**Designation: ~~F2901 – 12~~ F2901 – 13**

# Standard Guide for Selecting Tests to Evaluate Potential Neurotoxicity of Medical Devices[1]

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## 1. Scope

1.1 Medical devices may cause adverse effects on the structure and/or function of the nervous system. In this guide, these adverse effects are defined as neurotoxicity. This guide provides background information and recommendations on methods for neurotoxicity testing. This guide should be used with Practice F748, and may be helpful where neurotoxicity testing is needed to evaluate medical devices that contact nervous system tissue or cerebral spinal fluid (CSF).

1.2 *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]

F748 Practice for Selecting Generic Biological Test Methods for Materials and Devices

F1904 Practice for Testing the Biological Responses to Particles ***in vivo***

2.2 *Other Referenced Documents:*

ISO/AAMI/ANSI 10993-3 ~~:2003Biological~~ :2003 Biological Evaluation of Medical Devices—Part 3: Tests for Genotoxicity, Carcinogenicity, and Reproductive Toxicity[3]

ISO/AAMI/ANSI 10993-5 :2009 Biological Evaluation of Medical Devices—Part 5: Tests for In Vitro Cytotoxicity[3]

ISO 10993–11 : 2006 Biological Evaluation of Medical Devices—Part 11: Tests for Systemic Toxicity

ISO/AAMI/ANSI 10993-18 Biological Evaluation of Medical Devices—Part 18: Chemical Characterization of Materials[3]

ANSI/AAMI ST72 :2010 Bacterial Endotoxins—Test Methodologies, Routine Monitoring, and Alternatives to Batch Testing[3]

USP <151> Rabbit Pyrogen Test[4]

USP <161> Transfusion and Infusion Assemblies and Similar Medical Devices[4]



## 3. Summary of Guide

3.1 This is an informative guide and should be used with Practice F748.

3.2 The duration of contact between the tissue and medical device should be considered when determining the appropriate panel of testing. This guide may not address neurosurgical instruments or medical devices that have transient incidental contact with the nervous system due to the limited tissue contact duration.

3.3 The evaluation of neurotoxicity should be considered in conjunction with material characterization and other information such as non-clinical tests, clinical studies, post-market experience, and intended use.

## 4. Significance and Use

4.1 The objective of this guide is to recommend a panel of biological tests that can be used in addition to the testing recommended in Practice F748. This guide is designed to detect neurotoxicity caused by medical devices that contact nervous tissue.

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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.

[4] Available from U.S. Pharmacopeia (USP), 12601 Twinbrook Pkwy., Rockville, MD 20852-1790, http://www.usp.org.

4.2 The testing recommendations should be considered for new materials, established materials with different manufacturing methods that could affect nervous tissue response, or materials used in new nervous tissue applications.

4.3 Chemical characterization can be used to evaluate similarity for materials with a history of clinical use in a similar nervous tissue application.

## 5. Tests for Neurotoxicity

5.1 Testing should be performed on the final sterilized device, representative samples from the final sterilized device, or materials processed in the same manner as the final sterilized device. Testing of individual materials may be useful for research and development, but the definitive neurotoxicity evaluation should include all materials in the final version of the device. The test article should be exposed to all phases of manufacturing including processing, cleaning, sterilization, and packaging.

5.1.1 A complete description of all device materials and reagents used during manufacturing and processing should be provided with information on the source, purity, and toxicity profile. Chemical characterization studies can provide additional information on the device safety profile. See ISO/AAMI/ANSI 10993-18 for information on chemical characterization of materials.

5.2 The following tests should be considered to assess neurotoxicity of medical devices within the scope of this guide.

5.2.1 *Cytotoxicity*—Cytotoxicity assays are sensitive screening tools that generally serve as a starting point for evaluating medical device biocompatibility. See X1.4 for information on neuro-cytotoxicity testing.

