Designation: F1903 – 18

Standard Practice for Testing for Cellular Responses to Particles *in vitro*[1]

This standard is issued under the fixed designation F1903; 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 assessment of cellular responses to 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 cellular responses to particles and to determine if these correlate with *in vivo* responses. Since there are many possible and established ways of determining responses, a single standard protocol is not stated. However, well described protocols are needed to compare results from different investigators using the same materials and to compare biological responses for evaluating (ranking) different materials. For laboratories without established protocols, recommendations are given and indicated with an asterisk (*).

1.2 Since the purpose of the following test procedures is to predict the response in human tissues, the use of human (preferably macrophage lineage) cells is recommended. However, the use of non-macrophage cell lineage or the use of cells from non-human and non-primate sources may be acceptable. The source of the cells or the cell line used should be justified based on the cellular responses under test and/or tissue of interest. Non-human cells should not be used if there is evidence of possible cross-species difference for specific test results as the results of this *in vitro* test may not correspond to actual human response.

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

1.4 *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.*

1.5 *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]

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 Cellular responses to particles may be evaluated using specimens from animals being tested according to the Practice F748 matrix for irritation and sensitivity, or for implantation. Blood, organs, or tissues from the animals may be used.

3.2 Cellular responses to particles may be evaluated using materials or extracts according to Practice F619. These materials or extracts may be used for *in vivo* tests or for the *in vitro* tests. Particles generated by methods (for example, derived from *in vitro* mechanical testing or retrieved from *ex vivo* peri-implant tissues either from clinical retrievals or animal models) may also be used as long as they have characteristics similar to those produced by the implant or device being tested with appropriate justification.

3.3 The purpose of this practice is to assess the response of cells in direct contact with particles and therefore, this practice is primarily intended to cover the testing of particles placed into culture with the cells. This practice should be equally appropriate for the testing of the response to nanoparticles placed in culture, if particles of that size are the particles of interest. The size range of particles (among other particle characteristics) should be clearly defined and stratification of

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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.

the test results based on the particle size and other characteristics is recommended.

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 released from medical devices and biomaterials on macrophages or other cells.

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.

4.3 *Abbreviations:*

4.3.1 *FCS (FBS)*—Fetal Calf Serum (Fetal Bovine Serum)
4.3.2 *FGFs*—Fibroblast Growth Factors
4.3.3 *HBSS*—Hank's Balanced Salt Solution
4.3.4 *HEPES*—A buffering salt (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid)
4.3.5 *IL17*—Interleukin 17
4.3.6 *IL18*—Interleukin 18
4.3.7 *IL1β*—Interleukin 1 beta
4.3.8 *IL6*—Interleukin 6
4.3.9 *IL8*—Interleukin 8
4.3.10 *LAL*—Limulus Amebocyte Lysate
4.3.11 *LPS*—lipopolysaccharide (endotoxin)
4.3.12 *MCP1*—Monocyte Chemotactic Protein-1
4.3.13 *MMPs*—Matrix Metalloproteinases
4.3.14 *NO*—Nitric Oxide
4.3.15 *PBS*—Phosphate Buffered Saline
4.3.16 *PGE2*—Prostaglandin E2
4.3.17 *RPMI 1640*—Specific Growth Medium (Roswell Park Memorial Institute)
4.3.18 *TGFβ*—Transforming growth factor beta
4.3.19 *TNFα*—Tumor Necrosis Factor alpha
4.3.20 *VEGF*—Vascular Endothelial Growth Factor

5. Responses from Cells Grown *in vitro*

5.1 *Particles*—Define the nature of the particles used (see Practice F1877 for detailed particle characterization methodology):

5.1.1 Source;
5.1.2 Chemical composition;
5.1.3 Size (mean and range, refer to Practice F1877 for additional information regarding how size should be determined);
5.1.4 Shape;
5.1.5 Method of sterilization;
5.1.6 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 endotoxin units (EU) is recommended, see X1.5);
5.1.7 Concentration of particles used as weight, number, or surface area/106 cells;
5.1.8 Surface charge (if known);
5.1.9 Since some particles may have a density less than that of the culture medium being used, careful consideration should be given to determining appropriate procedures for ensuring that the particles and cells come into contact with each other. Techniques in which cells are grown on a substrate and then the substrate is inverted in culture may be appropriate.

5.2 *Cells*—Define the nature of the cells used:

5.2.1 *Established Cell Lines* (if not, go to 5.2.2)—The use of established cell lines provides a known cell type with a reproducible response. Although the correlation with the *in vivo* system may not be known at this time, careful studies with established cell lines could eventually allow determination of correlation between *in vivo* and *in vitro* systems.

5.2.1.1 Specify source of cell and identifying number or code.
5.2.1.2 Specify type of cells.
5.2.1.3 Specify special attributes of the cell line.
5.2.1.4 *Human macrophages such as U-937 are recommended; however, non-human murine macrophages such as RAW 264.7, J774A.1, P388D1, or IC-21 may be used.

5.2.2 *Primary Isolate:*

5.2.2.1 Specify source of cells including species and tissue origin (e.g., human, alveolar).
5.2.2.2 Specify type of cells.
5.2.2.3 Specify mechanism of isolation (e.g., lavage, enzymatic digestion).
5.2.2.4 Specify if stimulant used and if so, which one (e.g., mineral oil).
5.2.2.5 *Strain, age, and sex used should be specified; use of animals of both sexes should be considered for tests involving *in vivo* models.

5.3 *Culture Conditions:*

5.3.1 Specify source and type of medium. If from a commercial source, provide the catalog and/or reference number. If not from a commercial source, provide a list of ingredients and their sources.
5.3.2 Specify source of serum, and whether it was heat-inactivated. If the presence of LPS was determined, specify the amount present as well as the method and sensitivity of the method.
5.3.3 Specify culture conditions (for example, 37°C, humidified, 5 % CO_2 incubator).
5.3.4 Specify when and how the particles were added to the system.
5.3.5 Specify time of culture duration, test exposure, and/or time course of sampling of the culture medium.
5.3.6 If cell counts (in units of cell number/mL) were determined, specify as to when and how (for example, hemocytometer, Coulter counter).
5.3.6.1 *Cells should be cultured using the culture medium and serum specified/recommended by the supplier. LPS levels are generally provided or available from the distributor. Recommended culture conditions are 37°C, with 5 % CO_2, in a humidified incubator. Cell counts at the time of initial plating and at the termination of the culture are recommended. For adherent cell populations, cells should be detached from the culture surface and suspended either by gentle pipetting and/or scraping, or by using a cell dissociation solution, depending on the cell supplier recommendations. After the cells are resuspended, they should be washed with Ca- and Mg-free PBS, HBSS, or culture medium. During counting with a hemocytometer, it may be helpful to ascertain the percentage of