



Standard Test Method for Evaluation of the Effect of Nanoparticulate Materials on the Formation of Mouse Granulocyte-Macrophage Colonies¹

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1. Scope

1.1 This test method provides a protocol for quantitative analysis of the effect of nanoparticulate materials in physiologic solution on granulocyte-macrophage colony-forming units.

1.2 This test method employs murine bone marrow hematopoietic stem cells which proliferate and differentiate to form discrete cell clusters or colonies which are counted.

1.3 This test method is part of the in vitro preclinical characterization cascade for nanoparticulate materials for systemic administration in medical applications.

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

1.5 *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

F1903 Practice for Testing For Biological Responses to Particles *In Vitro*

2.2 ANSI Standard:³

ANSI/ AAMI ST72 Bacterial Endotoxins—Test Methodologies, Routine Monitoring, and Alternatives to Batch Testing

¹ This test method is under the jurisdiction of ASTM Committee E56 on Nanotechnology and is the direct responsibility of Subcommittee E56.03 on Environment, Health, and Safety.

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

3. Terminology

3.1 Abbreviations:

3.1.1 **BM**—bone marrow

3.1.2 **CFU-GM**—colony forming unit of granulocyte and macrophage

3.1.3 **Cisplatin**—positive control

3.1.4 **DMSO**—dimethyl sulfoxide

3.1.5 **DPBS**—Dulbecco's phosphate buffered saline

3.1.6 **FBS**—fetal bovine serum

3.1.7 **IMDM**—Iscove's media

3.1.8 **LPS**—lipopolysaccharide

3.1.9 **Physiologic Solution**—isotonic, pH 7.2 ± 0.2

4. Summary of Test Method

4.1 The effect of nanoparticulate materials on the formation of granulocyte and macrophage colonies is assessed. Bone marrow cells are obtained from mice and cultured in stimulatory media. The number of colony forming units following contact with nanoparticles is counted and compared to baseline and positive control. This determines if the nanoparticulate material in physiologic solution is stimulatory or inhibitory to bone marrow stem cells. Aseptic procedures are necessary.

5. Significance and Use

5.1 Stem cells of hematopoietic origin are pluripotent and may be particularly sensitive to the effects of stimulation by nanoparticulate materials.

5.2 The effect of particles on macrophage responses has an extensive history and can be assessed by Practice F1903. The test method described here will assess the effect on stem cells which can be progenitor cells to the macrophage line.

6. Reagents and Materials

6.1 **Purity of Reagents**—Reagent grade chemicals shall be used in all tests. Unless otherwise indicated, it is intended that all reagents conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society where