ISO 10993-1 and Biocompatibility

Conducting a Biological Evaluation of a Medical Device



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ISO 10993-1 – How to Conduct a Biological Evaluation

In order to place a medical device on the market in many regulated countries, manufacturers must systematically evaluate the product's biological safety to avoid any risk of bio-incompatibility with the human body. This introduces the ISO 10993 series as an international standard recognized in Europe, the United States, and many countries around the world. Some countries like Japan use their own regulation (e.g. MHLW) whereas other countries have developed specific guidelines to properly implement the ISO 10993 series (e.g. FDA guidance Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process" in the US).

The ISO 10993 series provides guidelines and requirements for manufacturers to appropriately mitigate the biological risks to an acceptable benefit/risk level, including testing to confirm biocompatibility. The process supporting the biological evaluation is consequently highly related to the risk management process, and can lead to conducting a pre-clinical testing program through material characterization or testing. ISO 14971:2007[1] also includes guidance in Annex I regarding the risk analysis addressing biological hazards.

Each manufacturer should be aware of its responsibilities, and should be able to properly define a program of biological evaluation generally supported by external experts. This paper outlines the general principles of biological evaluation according to the ISO 10993 series and offers an overview of each phase, as well as the basics of implementing a testing program and interpreting the tests results. This paper also includes the relative timelines and costs related to biological evaluation.

Process of Biological Evaluation

Manufacturers of medical devices must document their process of biological evaluation for a specific device or a device family. Consequently, a biological evaluation plan is expected to support the medical device assessment in regards to biological characteristics, selection of materials, material characterization and verification of biological safety through a biocompatibility testing program. The plan should also include the responsibilities, technical competencies, and expertise of any individual(s) involved in the evaluation. The results of the testing program should be documented in a biological evaluation report.

The biological evaluation is not a process frozen in place once conducted:

- Any design or manufacturing process change should be justified regarding the device biocompatibility.
- Post-market surveillance may induce the review of biological evaluation when a risk has been detected.
- As the "state of the art" evolves (e.g. standard update or new testing method), manufacturers must document that their device biological evaluation, including their testing program, complies with the state of the art.

The following chart is an example of a biological evaluation process, including the interrelations with the ISO 10993-X standards and risk management process. The chart also includes sections and content that may be used to write a Biological Evaluation Plan, as well as a Biological Evaluation Report.

ISO 10993-1 – How to Conduct a Biological Evaluation

Biological Evaluation Plan		
Steps	**Inputs of Biological Evaluation**	**Content of Biological Evaluation Plan**
Responsibilities	• ISO 14971 - Risk Management Plan	• Definition of technical competencies • Definition of responsibilities and authorities for biological evaluation
Material Selection	-	• Presentation or relevant characteristics of candidate materials (physical, chemical, mechanical, electrical, etc.) for the biological evaluation • Presentation of clinical experience of candidate materials • Presentation of advantages and disadvantages of candidate materials • Selection of most appropriate materials
Material Characterization	• ISO 10993-18 • ISO 10993-17(when applicable) • ISO 10993-19 (when applicable) • ISO 10993-9 (and ISO 10993-13,-14,-15)(when applicable)	• Presentation of manufacturing process • Presentation of manufacturing materials and intended process residues, contaminants • Materials characterization and quantification • Leachable substance characterization and quantification • Characterization of relevant physical characteristics (porosity, shape, etc.) • Degradable materials characterization
Literature Review	• ISO 10993-1 (Appendix C) • ISO 14971 - Risk Analysis	• Presentation of existing toxicological data of materials characterized according to the intended use and exposure conditions, nature, and duration • Determination of all possible biological hazards and associated risk levels
Testing Selection	• ISO 10993-1 (Appendix A) • ISO 14971 - Risk Analysis	• Select the recommended testing (Appendix A) to conduct when the risk level is no appropriate for the intended use

Biological Evaluation Report		
Steps	**Inputs of Biological Evaluation Report**	**Content of Biological Evaluation Report**
Biological Testing	-	• Implementation of testing program
Biological Results	-	• Presentation of results refering to test reports • Discussion
Benefits/Risk Ratio	• ISO 14971 - Risk Management Report	• Confirmation that the risk analysis and risk control have been implemented • Conclusion on Benefit / Risk Ratio for biological hazards

Selection of Material

The biological evaluation process involves the selection of the most suitable materials during the design of medical devices, instruments, and/or accessories used for a defined application (medical purpose). The evaluation is therefore related to the device's indications for use and the performances claimed by the manufacturer.

