



TESTING AND EVALUATION STRATEGIES FOR THE BIOLOGICAL EVALUATION OF MEDICAL DEVICES SUBMITTED FOR CE MARK AND FDA APPROVAL





Today, millions of medical devices are used worldwide to treat or support patients. Patients expect that each device has been rigorously tested for functionality, performance, sterility and safety prior to being sold. The biological safety (biocompatibility) of a device is established for both short-term (acute) and longtime (chronic) safety risks to patients and device users. Medical device manufacturers must investigate their devices thoroughly to meet these expectations and receive approval to market their devices.

Biological Safety

The biological safety of various categories of possible toxicological effects should be considered for a particular device. These include: cytotoxicity, irritation, acute systemic toxicity, hemocompatibility and thrombogenicity as short-term effects, and sensitization, genotoxicity, subchronic and chronic toxicity, carcinogenicity, and reproduction toxicity as long-term effects. In order to completely evaluate the biological safety of a medical device, the nature and duration of body contact must be considered. For such a biological safety evaluation, manufacturers most often use the ISO 10993 standard series “Biological evaluation of medical devices”. This standard is internationally accepted; however, many countries have additional requirements or interpret this standard differently.

According to ISO 10993-1 [1], the biological risk of a product should be evaluated and tested within the scope of risk management. The first step of this process is to determine if there is body contact. The standard is not applicable for products with no direct or indirect body contact. For products with direct or indirect body contact, the device components are categorized following

ISO 10993-1 for nature of contact (e.g., surface, externally communicating, implant), type of tissue contacted (e.g., skin, mucosal membrane, compromised/breached surfaces, tissue/bone/dentin, circulating blood), and contact duration (e.g., limited, prolonged, permanent), where the contact duration is the cumulative sum of single, multiple or repeated use contact.

Based upon the categorization of the device under evaluation, the

manufacturer should develop an appropriate testing strategy. The test strategy should also take into account where the device will be submitted for registration and marketed, e.g., EU, USA, Japan, China, etc., because countries often have specific requirements for the biological evaluation. This article focuses on approval aspects in the European Union (CE Mark) and United States (FDA Approval).





ISO 10993 Standard Series

ISO 10993-1 includes a table (see Table 1 below) that provides "... a framework for the development of an assessment program..." where, for the various intended uses, a general biological test strategy is described. Within the table, the toxicological effects to be considered based on the intended use of the device are marked with an "X". The manufacturer should consider if data are available which cover the marked biological effects. If the biological effect is determined to be not relevant for this device, or if adequate data are available, further testing in this category is not needed. Thus, an "X" in the aforementioned table does not mean that a test is a required. However according to ISO 10993-1, additional biological effects such as: chronic toxicity, carcinogenicity, biodegradation, toxicokinetics, immunotoxicity, reproductive/developmental toxicity or other organ-specific toxicities need to be considered depending on the intended use of the device and assessment of risk.

After it is determined which biological effects are relevant and should be considered, the safety risk of each of these effects needs to be addressed. This can be done with testing or a documented justification. A justification can take the place of testing in regulatory submissions when the device components are found to be equivalent to marketed device materials. To document these conclusions, the marketed device materials should be listed and the method of establishing equivalence identified (i.e. manufacturing equivalence memo or comparative analytical chemistry testing). An additional alternative to testing is to use raw materials that are known to be

biocompatible and have a long history of safe-use as a medical device material, i.e. stainless steel acc. to ISO 5832-1 [2] / ASTM F139 [3] or Ti6Al4V acc. to ISO 5832-3 [4] / ASTM F 136 [5]. For these materials, it is sufficient to show that removal of process related surface residues at the end of the manufacturing process has been performed.

Ideally, testing should be performed according to the standards recognized by the applicable regulatory bodies. The ISO 10993 series of standards is recognized in both the EU and US.

The test methods used for determining various biological effects are described in the individual parts of the ISO 10993 standards. Tests are typically performed using final packaged and sterilized devices, so that all possible effects of the multiple production steps are included in the test sample. However, when the device polymerizes in-situ or is biodegradable, the interim reaction and degradation products must also be investigated for potential biological risk.

In addition to biological tests, the ISO 10993 series includes several standards specifically for physico-chemical tests. The assumption is that the biological effects of a device are dependent not only on the device's chemical structure, but also on its physical and morphological characteristics. These may have a significant influence on the product's biocompatibility. For example, a change in the roughness of a surface (change of morphology) may directly influence the cytotoxicity of the material whereas a chemically safe material with rough edges or particulates that can physically harm patient causing irritation.

