



Standard Practice for Testing the Biological Responses to Particles *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 reappraisal. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reappraisal.

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 Medical Plastics](#)

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

Current edition approved March 1, 2014. Published May 2014. Originally approved in 1998. Last previous edition approved in 2008 as F1904–98 (2008). DOI: 10.1520/F1904-14.

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](#)

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.

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 amoebocyte 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.

ASTM International takes no position respecting the validity of any patent rights asserted in connection with any item mentioned in this standard. Users of this standard are expressly advised that determination of the validity of any such patent rights, and the risk of infringement of such rights, are entirely their own responsibility.

This standard is subject to revision at any time by the responsible technical committee and must be reviewed every five years and if not revised, either reapproved or withdrawn. Your comments are invited either for revision of this standard or for additional standards and should be addressed to ASTM International Headquarters. Your comments will receive careful consideration at a meeting of the responsible technical committee, which you may attend. If you feel that your comments have not received a fair hearing you should make your views known to the ASTM Committee on Standards, at the address shown below.

This standard is copyrighted by ASTM International, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959, United States. Individual reprints (single or multiple copies) of this standard may be obtained by contacting ASTM at the above address or at 610-832-9585 (phone), 610-832-9555 (fax), or service@astm.org (e-mail); or through the ASTM website (www.astm.org). Permission rights to photocopy the standard may also be secured from the ASTM website (www.astm.org/COPYRIGHT/).