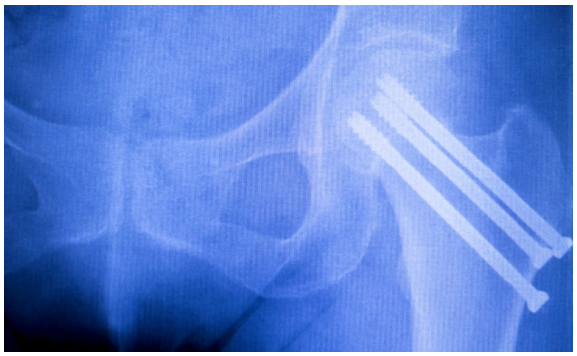
A review must be conducted and documented to determine whether the materials meet the requirements regarding device performance (e.g. elongation, lifetime, mechanical strength), biocompatibility (e.g. implantable, non-toxic), and the clinical suitability of materials for the application.

In consequence, the candidate materials should be determined according to their relevant characteristics (chemical, physical, electrical, mechanical, biological, etc.) and history of use. At this stage, it can be assumed that known materials are more adapted in terms of biocompatibility due to their long history of use, and their biological hazards are widely known. However, use of advanced technology applications like new materials may improve the device performance (e.g. mechanical strength, lifetime) and could be consequently important to consider in order to stay ahead of the competition. For the same application, innovative materials may have a limited history of use, and thus the mitigation of potential biological hazards may take longer and be more expensive than for better-known, well-established materials.

The final selection of materials for an application should be documented in the biological evaluation plan and all the materials should be clearly identified (complete identification, composition, supplier, part number, colorants, etc.).

There are multiple benefits to including a stage of material selection in your biological evaluation plan:

- Reduce the risks of bio-incompatibility for patients by using appropriate materials.
- Reduce the risks of biocompatibility testing failure during product development.
- Avoid testing due to the history of use (e.g. irritation testing may not be required for gloves made of a material known to be non-irritant).
- When the final device is made of well-known material(s) for an application (e.g. 316LVM stainless steel or Ti6Al4V titanium), FDA, Notified Bodies, or other international authorities may accept the biological risks with just a few tests or even with no tests due to the material's safe and long history of use. Examples include an implantable orthopedic screw made from Ti6Al4V or orthopedic guidewire made from 316L stainless steel. This may be especially important when the device is composed of a single material and with a known process of manufacturing.



Material Characterization

Though the medical device materials selected are defined, there is no assurance that the actual device is only composed of such materials due to supplier substances used in production. Indeed, the biological evaluation considers the impact of manufacturing processes and its potential residues or contaminants. All manufacturers should identify in their biological evaluation plans the manufacturing process as well as the manufacturing materials to establish a complete listing of substances that may be associated with the medical device during its use.

ISO 10993-1 requires an actual identification of device constituents and manufacturing residue through a chemical characterization according to ISO 10993-18. The tested medical device should have completed all steps of its manufacturing process to be considered a finished device, or must have a clear justification that supports the tested medical device is representative of the finished device.

ISO 10993-18 consists of qualitative and quantitative evaluations of materials present in the finished device through one of the appropriate testing methods listed in the standard. The substances detected are then compared with the "substances card." In light of the intended use, exposure conditions (invasiveness, duration), and dose, the substances detected must be justified clinically for their biocompatibility through a literature review, recognized standards, or supplemental testing. By this method, according to the dose, intended use, and exposure conditions, if a substance is clinically known as a non-irritant, then an irritation test may not be required for this substance due to the characterization.

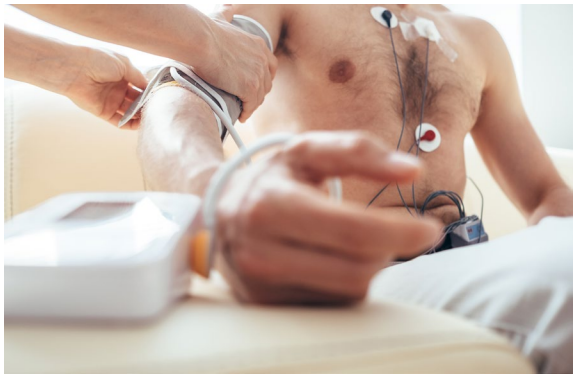
As well, depending on device features, other device characterizations have to be considered, if applicable:

- ISO 10993-9, if the device may be degraded during its lifetime
- ISO 10993-13, if the device includes polymers that may be degraded during its lifetime
- ISO 10993-14, if the device includes ceramics that may be degraded during its lifetime
- ISO 10993-15, if the device includes metals that may be degraded during its lifetime
- ISO 10993-19, if the device may have a physical effect that impacts the biocompatibility

Material Characterization

Many manufacturers do not perform the device characterization (according to ISO 10993-18) and perform all tests required by Appendix A of ISO 10993-1 (See Figure 1). By performing all the necessary tests of ISO 10993-1 Appendix A, they avoid having to justify the relevance of selected materials. The FDA, Notified Bodies, and other international authorities generally accept this approach. However, this leads to several inconveniences to the manufacturer:

- When a design change related to device materials (i.e. change of composition, supplier) is in process, the applicability of original testing data to the new device and/or the new materials may no longer be sufficient. Therefore, the writing of the associated rationale may be difficult and all applicable tests of ISO 10993-1 Appendix A have to be re-conducted to support the design change.
- Similarly, when the biocompatibility for a prototype device is tested, the equivalence with the final device is not obvious. Therefore, all applicable tests of ISO 10993-1 Appendix A may have to be re-conducted to support the equivalence.