In order to avoid unnecessary animal testing (requirement of ISO 10993-2), ISO 10993-1 specifies starting the biological evaluation with chemical and physical characterization of the materials (Clause 4.3). Therefore, the final device, including intermediate reaction and degradation products (see above) should be subjected to material characterization as described in ISO 10993-18. It is important to determine the identity and amount of leachable substances that may be bioavailable during the intended use of the device. Data on extractables and leachables should be cross-checked with toxicological databases, such as RTECS and TOXLINE, and an estimation of the benefits vs. risks for the final product determined. Identifying and estimating a potential health hazard early in the design and manufacturing process can avoid unnecessary animal testing, reduce costs and prevent delays in case material changes and retesting is indicated.

In the UL white paper, "Physical and Chemical Characterization of Medical Device Materials" [6] additional background information for applying ISO 10993-18 [7] is described.

Furthermore, ISO 10993-1 requires conducting *in vitro* test procedures before conducting *in vivo* tests. Also, before starting *in vivo* tests results of *in vitro* tests should be available.

Additionally, if the final packed device is sterilized using ethylene oxide, the levels of Ethylene Oxide (EO) and Ethylene Chlorohydrin (ECH) residuals and whether they are in compliance with the limits specified in ISO 10993-7 [8] should be determined.

Table 1: ISO 10993-1 Biocompatibility Testing Selection Criteria

Medical device categorization by			Biological Effect ^a							
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	Systemic toxicity (acute)	Subchronic toxicity (subacute toxicity)	Genotoxicity	Implantation	Haemocompatibility
Category	Contact									
Surface device	Skin	A	X	X	X					
		B	X	X	X					
		C	X	X	X					
	Mucosal membrane	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
	Breached or compromised surface	A	X	X	X					
		B	X	X	X					
		C	X	X	X		X	X		
External communicating device	Blood path, indirect	A	X	X	X	X				X
		B	X	X	X	X				X
		C	X	X		X	X	X		X
	Tissue/bone/dentin	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Circulating blood	A	X	X	X	X				X
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
Implant device	Tissue/bone	A	X	X	X					
		B	X	X	X	X	X	X	X	
		C	X	X	X	X	X	X	X	
	Blood	A	X	X	X	X	X		X	X
		B	X	X	X	X	X	X	X	X
		C	X	X	X	X	X	X	X	X

Note:
^a The "X" indicates data endpoint that can be necessary for a biological safety evaluation, based on a risk analysis. Where existing data are adequate, additional testing is not required.

Test Strategy for CE Mark

Before obtaining a CE mark, medical devices shall fulfill the essential requirements defined in the Medical Device Directive (MDD) 93/42/EEC as amended by the Directive 2007/47/EC [9], and in the Directive 90/385/EEC for active implantable medical devices (AIMD) [10]. Regarding the biocompatibility of a medical device, the essential requirements as defined in the MDD state “The devices must be designed and manufactured in such a way as to guarantee the characteristics and performance referred to in Section I on the ‘General requirements’. Particular attention must be paid to:

- the choice of materials used, particularly as regards toxicity and, where appropriate, flammability,
- the compatibility between the materials used and biological tissues, cells and body fluids, taking account of the intended purpose of the device, [...]”

A similar requirement can be found in the directive for active implantable medical devices 90/385/EEC.

In the region where the CE mark is used, the biological safety of a device is approved by adhering to the framework described in ISO 10993-1. Clause 6.2.1 of ISO 10993-1 describes particular conditions where animal experimental studies are not justifiable in terms of ISO 10993-2 [11]:

- study results and/or conclusive toxicological data are available for chemically identical products or materials,
- preclinical and clinical data (including a human history of safe use) exist for a chemically identical material or final product.

Without going into detail of the individual biological tests, we discuss below some requirements for obtaining the CE mark focusing on areas where the CE approach differs from that of the FDA.

Sensitization

For the CE mark, two tests are accepted for the evaluation of the sensitization potential (ISO 10993-10 [12]) of a device: the Guinea Pig Maximization Test (GPMT) according to Magnusson and Kligman, and the Local Lymph Node Assay (LLNA). The advantages of the LLNA test, compared to the GPMT, are that various doses can be evaluated, the test is a quantitative assay, and fewer animals are required. The disadvantages of the LLNA are that insoluble or systemically toxic materials may not test well and the test is not predictive for metals.

Genotoxicity

It is important to use various independent test models to evaluate conclusively the various genotoxic risks according to ISO 10993-3 [13]. Multiple tests are needed because no single test is able to detect all genotoxins.

