ISO 10993-1 and Biocompatibility

Conducting a biological evaluation of a medical device

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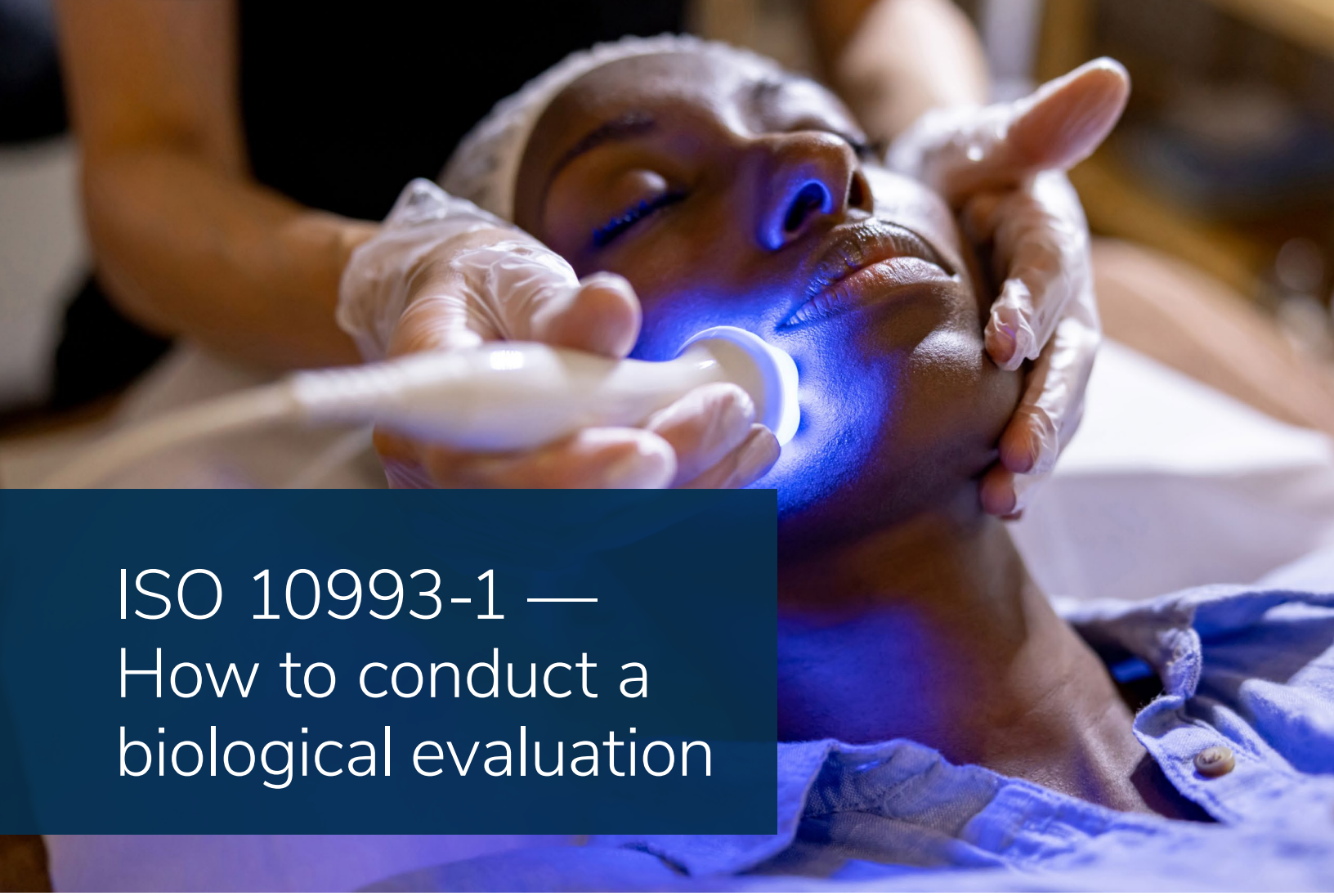


ISO 10993-1 — How to conduct a biological evaluation

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 establish their own standards, e.g., JIS T 0993-1, whereas other countries have developed specific guidelines to implement the ISO 10993 series, e.g., US Food and Drug Administration (FDA) guidance Use of International Standards ISO 10993-1, "Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing Within a Risk Management Process".