Conducting the characterization according to ISO 10993-18 may avoid the repetition of biocompatibility tests according to ISO 10993-1 Appendix A. When a change is implemented or an equivalency must be demonstrated, a new characterization may be conducted on the new device to scientifically support the equivalence or to show that the change is minor.

Literature Review and Risk Management

During material selection, there is key information to define the history of use, or during the material characterization to evaluate the clinical relevance. Therefore a literature review may be conducted according to the suggested procedure in Appendix C of ISO 10993-1.

The review should be conducted for intended use and exposure conditions (e.g. invasiveness, duration of contact) and should conclude on the clinical relevance of materials. A manufacturer may focus the literature search in regards to the applicable biological hazards identified in Appendix A of ISO 10993-1. However, the search should also be designed to identify the other potential hazards known in the literature.

Examples of potential hazards: *allergy, irritation, inflammation, cytotoxicity, neurologic toxicity, carcinogenicity, haemo-incompatibility, genotoxicity.*

The biological evaluation plan has to be consistent with the risk management process. The risk analysis has to reference the potential biological hazards identified in the literature review, implement a mitigation plan, and conduct biocompatibility tests when deemed necessary to verify that hazards are properly reduced to an appropriate level. The FDA, Notified Bodies, or other international authorities are mindful that all biological hazards are identified in the risk analysis and correctly mitigated.

Testing Selection

The reduction of biological hazards to an appropriate level of risk may be implemented via the recommended tests listed in Appendix A of ISO 10993-1. When a sufficient level of pre-clinical or clinical evidence is available regarding the safety of applicable biological hazards, there may be no need to conduct any tests. However, a rationale supported by preclinical tests, literature review, or recognized standards needs to be generated that support the decisions made by the organization.

When sufficient clinical evidences are available to support the safety of some of biological hazards, the corresponding tests are not required, but the remaining biological hazards must be mitigated by appropriate tests. Otherwise, when the clinical evidence is too weak to justify the use of materials solely on a rationale, all recommended tests must be conducted.

Tests are selected according to the table in Appendix A of ISO 10993-1, shown in **Figure 1** below, depending on two criteria: the nature of contact and the duration of contact.

Manufacturers must take care to consider similarly direct contact (dressing on a wound, for example) and indirect contact (such as a breathing tube connected to an endotracheal tube). As well, manufacturers must take care to consider duration as a cumulative time of contact with the patient. For instance, considering a wound that heals in one month and that requires two changes of dressings, the contact duration is 30 days and not 10 days. The cumulative time of contact does not consider the number of devices used but the time necessary to achieve the expected performance (e.g. protection of the wound during the healing).

Taking into consideration both criteria, the testing program can be designed and must include testing protocols for each type of test performed.

Medical device categorization by			Biological effect							
Nature of body contact (see 5.2)		Contact duration* (see 5.3)	Cytotoxicity	Sensitization	Irritation or Intracutaneous Reactivity	Systemic toxicity (acute)	Subchronic toxicity (subacute toxicity)	Genotoxicity	Implantation	Haemocompatibility
Category	Contact									
Surface device	-	A	✓[a]	✓	✓					
		B	✓	✓	✓					
		C	✓	✓	✓					
	Mucosal membrane	A	✓	✓	✓					
		B	✓	✓	✓					
		C	✓	✓	✓		✓	✓		
	Breached or compromised surface	A	✓	✓	✓					
		B	✓	✓	✓					
		C	✓	✓	✓		✓	✓		

Testing Selection

Medical device categorization by			Biological effect							
Nature of body contact (see 5.2)		Contact duration* (see 5.3)	Cytotoxicity	Sensitization	Irritation or Intracutaneous Reactivity	Systemic toxicity (acute)	Subchronic toxicity (subacute toxicity)	Genotoxicity	Implantation	Haemocompatibility
Category	Contact									
External communicating device	Blood path, indirect	A	✓	✓	✓	✓				✓
		B	✓	✓	✓	✓				✓
		C	✓	✓		✓	✓	✓		✓
	Tissue/bone/ dentin	A	✓	✓	✓					
		B	✓	✓	✓	✓	✓	✓	✓	
		C	✓	✓	✓	✓	✓	✓	✓	
	Circulating blood	A	✓	✓	✓	✓				✓
		B	✓	✓	✓	✓	✓	✓	✓	✓
		C	✓	✓	✓	✓	✓	✓	✓	✓
Implant device	Tissue/bone	A	✓	✓	✓					
		B	✓	✓	✓	✓	✓	✓	✓	
		C	✓	✓	✓	✓	✓	✓	✓	
	Blood	A	✓	✓	✓	✓	✓		✓	✓
		B	✓	✓	✓	✓	✓	✓	✓	✓
		C	✓	✓	✓	✓	✓	✓	✓	✓

Figure 1: *Biological Evaluation Tests (source: ISO 10993-1:2009 - Appendix A)*
**A - limited (≤24 h)*
B - prolonged (>24 h to 30 d)
C - permanent (>30 d)

Note: FDA relies on the following guidance: Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process"; the table of tests is slightly different as presented in Figure 2.