Genotoxic effects may be caused by very low concentrations of hazardous substances, which are released from the device. As a consequence, even if within the material characterization no relevant amounts of substances with known genotoxic potential are released, genotoxicity must be tested.



In October 2014, an updated version of ISO 10993-3 was published. It outlines the following *in vitro* testing strategy for evaluation of genotoxic potential:

Option: Gene mutations in bacteria (Bacterial Reverse Mutation Test, “Ames” Test)

AND EITHER

Clastogenicity in mammalian cells (*In Vitro* Mammalian Chromosome Aberration Test)

OR

Gene mutations in mammalian cells (e.g., *In Vitro* Mouse Lymphoma Assay)

OR

Clastogenicity in mammalian cells (*In Vitro* Micronucleus Test).

Quality System of Test Facility

To be accepted by the Notified Bodies, biocompatibility test reports prepared according to ISO 10993 should be generated in an accredited ISO/IEC 17025 [14] test facility.

Test Strategy for FDA Approval

The ISO 10993-1 testing strategy is supported by ISO/TIR 15499 [15] and ISO 14971[16]. ISO 10993-1 is within the FDA’s list of recognized standards. Nevertheless, in 1995 the FDA prepared a guideline on the use of ISO 10993 titled “FDA Blue Book Memorandum # G95-1: Use of International Standard ISO 10993, Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing” [17]. In this document, the FDA describes additional evaluation requirements regarding the biocompatibility evaluation of medical devices. As a consequence, additional check marks are found in the FDA Blue Book Memorandum evaluation table compared

to the ISO 10993-1 evaluation table.

In April 2013, the FDA published a “Draft Guidance for Industry and FDA Staff on the Use of International Standard ISO 10993, ‘Biological Evaluation of Medical Devices Part 1: Evaluation and Testing’” [18]. When finalized, this will replace the G95 memorandum. By the end of the comment period (three months after the draft version was issued), the FDA had received over 700 comments. Although we expect there will be some clarifications to the text of the guidance when finalized, the main strategy of the FDA regarding the evaluation of the biocompatibility of medical devices will not change. Thus, it is strongly recommended to follow the suggestions in this latest FDA draft guidance. Therefore, this article discusses the 2013 draft guidance document as opposed to the G95 Blue Book Memorandum.

This FDA draft guidance document presents some differences when compared to the ISO 10993-1 standard. One important difference is that the FDA requires additional effects to be evaluated for various devices. In the table, these additional effects are marked with “o”.

These additional effects include:

Use of final product or representative sample

Ideally, the final device product should be tested. If this is not possible, testing of a representative, fabricated test sample (e.g., coupon or dummy) is allowed; however the manufacturer should evaluate if they are identical in their biological behavior (e.g., amount and identity of leachable substances). If not, additional testing may be necessary to justify the strategy used.

Biological response resulting from device mechanical failure

When a device has a coating or multiple components, it is possible that the mechanical failure of these could alter the biological response. For example the breakdown of a coating could change the surface topology of the device, release particulates into the circulatory system blocking blood flow, expose the patient to an area of the device that was not intended to contact the patient, or allow unwanted leaching of chemicals. These may have unintended harmful effects.

Submicron or nanotechnology components

These components have unique properties, e.g., aggregation, agglomeration, immunogenicity or toxicity. It is advisable to consult relevant literature and standards during development of protocols, and to contact the FDA prior to initiation of the test. An additional ISO 10993 part is expected to be released in the future that addresses nanoparticles.

Sample Preparation for Extract Testing

- Use surface area to volume ratio, and polar and non-polar extraction vehicles.
- Extraction times and temperatures should be appropriate for the intended use of the device. The typical extraction temperature of 37 °C may not be sufficient for devices with prolonged or permanent body contact. Therefore, a justification for the selected extraction conditions should be provided.
- Condition of extract and of test article after extract needs to be described.
- Do not process the obtained extracts -

do not filter, centrifuge, or adjust the pH. If processing is needed, a justification should be made explaining why this does not affect the integrity of the test.

Inclusion of multiple components or materials in a single sample

Devices with components with different durations of contact should be extracted separately. The same should be done if a device consists of several materials, because if combined, some components will be diluted.

Use of animal studies to justify omission of specific biocompatibility tests

An efficacy study of the final finished device performed in a relevant animal model can be designed to include assessments that may be used to justify omission of some biocompatibility tests. For example, a well-designed implantation study at a clinically relevant site may justify omission of the standard tests for local implantation, *in vivo* thrombogenicity and chronic toxicity.

