

# Biocompatibility Testing for Medical Devices: "The Big Three"

With the tremendous growth of the implantable device market and continuous emergence of new medical device technologies, the FDA has established a renewed concern regarding medical device biocompatibility. In June 2016, the FDA released an updated Industry Guidance for the Use of International Standard ISO 10993. Among the updates in this document is an expanded table of Biocompatibility Evaluation Endpoints, which can be seen in Figure 1. Previous versions of the guidance only listed the ISO 10993-1 recommended endpoints based on the type of medical device, the type of patient contact, and the duration of patient contact (denoted by X's in Figure 1). However, an updated version of this guidance now includes additional FDA recommended endpoints to consider (denoted by O's in Figure 1). Hence, it is important to have an understanding of medical device biocompatibility testing as outlined in ISO 10993, and which tests need to be considered for a given device.

In terms of biocompatibility, one will often hear reference to "The Big Three." This refers to cytotoxicity, sensitization, and irritation testing. Testing these three biological effects are required on most medical devices regardless of category, patient

contact, and duration of use. Cytotoxicity testing (ISO 10993-5) is currently the only *in vitro* test of the big three and assesses the effects of leachables, which can be drawn out of the device, on living cells. This testing uses L929 mouse fibroblast cells, and results can be evaluated via quantitative methods (e.g., Neutral Red Uptake, MTT, XTT, and Colony Formation) and qualitative methods (e.g. Qualitative morphological grading of cytotoxicity of extracts). Analysts can record the presence of granules, signs of apoptosis or cell death, and cell proliferation or

growth when evaluating cytotoxicity.

Sensitization testing (ISO 10993-10) is an *in vivo* test that evaluates the ability of leachables to cause Type IV Hypersensitivity (i.e., delayed hypersensitivity). The tests are designed to determine if a patient will develop a reaction with repeated exposure to a medical device. Type IV Hypersensitivity is a cell-based immune reaction that results in edema and erythema, or swelling and redness respectively.

The gold standard for sensitization testing is the Magnusson &

Figure 1

Medical device categorization by			Biological effect												
Nature of Body Contact		Contact Duration  A – limited (≤24 h)  B – prolonged (>24 h to 30 d)  C – permanent (> 30 d)	Cytotoxicity	Sensitization	Irritation or Intracutaneous Reactivity	Acute Systemic Toxicity	Material-Mediated Pyrogenicity	Subacute/Subchronic Toxicity	Genotoxicity	Implantation	Hemocompatibility	Chronic Toxicity	Carcinogenicity	Reproductive/Developmental Toxicity	Degradation
Category	Contact														
Surface device	Intact skin	A	X	X	X										
		B	X	X	X										
		C	X	X	X										
	Mucosal membrane	A	X	X	X										
		B	X	X	X	O	O	O		O					
		C	X	X	X	O	O	X	X	O		O			
	Breached or compromised surface	A	X	X	X	O	O								
		B	X	X	X	O	O	O		O					
		C	X	X	X	O	O	X	X	O		O	O		
External communicating device	Blood path, indirect	A	X	X	X	X	O				X				
		B	X	X	X	X	O	O		X					
		C	X	X	O	X	O	X	X	O	X	O	O		
	Tissue/bone/dentin	A	X	X	X	O	O								
		B	X	X	X	X	O	X	X	X					
		C	X	X	X	X	O	X	X	X		O	O		
	Circulating blood	A	X	X	X	X	O		O		X				
		B	X	X	X	X	O	X	X	X	X				
		C	X	X	X	X	O	X	X	X	X	O	O		
Implant device	Tissue/bone	A	X	X	X	O	O								
		B	X	X	X	X	O	X	X	X					
		C	X	X	X	X	O	X	X	X		O	O		
	Blood	A	X	X	X	X	O		O	X	X				
		B	X	X	X	X	O	X	X	X	X				
		C	X	X	X	X	O	X	X	X	X	O	O		

nonvolatile residue was less than 10% of the weight of the original nonvolatile residue, at which point the extraction would be deemed exhaustive.

Once the samples are prepared the extracts are tested by several orthogonal analytical techniques in order to generate a broad extractables profile in accordance with ISO 10993-18. Liquid chromatography mass spectrometry is used to detect nonvolatile organic extractable compounds. Eurofins Medical Device Testing utilizes accurate mass time-of-flight (TOF) mass spectrometers and quadrupole time-of-flight (QTOF) mass spectrometers for this analysis. “Soft” ionization techniques, such as electrospray ionization (ESI), atmospheric chemical ionization (APCI), or a multimode source that combines the two types of ionization, are used often resulting in the mass spectrum displaying the molecular mass. The resulting mass spectra for each observed extractable compound must then be evaluated to determine identifications so that an accurate toxicological safety assessment can be performed. However, there are a limited number of choices of commercially available databases that are suitable for rapid identification of the observed peaks. Therefore, Eurofins Medical Device Testing has developed its own in-house proprietary database to aid in identification. The Eurofins Extractables Index (EEI) contains over 1,500 nonvolatile organic compounds that are commonly used in the production of medical devices and their materials of

construction, such as plastics, polymers, elastomers, inks, and adhesives.

Gas chromatography-mass spectrometry (both headspace and direct injection sample introduction) is also used to evaluate the presence of both volatile and semi-volatile organic compounds. Eurofins Medical Device Testing utilizes the NIST and Wiley databases to assist in the identification of observed extractable compounds. Finally, Inductively Coupled Plasma (ICP) with either mass spectrometry or optical emission spectroscopy detection is used to monitor the presence of metals. Eurofins Medical Device Testing typically evaluates the metals listed in USP <232> and ICH Q3D and has the ability to evaluate additional metals.

When seeking approval for a device for which a previously approved predicate exists, a fingerprint study to compare the extractables profile of the new device to that of the predicate may be appropriate in lieu of a full chemical characterization study. This fingerprint analysis consists of conducting chemical characterization of both the new and predicate devices and comparing the results. Only those compounds detected at higher concentrations in the analysis of the new device would require further toxicological evaluation to establish patient safety. Ideally this comparison study, included in the 510(k) submission to the FDA, would support the substantial equivalency of the device to the predicate. Using the fingerprint

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analysis is also an option when making minor changes to the material supply chain for a given device in order to show that the device is equivalent following the material change.

With the increasing concern for animal welfare, there is a push to reduce the burden of *in vivo* animal studies, which may be achieved by relying on chemical characterization data and the corresponding toxicological safety assessment. Eurofins Medical Device Testing performs more than 400 extractables and chemical characterization studies per year, designing each study to meet the client’s project objectives. Eurofins Medical Device Testing has the experience and expertise in designing and performing these studies to surpass expectations.

#### References:

<sup>1</sup>Use of International Standard ISO 10993 -1, Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing within a Risk Management Process. N.p.: Food and Drug Administration, 16 June 2016. PDF.