Testing Selection

Medical device categorization by			Biological effect												
Nature of body contact															
Category	Contact	Contact duration*	Cytotoxicity	Sensitization	Irritation or Intracutaneous Reactivity	Acute Systemic Toxicity	Material-Mediated Pyrogenicity	Subacute/ Subchronic Toxicity	Genotoxicity	Implantation	Haemocompatibility	Chronic Toxicity	Carcinogenicity	Reproductive/ Developmental Toxicity#	Degradation@
Surface device	Intact skin	A	■	■	■										
		B	■	■	■										
		C	■	■	■										
	Mucosal membrane	A	■	■	■										
		B	■	■	■	O	O	O		O					
		C	■	■	■	O	O	■	■	O		O			
	Breached or compromised surface	A	■	■	■	O	O								
		B	■	■	■	O	O	O		O					
		C	■	■	■	O	O	■	■	O		O	O		
External communicating device	Blood path, indirect	A	■	■	■	■	O				■				
		B	■	■	■	■	O	O			■				
		C	■	■	O	■	O	■	■	O	■	O	O		
	Tissue+/bone/ dentin	A	■	■	■	O	O								
		B	■	■	■	■	O	■	■	■					
		C	■	■	■	■	O	■	■	■		O	O		
	Circulating blood	A	■	■	■	■	O		O		■				
		B	■	■	■	■	O	■	■	■	■				
		C	■	■	■	■	O	■	■	■	■	O	O		
Implant device	Tissue+/bone	A	■	■	■	O	O								
		B	■	■	■	■	O	■	■	■					
		C	■	■	■	■	O	■	■	■		O	O		
	Blood	A	■	■	■	■	O		O	■	■				
		B	■	■	■	■	O	■	■	■	■				
		C	■	■	■	■	O	■	■	■	■	O	O		

■ = *ISO 10993-1:2009 recommended endpoints for consideration*
O = *Additional FDA recommended endpoints for consideration*

Figure 2: *Biological Evaluation Tests (source: FDA guidance Use of International Standard ISO 10993-1, "Biological evaluation of medical devices - Part 1: Evaluation and testing within a risk management process" - Appendix A-1)*

Constraints for Testing Laboratories

The ISO 10993-1 standard requires the implementation of testing according to the recognized current and valid best laboratory and quality practices, e.g. Good Laboratory Practice (GLP) or ISO/IEC 17025, and the data must be evaluated by competent professionals. In consequence, biological testing is usually subcontracted to specialized entities or testing laboratories able to meet these requirements.

Biocompatibility tests may be conducted *in vitro* and/or *in vivo*. Therefore, some tests require the use of animals to provide the evidence of biological safety. When animals are involved in a study, the requirements from ISO 10993-2 regarding animal welfare are applicable. ISO 10993-2 establishes the ethical framework for using animals for experimental purposes; requires minimizing the number of animal tests by using alternative methods (e.g. literature searches); requires minimizing any pain, suffering, distress, and lasting harm caused to animals during experimental tests; and promotes a high standard of accommodation and care to safeguard the animal welfare.

Though a device should be used in its finished state for testing, biological tests are not generally conducted with the finished device "as is," but may be decomposed as necessary for the testing requirements. If the method constraints are too high or the device is too complex, device extracts or representative samples must be utilized. ISO 10993-12 provides a framework to prepare the appropriate samples of tests, when required.

Fortunately, manufacturers do not have to directly take these requirements into consideration except if they conduct testing themselves. But they must consider the feasibility of testing implementation according to the test standard when selecting their testing laboratory(ies) sites. In addition, manufacturers must consider ISO 10993-2, ISO 10993-12, and current recognized best laboratory practices (i.e. GLP, ISO 17025).

Communication with Testing Laboratories

The manufacturers should take care to properly select the testing method to obtain evidence of biocompatibility. Though ISO 10993-1 is recognized internationally, not all testing methods are internationally recognized (e.g. some of the ISO 10993 series in the EU or in Japan). Depending on the targeted markets, the manufacturer should discuss with their testing laboratory a potential adaptation of the test method to comply with the recognized standards (e.g. test method compliant with ISO 10993-3 as well as MHLW part 3 for an in vitro chromosomal aberration study to access EU and Japanese markets). When the adaptation cannot be done, individual tests must be implemented in compliance with each standard.