Assessment of known or potentially toxic chemical entities

If a manufacturer uses chemicals within a device for the first time or it is known that some of the chemicals used have known or potential toxicity (e.g., color additives), additional information should be provided to determine whether toxicology evaluations beyond standard biocompatibility testing is needed

Test report

Full test reports, not just a declaration of conformity, must be submitted for all tests performed because ISO

10993 includes general test methods with multiple options. The reports should include sections describing the sample preparation, test methods, test parameters and acceptance criteria, analysis of results, and conclusions.

Sensitization

The FDA prefers the use of the Guinea Pig Maximization Test (GPMT). When the Local Lymph Node Assay (LLNA) is used to evaluate the sensitization potential of a device, the FDA will evaluate whether to accept such reports on a case-by-case basis.

Genotoxicity

If genotoxicity testing is performed for an FDA submission, the following three test models are recommended:

- Gene mutations in bacteria (*in vitro* Bacterial Reverse Mutation Test, “Ames” Test)
- An *in vitro* mammalian genotoxicity assay. A choice of one of the following

is recommended:

- a. the Mouse Lymphoma gene mutation assay, which is preferred since it detects the broadest set of genotoxic mechanisms associated with carcinogenic activity;
 - b. an *in vitro* chromosomal aberration (CA) assay; or
 - c. an *in vitro* micronucleus assay.
- An *in vivo* cytogenetics assay. A choice of one of the following is recommended:
 - a. a bone marrow micronucleus (MN) assay;
 - b. a bone marrow chromosomal aberration (CA) assay; or
 - c. a peripheral blood MN assay.

Quality System of Test Facility

Any *in vitro* and *in vivo* tests should be conducted in accordance with Good Laboratory Practice (GLP). Test reports should include a compliance statement regarding GLP regulation.





Table 2: FDA Draft Guidance Document - Initial & Supplementary Evaluation Test for Consideration

Medical device categorization by			Biological Effect ^a											
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	Systemic Toxicity (acute)	Subchronic Toxicity (subacute toxicity)	Genotoxicity	Implantation	Haemocompatibility	Chronic Toxicity	Carcinogenicity	Reproductive/Developmental	Biodegradation
Category	Contact													
Surface device	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					
		C	X	X	X	O	X	X	O		O			
	Breached or compromised surface	A	X	X	X	O								
		B	X	X	X	O	O		O					
		C	X	X	X	O	X	X	O		O			
External communicating device	Blood path, indirect	A	X	X	X	X				X				
		B	X	X	X	X	O			X				
		C	X	X	O	X	X	X	O	X	O	O		
	Tissue/bone/dentin +	A	X	X	X	O								
		B	X	X	X	X	X	X	X					
		C	X	X	X	X	X	X	X		O	O		
	Circulating blood	A	X	X	X	X		O [^]		X				
		B	X	X	X	X	X	X	X	X				
		C	X	X	X	X	X	X	X	X	O	O		
Implant device	Tissue/bone	A	X	X	X	O								
		B	X	X	X	X	X	X	X		O	O		
		C	X	X	X	X	X	X	X		O	O		
	Blood	A	X	X	X	X	X		X	X				
		B	X	X	X	X	X	X	X	X				
		C	X	X	X	X	X	X	X	X	O	O		
Notes:														
X ISO Evaluation tests for consideration														
O Additional categories which should be addressed in FDA submissions, either by inclusion of the testing or a rationale for its omission														
+ Tissue includes tissue fluids and subcutaneous spaces														
[^] For all devices used in extracorporeal circuits														
^a The "X" indicates data endpoint that can be necessary for a biological safety evaluation, based on a risk analysis. Where existing data are adequate, additional testing is not required.														



Overall Biological Evaluation Document

Within the risk management process according to the ISO 14971 [16], every final, finished medical device that is going to be CE marked, or receive a 510(k) clearance or other regulatory approval to market needs to undergo a complete biological safety assessment according to the ISO 10993-1 [1].

The ISO 10993-1 standard should be considered at the earliest phase of the product life cycle, starting with idea conception, continuing throughout the R&D phase and early production, to launch of the final medical device.

Due to the diversity of medical devices and the different interpretations of regulators in different countries, even if the requirements seem similar for various regulatory regions, the biological testing strategy and demonstration of the biocompatibility should be developed for each region individually.