5.2.2 *Genotoxicity*—Nervous tissue contains proliferating cell populations, and can respond to device implantation with a proliferative response. Nervous tissue is also known to give rise to various tumor types. To ensure that medical devices do not include genotoxic chemicals, the use of a panel of genotoxicity tests is recommended. The panel of genotoxicity tests should include a ~~bacterial assay~~ test for gene mutation in bacteria, an *in-vitro* test with cytogenetic evaluation of chromosomal damage with mammalian cells or an *in-vitro* ~~and mammalian~~ mouse lymphoma tk assay, and an ~~*assays*~~*in-vivo* ~~capable of detecting both gene level and clastogenic mutations.~~ test for chromosomal damage using rodent hematopoietic cells. See ISO/AAMI/ANSI 10993-3 for additional information on genotoxicity testing.

5.2.3 *Implantation*—The use of a clinically relevant implantation study is recommended. The implantation site and animal model should be selected and justified according to the intended clinical use of the medical device. The study should include both histopathology and neurobehavioral assessments. In addition to the use of hematoxylin and eosin (H&E), more sensitive and specific histopathological assessments should be considered, including methods that are capable of enhancing and quantification of neurodegeneration, astrogliosis, microglia activation, and myelinopathy. See Polikov et al. (**1**)[5], Schmued et al. (**2**), and O'Callaghan et al. (**3**) for examples of detection methods using Fluor-Jade for detection of neurodegeneration, glial fibrillary acidic protein (GFAP) for detection of astrogliosis, and Macrophage-1 antigen (MAC-1), Isolectin IB4, or ionized calcium binding adaptor molecule 1 (IBA-1) for detection of microglia activation. Tissue sectioning should include the implant-tissue interface and include sectioning of sufficient area around the implant to ensure that the potential effects of diffusion of degradable and/or leachable materials are captured in the histological analysis. Consideration should be given to whether cross or transverse sectioning best captures the area of interest depending on the anatomical region. The test period should be determined by the clinical exposure time, or go beyond the point where a tissue response steady state has been reached. The time course of the study should be designed and justified based on the intended clinical use. Finally, a functional observation battery designed to detect signs of neurobehavioral dysfunction is recommended to compliment the histopathological assessments.

5.2.4 *Pyrogen Testing*—Pyrogen testing on the final sterilized medical device is recommended to reduce the likelihood of a neuroinflammatory response to the device. Material-mediated pyrogen testing should be conducted using the rabbit pyrogen ~~test.~~ test (see USP <151> and ISO 10993–11, Annex F). Endotoxin testing with an assay such as the Limulus Amebocyte Lysate (LAL) assay should be conducted in compliance with ANSI/AAMI ST72 and USP <161>.

5.2.5 *Wear Particle Testing*—The proximity of orthopedic spine devices to the spinal cord and nerve roots may warrant evaluation of potential neurotoxicity. Devices capable of generating wear particles should be evaluated if the wear particles have not already been adequately tested for potential neurotoxicity. If particle testing is warranted, an appropriately justified animal study is recommended. See Practice F1904 and reference by Cunningham (**4**) for examples of particle-mediated neurotoxicity evaluation methods. The animal study should be designed to evaluate local, systemic, and neurobehavioral responses to particles. The spinal cord, nerve roots, surrounding tissue, and distant tissues should be evaluated for signs of toxicity and inflammation. The size, shape, and dose of particles used in the animal study should be representative of the particles expected to be generated clinically.

5.2.6 *Developmental Neurotoxicity*—Neurodevelopment includes critical periods that can have increased sensitivities to neurotoxicants, and neurodevelopment continues into the postnatal period. Therefore, developmental neurotoxicity studies ~~should~~may be considered for medical devices that ~~may~~ both potentially leach chemicals of developmental neurotoxicity concern and are indicated for pregnant women with the potential for fetal exposure or are indicated for pediatric patient populations. For guidance on developmental neurotoxicity study design, see Raffaele et al (**5**).

[5] The boldface numbers in parentheses refer to the list of references at the end of this standard.