Additionally, various testing methods may be conducted to mitigate the same hazard for any biological concern or biocompatibility. For instance, maximization sensitization testing and Buehler testing are two methods addressing sensitization testing. However, depending on the medical device, its intended use, contact nature or duration, one or both may be more suitable due to its sensibility or testing constraints (e.g. sample size).

Similarly, a single test may be conducted to meet the requirements of multiple biological hazards or combinations of biocompatibility interactions. For instance, the risk of local effect after device implantation and the risk of systemic toxicity may sometimes be addressed through a single test compliant with ISO 10993-6 and ISO 10993-10.

In conclusion, due to the expense of biocompatibility tests, manufacturers must maintain good and interactive communication with their testing laboratories to define the most appropriate testing program for their devices.

Sample Preparation

Standard: ISO 10993-12

Though the samples are generally prepared by the testing laboratory, the manufacturer must understand the principles of preparation. A significant revision to the sample preparation standard was published recently (e.g. ISO 10993-12:2012) that may potentially impact a large range of testing already conducted. The manufacturer bears the accountability to measure the impact of new standard revisions, implement the associated action plans, or justify that current testing is still applicable.

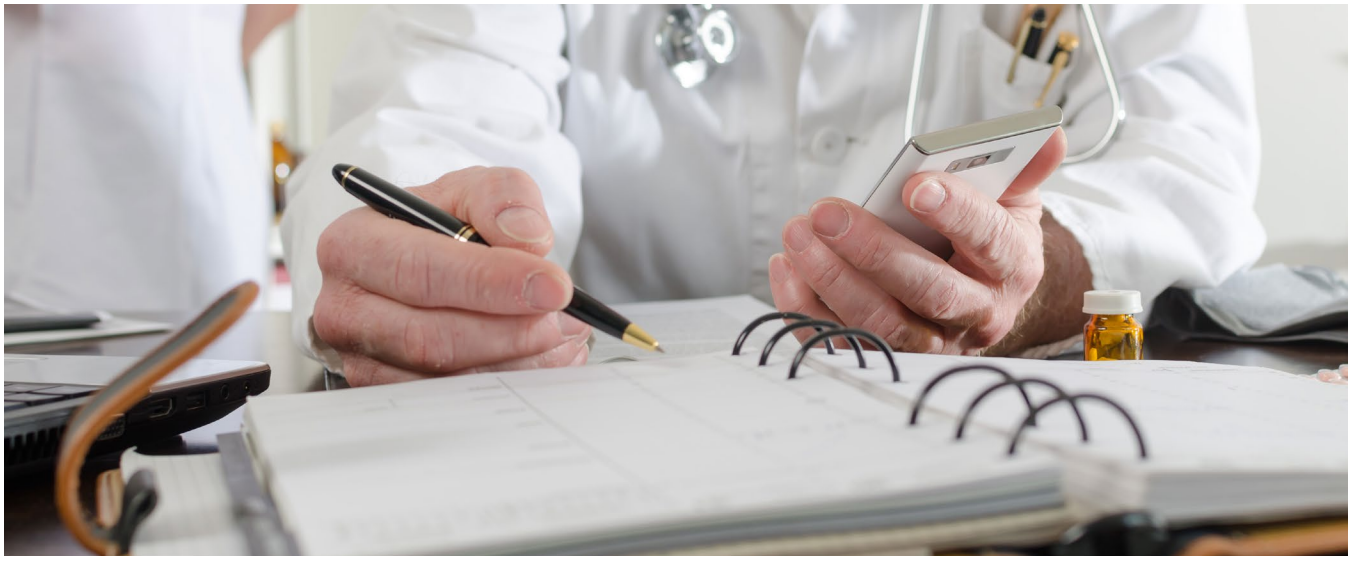
Extracts of device

This preparation is applicable when required by the test procedure. An extraction is used to collect the residues issued from a manufacturing process (e.g. oil or grease) or from raw materials. Under specific extraction conditions, the medical device is submerged in an extraction vehicle able to detach the residues. The extraction conditions must be justified in regards to the nature and use of the final device and the purpose of the test. The extract can then be used during the test procedure.

- Extraction conditions: 37±1°C for 72±2 hours; 50±2°C for 72±2 hours; 70±2°C for 24±2 hours; 121±2°C for 1±0.1 hours; or other conditions described and justified.
- Vehicle extraction: polar (e.g. NaCl) and non-polar (e.g. sesame oil) at specific justified ratio (e.g. 3cm²/mL or 0.2g/mL – see table 1 of ISO 10993-12)

Samples

This preparation is applicable when required by the test procedure. When the device cannot be used in its natural state (e.g. too large), samples must be created by cutting the original device or producing a sample representative of the original device. The sample must undergo the same manufacturing process (e.g. coating, sealing, cleaning, sterilization) to be considered equivalent to the original device. Similarly, each individual material must be represented proportionally in the sample.