The ISO 10993-1 [1] standard highlights repeatedly that a “...biological evaluation shall be planned, carried out, and documented by knowledgeable and experienced professionals, appropriately qualified by training and experience.” UL can provide all of these services.

About UL MDT:

In order to successfully gain market access, you will need a partner to support you with strategic and targeted testing to fulfill the regulatory requirements of the global market.

UL MDT (Medical Device Testing) can be your partner within the UL family; we can help you with testing services to fulfill the specific requirements for specific market entry. Our effective testing strategy will help you save valuable time and resources.

UL MDT can support you starting from the idea of your new medical device to far beyond market entry. We evaluate your production procedures, products or product components with internationally accepted biological, microbiological, virological, physical and chemical test methods. Besides our standardized test methods according to international standards and guidances we can also help you with custom test methods for special applications and products.

Our standardized testing services for all medical devices and ophthalmic products include the following:

- Production processes, raw and auxiliary materials;
- Biocompatibility, including comprehensive material characterization;
- Cleaning, disinfection and sterilization within the scope of reprocessing;
- Transport safety;
- Shelf life of devices and packages.

UL MDT is has an ILAC accreditation according to ISO/IEC 17025 and is GLP certified. Learn more about UL MDT non-clinical testing services at www.ul-mdt.com.



About UL - Medical Regulatory Advisory Services (MRAS):

UL's MRAS team provides qualified experts and assessors with experience in biocompatibility to support manufacturers in fulfilling this role. UL can assist medical device manufacturers with the justifications of the biological safety of their final products, components, or raw materials, and make overall biological safety conclusions by:

- Appropriately categorizing medical devices in terms of their type and duration of body contact,
- Selecting appropriate testing strategies and evaluation procedures in line with a risk management plan,
- Providing a rationale for the selection or waiving of tests,
- Assessing and interpreting biocompatibility data received from performed test reports as the testing institute UL MDT or other sources, and
- Reviewing scientific literature and clinical data.

With UL's Medical Regulatory Advisory Services team, you have a regulatory partner who can help you understand the specific requirements needed for market entry and also determine the most efficient path to save valuable time and resources along the way. Learn more about UL's MRAS services at www.ulmedicaladvisory.com.

To learn more about UL services to support global submissions, please contact us at Medical.Inquiry@ul.com or visit www.ul.com/medical.

Authors:

Jan Peeters, UL MDT,
Germany

Claudine Kos, UL MDT, US

Paul Malinowski, UL,
MRAS team, Germany



References

1. ISO 10993-1:2009, Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process
2. ISO 5832-1:2007, Implants for surgery - Metallic materials - Part 1: Wrought stainless steel
3. ASTM F139:2012, Standard Specification for Wrought 18Chromium-14Nickel-2.5Molybdenum Stainless Steel Sheet and Strip for Surgical Implants (UNS S31673)
4. ISO 5832-3:1996, Implants for surgery - Metallic materials - Part 3: Wrought titanium 6-aluminium 4-vanadium alloy
5. ASTM F 136:2013, Standard Specification for Wrought Titanium-6Aluminum-4Vanadium ELI (Extra Low Interstitial) Alloy for Surgical Implant Applications (UNS R56401)
6. Peeters J (2013) Physical and Chemical Characterization of Medical Device Materials, UL White Paper
7. ISO 10993-18:2005, Biological evaluation of medical devices - Part 18: Part 18: Chemical characterization of materials
8. ISO 10993-7:2008 Biological evaluation of medical devices - Part 7: Ethylene oxide sterilization residuals
9. Council Directive 93/42/EEC of 14 June 1993 concerning medical devices as amended by Directive 2007/47/EG
10. Council Directive of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices (90/385/EEC)
11. ISO 10993-2:2006, Biological evaluation of medical devices - Part 2: Animal welfare requirements
12. ISO 10993-10:2010, Biological evaluation of medical devices - Part 10: Tests for irritation and skin sensitization
13. ISO 10993-3:2014, Biological evaluation of medical devices - Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity
14. ISO/IEC 17025:2005, General requirements for the competence of testing and calibration laboratories
15. ISO/TIR 15499:2012, Biological evaluation of medical devices - Guidance on the conduct of biological evaluation within a risk management process
16. ISO 14971:2007, Medical devices - Application of risk management to medical devices
17. FDA Blue Book Memorandum # G95-1: Use of International Standard ISO 10993, Biological Evaluation of Medical Devices—Part 1: Evaluation and Testing”
18. FDA Draft Guidance (2013) Use of International Standard ISO 10993, "Biological Evaluation of Medical Devices Part 1: Evaluation and Testing"