The ISO 10993 series provides guidelines and requirements for manufacturers to appropriately assess the biological safety of a medical device, 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. Biological risks should be addressed in alignment with ISO 14971.[1]

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 test results. This paper also includes relative timelines related to biological evaluation.

Process of biological evaluation

Manufacturers of medical devices must document their process of biological evaluation for a specific device or device family. Consequently, a biological evaluation plan is expected to support a medical device assessment regarding 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 testing program results should be documented in a biological evaluation report.

Analogous to a quality management system or risk management system, biological evaluation is an ongoing process. The overall biological safety of a medical device and its materials should be re-evaluated via a biological risk assessment in the event of a change, such as:

- Change in material (e.g., source, specification) or manufacturing process (e.g., formulation, processing, packaging, sterilization).
- Change in intended use or storage instructions/requirements (e.g., transport, shelf life).
- Data indicating an adverse biological effect when in human use (e.g., post-market surveillance data).

Additionally, manufacturers often market their device where the state of the art is to be taken into account. As the state of art evolves (e.g., revisions to standards, new test methods), manufacturers must assess whether their biological evaluation and testing program complies with the state of the art, and document it.

Biological evaluation plan and report

Biological evaluation is a form of risk management. In some markets, like the EU, a biological evaluation plan is required and considered part of the device's risk management plan. The following table summarizes the potential elements of a biological evaluation plan.

Description	Potential content of biological evaluation plan
Responsibilities	• Definition of technical competencies • Definition of responsibilities and authorities for biological evaluation • CVs of responsible individuals
Description of device and manufacturing process	• Description of device, e.g., intended use, device lifetime, shelf life • Representative drawing/image of device • Identification of device variants • Presentation of materials of construction (identity, manufacturer, contact type and duration) • Presentation of manufacturing process • Presentation of manufacturing materials and intended process residues, contaminants • Device category per ISO 10993-1, Clause 5.2 (Nature of contact) and 5.3 (Duration of contact) • Relevant biological endpoints per ISO 10993-1, Annex A • Relevant biological endpoints per FDA guidance (as applicable)
Biological risk estimation	• Relevant health-based endpoints, e.g., no observed adverse effect level (NOAEL), tolerable intake (TI), tolerable exposure (TE), margin of safety (MOS) • Literature review results for known toxicological hazards of device and manufacturing materials (ISO 10993-1, Annex C)
Biological risk analysis	• Biological risk analysis of patient-contacting raw materials • Biological risk analysis of manufacturing process • Review existing biocompatibility studies — e.g., test method, extraction conditions, results — as applicable, including chemical, toxicological, analyses • Gap analysis of existing biocompatibility studies to identify and address any potential gaps between current version of standard and version applied for testing • Review existing clinical and PMS data as applicable
Biological risk evaluation/risk control	• Determination to conduct chemical characterization testing (ISO 10993-18) • Recommended biological endpoints (testing) per ISO 10993-1, Annex A • Rationale for omitted testing/biological endpoints • Select representative device(s) for testing • Summarize conditions to reassess biological risk

Table 1: Potential Content of a Biological Evaluation Plan

A biological evaluation report summarizes the findings of the biological evaluation. While the report may duplicate information from the biological evaluation plan, the biological evaluation report may also include the following, as provided in Table 2.

Description	Potential content of biological evaluation report
Description of device and manufacturing process	• General description or representative drawing/image of device • Presentation of materials composition/formulations • Information on physical characteristics of device components • Presentation of manufacturing process • Presentation of manufacturing materials and intended process residues, contaminants
Endpoint assessment	• Presentation of existing data, newly conducted additional testing or rationale for why additional testing was not warranted • Discussion/assessment of data
Conclusion	• Confirmation that risk analysis and risk controls have been implemented • Conclusion on biological safety of device under evaluation with consideration for contact type and duration

Table 2: Potential Content of a Biological Evaluation Report

Material selection and characterization

The design process involves selecting the most suitable materials when developing medical devices, instruments and/or accessories for a defined application (intended use). 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, nontoxic; and the clinical suitability of materials for the application.

Consequently, candidate materials should undergo assessment according to their relevant characteristics (chemical, physical, electrical, mechanical, biological, etc.) and history of use. At this stage, one can assume that known materials are more suitable than novel ones in terms of biocompatibility due to their long history of use, and their potential biological hazards are widely known. However, using advanced technology applications like new materials may improve the device's performance — e.g., mechanical strength, lifetime — and could be important to consider. For the same application, innovative materials may have a limited history of use; thus, mitigating potential biological hazards may require more time and financial investment than 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.).