Cytotoxicity

Standard: ISO 10993-5
Type: *In vitro*
Description: Cytotoxicity tests measure the effects of medical devices on cells (e.g. lysis, inhibition of cell growth, colony formation) through observations with a microscope. The test selection should consider the nature of the medical device (e.g. liquid, solid, gel).

Results: Qualitative evaluation (grade 0 to 4 respectively, from no reactivity to severe reactivity); Quantitative evaluation (reduction of cell viability by more than 30% is considered cytotoxic).

Examples of tests: Agar Overlay, MEM Elution, Direct Contact, MTT cytotoxicity, Colony formation cytotoxicity, Neutral Red Uptake (NRU) cytotoxicity.

Sensitization

Standard: ISO 10993-10
Type: *In vivo*
Description: Sensitization tests measure the effects of medical devices on sensitization of contact (e.g. allergic or sensitization reactions). The tests consist of an induction phase to make an animal sensitive and a challenge phase by placing the extract or solution in contact with the skin. The animals are observed compared to control group(s) to score the delayed allergic response.

Results: The observations are scored between 0 (no visible change) to 3 (intense erythema and swelling). More than grade 0 indicates sensitization.

Examples of tests: Buehler Sensitization Method, Local Lymph Node Assay (LLNA), Maximization (Magnus-Kligman).

Irritation

Standard: ISO 10993-10
Type: *In vitro* or *in vivo*

Note: for products where humans are highly exposed and where animal testing is not relevant, the standard ISO 10993-10 recommends testing on human skin.

Description: Irritation tests evaluate the risk of irritation when skin, eye, or mucous membranes come in contact with the medical device. Usually, a medical device, its sample, or its extract is applied on rabbit skin and the skin reaction is scored (edema, erythema) at 24, 48, and 72 hours.

Alternatively when medical devices are implanted or have contact with blood, the intracutaneous reactivity is measured by injecting an extract to determine the local reaction of tissues.

Results: The test result obtained is a score between 0 and 8 calculated from the various observations, considered as negligible (0-0.4), slight (0.5-1.9), moderate (2-4.9), or severe (5-8).

Examples of tests: Transcutaneous electrical resistance (TER), EPISKIN study, Primary Skin Irritation, Intracutaneous Reactivity, Ocular Irritation, Oral Mucosal Irritation, Vaginal Irritation.

Acute/Subacute toxicity

Standard: ISO 10993-1
Type: *In vivo*
Description: Acute/subacute toxicity tests are conducted to mitigate the risk of potential absorption of toxic leachable and degradation products during a period of less than 24 hours (acute systemic toxicity), and during a period not less than 24 hours and until 28 days (subacute toxicity). Usually, the test consists of a single injection (acute toxicity) or repeated injections (subacute toxicity) of extract in rats (e.g. intravenous, intraperitoneal according to the intended clinical route) to determine the toxic impact on the remote organs at various checkpoints. The evaluation is made through measuring animal weight and clinical observations (e.g. change in skin, respiratory, mortality).

Note: The animal model is selected depending on the medical device type and intended use. The dose is calculated with a safety factor and the maximum dose is determined according to the standard or literature for a type of animal model.

Results: A review of observations (e.g. lesions, change of body or organ weight, clinical pathology, gross pathology, histopathology) is made and recognized, and accepted statistical methods are used to conclude on the toxicity.

Examples of tests: Acute systemic toxicity test and Subacute Toxicity.

Sub-chronic / chronic toxicity

Standard: ISO 10993-11
Type: *In vitro* or *in vivo*
Description: Sub-chronic and/or chronic toxicity tests are conducted to mitigate the risks of potential accumulation of chemicals in tissues. The tests are implemented during:

- a period not less than 10% of life-span of animal model[2] (subchronic toxicity) and
- a major period of the life-span of the animal model[3] (chronic toxicity).

The purpose is to determine the toxicological mode of actions and toxic effect of medical device chemicals on organs when injected by the intended clinical route. Usually, the test consists of repeated injections of extracts in rats (e.g. intravenous, intraperitoneal according to the intended clinical route) to determine the toxic impact on the remote organs at various checkpoints. The evaluation is made through animal weight and clinical observations (e.g. change in skin, respiratory, mortality).

Note: The animal model is selected depending on the medical device type and intended use. The dose is calculated with a safety factor and the maximum dose is determined according to the standard or literature for a type of animal model.

Results: A review of observations (e.g. lesions, change of body or organ weight, clinical pathology, gross pathology, histopathology) is made and recognized, and accepted statistical methods are used to conclude the systemic toxicity.

