studies) may also be used, if appropriately justified. The method of generation must be described.

5. Significance and Use

5.1 This standard guide is to be used to help assess the biocompatibility of materials used in medical devices (for example, externally communicating, implants, and other body contact medical devices). It is designed to test the effect of particles and other wear debris and/or degradation products on the generation of FBR and other (local and systemic) host responses of immune/inflammatory origin.

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

5.2 The appropriateness of the selected testing methods should be carefully considered by the user since not all materials or applications need to be tested by this guide. Existing biocompatibility screening methods may not be fully predictive of the human response, and testing approaches such as those described here are needed for continuous improvement of the predictability of biocompatibility testing. The effectiveness of animal testing in terms of its predictability of human outcomes is dependent on the study design. If possible, study endpoints should be chosen to minimize interspecies variability and to investigate clinically relevant biological responses. While testing approaches should remain at the user's discretion, the following should be taken into consideration when selecting most appropriate tests and study endpoints.

5.2.1 Device-induced responses usually involve both innate and adaptive immunities, which raises possible need for specific testing for each of these immune response types.

5.2.1.1 Device-related adaptive immune responses are mostly due to lymphocyte-mediated delayed-type hypersensitivity. *In vivo* allergenicity to a test material (which can be introduced via different routes) should be assessed by monitoring for any signs of allergic and acute toxicity reactions, for example, scratch, tremor, and dyspnea. In addition, *ex vivo* analysis on immunophenotyping of the isolated splenocytes/lymphocytes from the same studies should be considered.

5.2.1.2 Device-related innate immune responses are mostly mediated by macrophages and can be assessed by histopathological assessment of the extent of FBR including macrophage accumulation around the test material. Supplementary *ex vivo* / *in vitro* assessment can be used for additional macrophage-based testing such as macrophage immunophenotyping (proinflammatory M1 and anti-inflammatory/wound healing M2) as well as debris uptake by phagocytes (phagocytozability) involving the entire range of test material characteristics.

5.2.2 Due to the role of inflammation in extending device-related FBR and promoting the resultant tissue remodeling, histopathological assessment should include identification of immune/inflammatory cell infiltration (with separate counts for the individual cell types representing both innate and adaptive responses) as well as corresponding tissue changes (for example, fibrosis, necrosis, ossification or osteolysis, angiogenesis). Identification of immune/inflammatory cells may involve different approaches including IHC phenotyping as needed. Supplementary *ex vivo* / *in vitro* assessment should be considered for assessing the balance in release of pro-inflammatory versus anti-inflammatory cytokines as well as generation of hyper-proliferative versus hypo-proliferative tissue responses.

5.2.2.1 Since the signs of inflammation and post-inflammatory tissue changes may not be always apparent, special attention should be given to the assessment of debris-related inflammogenic and tissue remodeling potentials using *ex vivo* specimens and supplementary *in vitro* assessment when needed. Pro-inflammatory cell death (necrosis) should be distinguished from programmed cell death (apoptosis usually associated with anti-inflammatory responses) by using cell viability and cytotoxicity testing involving cellular staining and flow cytometry. Given the importance of phagocytes in proper clearance of dying cells, normal non-phlogistic phagocytosis of cells undergoing apoptosis should be distinguished from "frustrated" phlogistic phagocytosis which may result in further cell/tissue damage due to the release of damage-associated molecular patterns (DAMP). See X1.10 for more details.

5.2.3 Due to the role of the device-tissue interface in shaping biological responses, *in vivo* models as well as supplementary testing should be aimed to simulate (as much as possible) device-specific use environments. *In vivo* animal models with intra-articular applications of a test material may be beneficial for testing of orthopedic materials, while intracardiac/intravenous applications may be more beneficial for testing of cardio/endovascular materials.

5.2.3.1 Since many implantable materials come in contact with blood during their clinical use, the need for hemocompatibility testing should be considered, especially when developing new materials. Development of new materials for cardiovascular applications may benefit from a more detailed hemocompatibility assessment, which could include microcirculation, cell adhesion, and leukocyte-endothelial interactions.

5.2.4 The predictability of testing for a certain material, including its debris, may benefit from the choice of study endpoints and testing approaches that incorporates clinical experience from known therapeutic applications and safety issues of similar materials.

5.2.4.1 In general, the study endpoints should be selected per their ability to measure immunomodulatory, pro/anti-inflammogenic, and tissue remodeling effects. As the examples of more specific choices, testing for an orthopedic material should take into consideration potential tissue changes such as periprosthetic osteolysis and pseudotumors, while testing for a cardiovascular material should take into consideration potential hemolytic, thrombolytic/thrombogenic, and pro-angiogenic effects.