Though the medical device materials selected are defined, there is no assurance that the actual device is only composed of such materials due to substances used in production. Indeed, biological evaluation considers the impact of manufacturing processes and their potential residues or contaminants. All manufacturers should identify 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.

Characterization

ISO 10993-1 requires an actual identification of device constituents and manufacturing materials. If a material characterization is performed, it is to be performed in accordance with ISO 10993-18 (ISO/TR 10993-2 applies if nanomaterials are involved). The tested medical device should have completed all steps of its manufacturing process to be considered a finished device, or the manufacturer must have a clear justification that supports that the tested medical device is representative of the finished device.

ISO 10993-18 provides a stepwise process for conducting a chemical characterization, starting with a description of the medical device — e.g., intended use — and material data such as composition (identity, nature of contact); proportion, e.g., by surface area or weight; physical structure, e.g., surface properties; and geometric distribution (configuration). If this information can demonstrate material/chemical equivalence to an equivalent clinically established device, no further testing or analysis may be required for some markets, like the EU. Note that in the US, this is generally only considered acceptable if the equivalent device is manufactured by the same manufacturer using the same process. Otherwise, the hypothetical worst-case chemical release — i.e., the entire device composition transferred during clinical use — is established and assessed. If acceptable when compared with an established safety threshold, the chemical characterization process is complete. However, if potentially unacceptable, the process can continue with an extraction study for a toxicological risk assessment (ISO 10993-17) and potentially a leachables study. Alternatively, a biological endpoint evaluation (ISO 10993-1, Annex A) may be appropriate if additional characterization is unlikely to provide further utility.

Depending on device features, other device characterizations are to be considered, including:

- ISO 10993-9 if the device may degrade during its lifetime
- ISO 10993-13 if the device includes polymers that may degrade during its lifetime
- ISO 10993-14 if the device includes ceramics that may degrade during its lifetime
- ISO 10993-15 if the device includes metals that may degrade during its lifetime
- ISO/TR 10993-19 if the device may have a physical effect that impacts its biocompatibility
- ISO/TR 10993-22 if the device includes particulate degradation products such as nanomaterials

Literature review and risk management

Obtaining physical and chemical data regarding material characterization is an initial step in the biological evaluation process, as demonstrated in Figure 1 of ISO 10993-1. Such material data may be obtained from sources including suppliers, internal data and literature.

ISO 10993-1, Annex C, suggests a literature review procedure. The literature review should account for the intended use and exposure conditions — e.g., nature and duration of contact — of the medical device and have clearly identified objectives, e.g., identify known biological and toxicological risks. Chemical characterization data (ISO 10993-18) may also be obtained by reviewing the chemical literature, e.g., ToxPlanet, ChemFinder. Note that in the US, this may be skipped, as the FDA rarely allows justifications and, generally, testing is required.

The biological evaluation plan is to be consistent with the risk management process. The risk analysis should identify physical and chemical material properties relevant to biological safety, estimate risk from exposure and, when necessary, implement mitigation such as a design change or assess biological endpoints (via existing data, endpoint-specific testing or rationale for not testing). European Notified Bodies, the US FDA and other regulatory authorities are mindful that all biological hazards are identified in the risk analysis and correctly mitigated.

Endpoint-specific testing

The reduction of biological hazards to an appropriate level of risk may be implemented via endpoint-specific testing, for which a framework is provided in Annex A of ISO 10993-1. If existing data are sufficient to demonstrate that risks are acceptable, additional biological safety testing may not be necessary in some regions.

Physical and chemical information is considered prerequisite information for risk assessment. If testing is necessary to obtain such data, then it is to be conducted in accordance with ISO 10993-18. The remaining endpoints are to be selected for evaluation according to the table in Annex A of ISO 10993-1, shown in Table 3 below, depending on two criteria: the nature and duration of contact.

Manufacturers must take care to consider direct contact, e.g., dressing on a wound, and indirect contact, e.g., a breathing tube connected to an endotracheal tube. Also, manufacturers must consider duration as the cumulative time of contact with the patient. For instance, for a wound intended to heal in one month and requiring two dressing changes, the contact duration is 30 days, not 15 days. The cumulative contact time does not consider the number of devices used but the accumulated time necessary to achieve the expected performance, i.e., wound protection during healing.