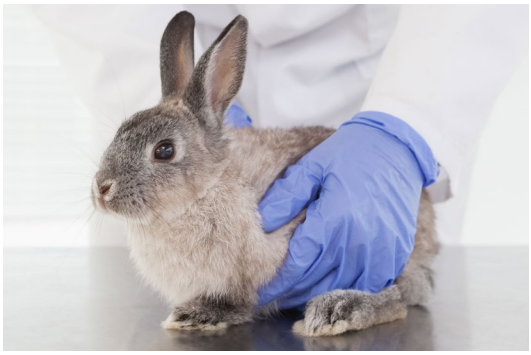
Example of tests: Subchronic Toxicity and Chronic Toxicity.

Pyrogenicity

Standard: ISO 10993-11
Type: *In vitro* and *in vivo*
Description: Pyrogenicity test through ISO 10993-11 is conducted to mitigate the risk of material-mediated[4] pyrogenic response. This test is required when the device is composed of material(s) that may be known to potentially induce pyrogenic responses. Otherwise, the test does not need to be considered. It should be noted that commonly sterile products must have pyrogenicity testing performed, as these are typically placed into the body or come in contact with the bloodstream.

When a risk assessment indicates potential presence of non-endotoxin pyrogen, it may be more appropriate to use the rabbit pyrogen test. When the risk assessment indicates potential presence of endotoxin pyrogen test, it may be more appropriate to use the LAL test.

For combined products with drugs or long-term implantable devices, pyrogenicity tests in rabbits are usually conducted according to USP Chapter <151> and consist of an injection of extract in the ear vein of three rabbits to observe the temperature rise.



Otherwise, a pyrogenicity test is usually conducted according to USP Chapter <85> and/or AAMI ST72 with LAL (Limulus amebocyte lysate) that can coagulate when in contact with bacterial endotoxin.

Results:
***In vivo* rabbit test:**
The medical device is non-pyrogenic when the temperature of three rabbits does not rise above 0.5°C. If one rabbit increases its temperature beyond 0.5°C, five new rabbits are tested. The medical device is non-pyrogenic if not more than three rabbits (out of eight) increase their temperatures beyond 0.5°C and if the sum of temperatures rises does not exceed 3.3°C.

***In vitro* LAL test:**
The acceptance limits are described in the USP Chapter <161> and depend on the intended use and contact type. For instance, 20EU/device is the limit for products that directly or indirectly contact the cardiovascular system and lymphatic system. 2.15EU/device is the limit for products in contact with cerebrospinal fluid.

Examples of tests: Pyrogenicity test in rabbits; LAL test

Implantation

Standard: ISO 10993-6
Type: *In vivo*
Description: An implantation test is conducted to mitigate the risk of local intolerance after medical device implantation. The test consists of an implantation by surgical procedure in an appropriate number of animals. The animal model is selected based on the implant type and size, intended duration of test, and biological animal responses.

Special considerations must be taken into account for degradation products by assessing the local tolerance at the beginning of the degradation, when the degradation is taking place and when a steady state has been reached. The macroscopic and histopathologic responses are evaluated and documented in functions of time by comparing the results obtained from the medical device with those obtained from a control sample. When feasible, acute systemic toxicity tests may be combined with subacute, subchronic toxicity, and implantation test protocols.

Results: Various scoring systems may be used and are proposed in Appendix E of ISO 10993-6 or in the literature. Usually, for the scoring described in Appendix E.3, results are considered as non-irritant (0.0 to 2.9), slightly irritant (3.0 to 8.9), moderately irritant (9.0 to 15.0), or severely irritant (>15).

Examples of tests: Intramuscular, subcutaneous, or bone implantations, etc.

Haemocompatibility

Standard: ISO 10993-4
Type: *In vitro* or *in vivo*
Description: Haemocompatibility tests are conducted to mitigate the risk of medical device intolerance with blood. In vitro testing with human blood is preferred, but in vivo testing should be considered for medical devices intended to be in contact with blood for prolonged, repeated, or permanent exposure. The standard provides a list of tests to implement according to the type of medical device. The tests are designed to evaluate the risks of thrombosis as well as the impact on platelets, coagulation, hematology, and complementary systems.

According to the type of test conducted, the appropriate animal models must be chosen and justified. For instance, a mechanical cardiac valve should be tested for thrombosis (e.g. occlusion percentage test) and hematology (e.g. hemolysis test).

Note: Considering a device made of materials already known for the intended use, FDA recommends the implementation of hemolysis, complement activation, and thrombogenicity tests for direct blood-contacting devices and hemolysis tests only for indirect blood-contacting devices.

Examples of tests: Hemolysis, PTT, Complement Activation, Dog/Sheep Thrombogenicity, etc.

Genotoxicity

Standard: ISO 10993-3
Type: *In vitro* or *in vivo*

Note: Manufacturer must carefully select the genotoxicity strategy. While the ISO 10993-3:2014 has been applied in EU since January 1st, 2016, the ISO 10993-3:2003 (FDA Recognition Number 2-175) is still the recognized standard in US.

