Designation: ~~F1904 – 98 (Reapproved 2008)~~ F1904 – 14

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 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 ~~debris~~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
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 ~~according to~~ in accordance with the Practice F748 matrix for irritation and sensitivity, or for implantation. ~~Blood, organs, or tissues from the animals may be used. Procedures according to~~If particles were implanted during the testing procedures or generated during the experimental ~~F561 may be used to assess the cellular response.~~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 ~~according to~~ 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 ~~must~~shall be described.

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[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.

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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.

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 ~~*Abbreviation*~~*Abbreviations Used:*

4.3.1 ~~*LPS—CD—*Lipopolysaccharide (endotoxin).~~Cluster differentiation.

4.3.2 ~~*LAL—DNA—*Limulus amebocyte lysate.~~Deoxyribonucleic acid.

4.3.3 ~~*PCR—EDS—*Polymerase chain reaction.~~Energy dispersive X-ray spectroscopy.

4.3.4 ~~*CD—EU—*Cluster differentiation.~~ 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 ~~should~~may be used as ~~a~~ reference ~~particle.~~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 ~~an emerging~~ 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 ~~must~~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 Determined—~~Response*—One or more of the ~~following:~~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