Designation: ~~F1903 – 10~~ F1903 – 18

Standard Practice for Testing ~~For Biological~~for Cellular Responses to Particles ~~*In Vitro*~~*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 ~~production of wear debris~~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 ~~there is correlation with the~~ 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*.~~asterisk (*).

1.2 Since the purpose of ~~these studies~~ the following test procedures is to predict the response in ~~humans,~~ human tissues, the use of human ~~cells would provide much information. However, in this practice,~~ (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 ~~cells is described. If the user should wish to employ cell lines from humans, cell lines are available from ATCC and most of the information and recommendations will still apply.~~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~~ safety, health, and ~~health~~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 ~~Biological~~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.

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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.

3.2 ~~Biological~~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 ~~other methods~~ 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.~~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,~~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 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 ~~from the materials on macrophages. The use of nonhuman, nonprimate cells is recommended in this practice. For laboratories equipped and approved to work with human blood and tissue, the use of these same protocols would be advantageous for development of understanding of the interaction of cells and 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 *LPS*—lipopolysaccharide (endotoxin).~~
~~4.3.2 *LAL*—Limulus Amebocyte Lysate.~~
~~4.3.3 *ATCC*—American Type Culture Collection.~~
4.3.1 *FCS (FBS)*—Fetal Calf ~~Serum.~~Serum (Fetal Bovine Serum)
4.3.2 ~~*NCS*—~~*FGFs*—~~Newborn Calf Serum.~~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.~~Saline
4.3.16 ~~*HANKS*—~~*PGE2*—~~A balanced salt solution.~~Prostaglandin E2
~~4.3.8 *MMPS*—Matrix Metallo Proteases.~~
4.3.17 *RPMI ~~1640~~1640*—~~—~~Specific Growth Medium (Roswell Park Memorial ~~Institute).~~Institute)
4.3.18 ~~*HEPES*—~~*TGFβ*—~~A buffering salt.~~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*~~*in vitro*

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

5.1.1 ~~Source,~~Source;
5.1.2 ~~Chemistry,~~Chemical composition;
5.1.3 Size (mean and ~~range),~~range, refer to Practice F1877 for additional information regarding how size should be determined);
5.1.4 ~~Shape,~~Shape;
5.1.5 Method of ~~sterilization,~~sterilization;
5.1.6 If the presence of bacterial lipopolysaccharide (LPS) was determined, specify how this was done and the sensitivity of the ~~method.~~method (LAL testing with a sensitivity of at least 0.06 ~~EU is recommended),~~endotoxin units (EU) is recommended, see X1.5);
5.1.7 Concentration of particles used as ~~weight or number or surface area/10~~weight, number, or surface area/10^6 ~~cells, and~~cells;
5.1.8 Surface charge (if ~~known).~~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 ~~are in contact.~~ 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 ~~Line~~Lines* (if not, go to 5.2.2)~~ (if not, go to~~ The ~~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 ~~Source~~ Specify source of cell and identifying number or ~~code;~~code.

5.2.1.2 ~~Nature of the cell (for example, macrophage), and~~Specify type of cells.

5.2.1.3 ~~Special~~ Specify special attributes of the cell ~~line (for example, nonphagocytic),~~line.

5.2.1.4 ~~*ATCC~~ *Human macrophages such as U-937 are recommended; however, non-human murine macrophages such as RAW 264.7, ~~J774A,~~J774A.1, P388D1, or IC-21 ~~are recommended.~~may be used.

5.2.2 *Primary ~~Isolate~~Isolate:* ~~(if not, go to 1.2.1):~~

5.2.2.1 ~~Source~~ Specify source of ~~cell~~cells including species and ~~location (for example, murine, alveolar),~~tissue origin (e.g., human, alveolar).

5.2.2.2 ~~Nature of the cell (for example, macrophage),~~Specify type of cells.

5.2.2.3 ~~Mechanism~~ Specify mechanism of isolation ~~(for example, lavage), and~~(e.g., lavage, enzymatic digestion).

5.2.2.4 Specify if stimulant used and if so, which one ~~(for example,~~ (e.g., mineral oil).

5.2.2.5 ~~*Mouse (specify strain,~~ *Strain, age, and sex ~~used) peritoneal exudate cells are recommended with a mild stimulant such as nutrient broth.~~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 ~~not~~ from a commercial source, provide the catalog and/or reference number. If not from a commercial source, provide a list of ingredients and ~~sources of ingredients.~~their sources.

5.3.2 Specify source ~~and type~~ of serum, and whether it was ~~heat inactivated.~~ 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 ~~termination of culture or sampling of~~ culture duration, test exposure, and/or time course of sampling of the culture medium.

5.3.6 If cell counts ~~were determined~~(in units of cell number/mL) were determined, specify as to when and ~~how. If estimates of cell number/mL specify when and how.~~how (for example, hemocytometer, Coulter counter).

5.3.6.1 ~~*Medium and serum specified by the supplier of the cells are recommended. Generally RPMI 1640 with heat inactivated 10 % newborn or fetal calf serum are recommended.~~ *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 using a hemocytometer with monolayer cells resuspended by trypsin solution (not recommended for macrophages), washing with Ca and Mg free PBS or Hanks, or scraping in 1 mL of Ca and Mg free PBS or Hanks. The addition of trypan blue is helpful. The supernatant of the medium from~~ 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 dead cells in the population using trypan blue viability staining. The responses of the macrophages exposed to particles for specified time periods ~~is assayed.~~should be assayed as described in 5.4.

5.3.7 *Controls:*

5.3.7.1 Cells not stimulated with ~~particles~~ particles, but otherwise cultured under the same experimental culture conditions, should be maintained ~~at the same time under the same conditions.~~in parallel with test condition(s), positive control, and/or reference standard.

5.3.7.2 Polystyrene ~~particles, spherical, size range 1 to 5 μm, should~~ (PS) particles may be used as a reference control. The choice of control may be affected by the size range, chemical composition, and other characteristics of particles of interest (e.g., special consideration should be given if nanoparticles are relevant to the device).

5.3.7.3 LPS is a known cell stimulant and ~~is a good positive control.~~ may be used as a positive control for certain immune responses. A concentration between 0.25 and 1 ng/mL of culture medium is sufficient.

5.3.7.4 Culture medium is a recommended diluent for the assays.

5.3.8 *Test Conduct:*

5.3.8.1 Culture cells for a dose ranging study to determine the Lethal Concentration (LC50) and investigate dosing effects on cellular uptake of particles and corresponding cellular response.

5.3.8.2 Record when and how particles are added to the test system, and explain how the doses for the study were chosen to include how the dosing concentration compares to a proposed clinical dose, if applicable.