Description: Genotoxicity tests are conducted to mitigate the risk of gene mutations, chromosome structure, and other DNA or gene toxicities caused by medical devices. In vitro tests are firstly preferred according to various methods (OECD guidelines). In vivo tests may be conducted when an in vitro test fails (ISO 10993-3:2003 approach).

The ISO 10993-3:2014 also introduces the implementation of an in vivo study and a follow-up evaluation if one or more in vitro tests are positive. Moreover, the ISO 10993-3:2014 adds requirements regarding the sample preparation with three proposed methods: direct method for solution and suspension (A), extract method according to ISO 10993-12 (B), or exaggerated extract (C). The appropriate method should be selected in regards to the composition of the medical device.

Special attention: Genotoxicity is evaluated through either two or three in vitro tests according to the strategy selected that essentially depends on their recognition by the countries where the device will be marketed. For instance, the US recognizes the implementation of three tests to clear a medical device (e.g. Ames test, chromosomic aberration in mammalian cells test, and micronucleus study) whereas Europe recognizes the implementation of two tests to clear the medical device (e.g. Ames test, chromosomic aberration in mammalian cells test). Similarly, Japan recognizes MHLW part 3 to conduct genotoxicity tests.

Examples of tests: Ames Mutagenicity, Chromosomal Aberration, Mouse Lymphoma, Mouse Micronucleus, etc.

Other

When detected in the literature (e.g. toxicity for reproduction, carcinogenicity), other potential biological hazards are considered by the ISO 10993 series and should be evaluated during the risk management process.

Biological Testing - Costs and Timeline

The following table details the estimated costs for some specific scenarios as well as for specific testing. The range of estimation depends on testing laboratory choice, type of device, selection of appropriate testing (according to testing sensibility required, type of device, etc.), and laboratory practices used (e.g. ISO 17025, GLP).

Note: The duration of in vivo testing requiring implantation is usually long (acute systemic toxicity ">24hours", subacute toxicity "24h-28 days", subchronic toxicity in rats "90 days", chronic toxicity in rats "6 months"), therefore the cost is quite high.

Biological Evaluation	Estimated Cost	Estimated Timeline
Short term contact device	~8,000 – 10,000€ (9,100 – 11,400$)	~2 months
Device (>24h to 30 days) with new materials	~60,000€ (68,200$)	~6 - 15 months
Implantable device (>30 days) with well-known materials	~10,000 – 20,000€ (11,400 – 22,800$)	~6 months
Implantable device (>30 days) with new materials	~150,000€ (170,000$)	~15 months
In detail:		
Cytotoxicity test	~150 - 700€ (170 - 800$)	~5 - 30 days€
Sensitization	~5,000-8,000€ (5,700-9,100$)	~25-80 days
Irritation	~500-2,000€ (570-2,280$)	~20-40 days
Genotoxicity (Ames)	~4,000€ (4,550$)	*Testing program timeline detailed above is not affected by the following tests*
Genotoxicity (Chromosomic Aberration)	~20,000€ (22,800$)	
Genotoxicity (Micronucleus)	~20,000€ (22,800$)	
Haemocompatibility	~1,500-15,000€ (1,700 – 17,000$)	
Carcinogenicity	~150,000€ (170,000$)	
Toxicity for reproduction	~150,000€ (170,000$)	

Table 1: Costs and Timelines

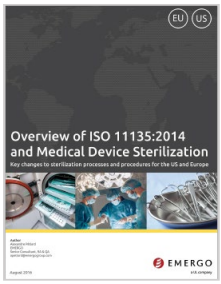
Conclusion

The evaluation of biological safety is a huge process that begins during a device's design stage. It involves the review of clinical literature, review of technical literature, and applicable standards, as well as the risk management process.

A biological evaluation plan and report should document the material selected for the intended use, and the implementation of testing programs.

Fortunately, manufacturers usually rely on the expertise and experience of testing laboratories to define and implement appropriate testing programs and make the suitable rationale supporting the acceptability of test results. However, manufacturers must have sufficient knowledge to establish the requirements of testing as well as to review the testing results and conclusions since they are accountable for the testing program and approval of the test results.

The biological evaluation is not a one-time action, but must be reviewed regularly for suitability when a change is implemented, a standard is revised, or when the results of post-market surveillance affect the biological risks.



Learn more about medical device testing standards

If you enjoyed this white paper, our white paper about medical device sterilization standard ISO 11135 may be useful to you. We discuss ISO 11135:2014, the most recent version of the standard, and key changes to medical device sterilization procedures and processes in the US and Europe.

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References:

1 ISO 14971:2007 "Medical devices — Application of risk management to medical devices"

2 90 days in rats

3 6 months in rats

4 Pyrogenic response may be material-mediated, endotoxin-mediated or mediated by other substances (e.g. gram-positive bacteria). The endotoxin (gram-negative bacteria) contamination is generally due to the manufacturing process and is tested by LAL (Limulus Amebocyte Lysate) test (AAMI/ST72).