



Designation: F1904 – 23

Standard Guide for Testing the Biological Responses to Medical Device Particulate Debris and Degradation Products *in vivo*¹

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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 biocom-

patibility 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

¹ This 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.

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.

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 (phagocytosability) involving the entire range of test material particle sizes.

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

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-inflammatory, 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.

5.2.4.2 Some endpoints currently used in effectiveness assessments can be applied to the safety assessment of adverse tissue remodeling (examples of osteogenesis-related study endpoints can be found in X1.12).

5.2.4.3 While not all possible clinical complications can be accurately replicated in animal testing models, the properly selected study endpoints for *in vivo* and supplementary *in vitro* testing can enhance the overall predictability of biocompatibility testing (more details on the choice of measurable study endpoints are provided in X1.5).

5.2.5 Rodents and other small animals (for example, rabbit, guinea pig) are traditionally used for *in vivo* biocompatibility testing models. Use of larger animal models is usually limited due to ethical and other concerns and may be reserved for models in higher need for imitating similarities with humans (weight, bone and joint structure, etc.).

5.3 Abbreviations Used:

5.3.1 **ALVAL**—Aseptic lymphocyte-dominated vasculitis-associated lesion.

5.3.2 **CD**—Cluster differentiation.

5.3.3 **DAMP**—Damage-associated molecular pattern.

5.3.4 **EDS/EDAX**—Energy dispersive X-ray spectroscopy.

5.3.5 **ELISA**—Enzyme-linked immunosorbent assay.

5.3.6 **FBGC**—Foreign body giant cell.

5.3.7 **FBR**—Foreign body response.

5.3.8 **FTIR**—Fourier-transform infrared (spectroscopy).

5.3.9 **H&E**—Hematoxylin and eosin.

5.3.10 **HMGB1**—High-mobility group box 1.

5.3.11 **HSP**—Heat shock protein.

5.3.12 **ICAM1**—Intercellular adhesion molecule-1.

5.3.13 **ICP-MS**—Inductively coupled plasma-mass spectrometry.

5.3.14 **Ig**—Immunoglobulin.

5.3.15 **IL**—Interleukin.

5.3.16 **LAL**—Limulus amoebocyte lysate.

5.3.17 **LPS**—Lipopolysaccharide (endotoxin).

5.3.18 **MMP**—Matrix metalloproteinase.

5.3.19 **NO**—Nitric oxide.

5.3.20 **NOS/iNOS**—Nitric oxide synthase / Inducible nitric oxide synthase.

5.3.21 **PCR**—Polymerase chain reaction.

5.3.22 **ROS**—Reactive oxygen species.

5.3.23 **SAA**—Serum amyloid A.

5.3.24 **SEM**—Scanning electron microscopy.

5.3.25 **α -SMA**—Alpha-smooth muscle actin.

5.3.26 **TBARS**—Thiobarbituric acid reactive substances.

5.3.27 **TGF- β** —Transforming growth factor-beta.

5.3.28 **TLR**—Toll-like receptor.

5.3.29 **TNF- α** —Tumor necrosis factor-alpha.

5.3.30 **TRAP**—Tartrate-resistant acid phosphatase.

5.3.31 **VEGF**—Vascular endothelial growth factor.