Some limited-exposure medical devices having transitory contact with the body, e.g., less than one minute, may not require biocompatibility testing. However, cumulative use and any materials — e.g., lubricants, coatings — that may remain following device contact require a more detailed assessment. Additionally, if more than one contact duration may apply, a more rigorous biological evaluation shall apply.

Considering both criteria, biological evaluation endpoints can be selected for evaluation in the risk assessment. Endpoints may be addressed through existing data, additional testing or a rationale for why additional testing is not warranted. Variations from the framework in ISO 10993-1, Annex A, should be justified. Device-specific standards addressing biocompatibility should also be considered.

Medical device categorization by			Endpoints of biological evaluation												
Nature of body contact		Contact duration*	Physical and/or chemical information	Cytotoxicity	Sensitization	Irritation or intracutaneous reactivity	Material-mediated pyrogenicity	Acute systemic toxicity	Subacute toxicity	Subchronic toxicity	Chronic toxicity	Implantation	Hemocompatibility	Genotoxicity	Carcinogenicity
Category	Contact														
Surface device	Intact skin	A	X	✓	✓	✓									
		B	X	✓	✓	✓									
		C	X	✓	✓	✓									
	Mucosal membrane	A	X	✓	✓	✓									
		B	X	✓	✓	✓		✓	✓			✓			
		C	X	✓	✓	✓		✓	✓	✓	✓	✓		✓	
	Breached or compromised surface	A	X	✓	✓	✓	✓	✓							
		B	X	✓	✓	✓	✓	✓	✓			✓			
		C	X	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
External communicating device	Blood path, indirect	A	X	✓	✓	✓	✓	✓					✓		
		B	X	✓	✓	✓	✓	✓	✓				✓		
		C	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	Tissue/bone/ dentin	A	X	✓	✓	✓	✓	✓							
		B	X	✓	✓	✓	✓	✓	✓			✓		✓	
		C	X	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
	Circulating blood	A	X	✓	✓	✓	✓	✓					✓	✓	
		B	X	✓	✓	✓	✓	✓	✓			✓	✓	✓	
		C	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Implant device	Tissue/bone	A	X	✓	✓	✓	✓	✓							
		B	X	✓	✓	✓	✓	✓	✓			✓		✓	
		C	X	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
	Blood	A	X	✓	✓	✓	✓	✓				✓	✓	✓	
		B	X	✓	✓	✓	✓	✓	✓			✓	✓	✓	
		C	X	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Table 3: Endpoints/Tests to Address in a Biological Risk Assessment (source: ISO 10993-1:2018, Table A.1)

X = Prerequisite for risk assessment (not required for US)
✓ = Endpoint to be evaluated
*A = Limited (≤ 24 h)
B = Prolonged (> 24 h to 30 d)
C = Long-term (> 30 d)

Note: The US FDA relies on the guidance, Use of International Standards ISO 10993-1, "Biological Evaluation of Medical Devices - Part 1: Evaluation and Testing Within a Risk Management Process" which is based on ISO 10993-1:2009.

Medical device categorization by			Biological effect											
Nature of body contact		Contact duration*	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	■	■	■										
		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		

Table 4: Biological Evaluation Endpoints (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" issued Sept. 4, 2020, Table A.1

■ = ISO 10993-1:2009 recommended endpoints for consideration (FDA guidance is based on ISO 10993-1:2009)
O = Additional FDA-recommended endpoints for consideration
*A = Limited (≤ 24 h)
B = Prolonged (> 24 h to 30 d)
C = Permanent (> 30 d)

Testing laboratories

Constraints for testing laboratories

The ISO 10993-1 standard requires testing according to the recognized current laboratory and quality practices, e.g., Good Laboratory Practice (GLP) per 21 CFR Part 58 or ISO/IEC 17025, and competent professionals must evaluate the data. Consequently, biological testing is usually subcontracted to specialized entities or testing laboratories that can meet these requirements.

Biocompatibility tests may be conducted *in vitro* and/or *in vivo*. Therefore, some tests require the use of animals to provide evidence of biological safety. When animals are involved in a study, the requirements from ISO 10993-2 regarding animal welfare apply. 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 pain, suffering, distress and lasting harm caused to animals during experimental tests; and promotes a high standard of accommodation and care to safeguard the animals' welfare.

Though a device should be used in its finished state for testing, biological tests are not generally conducted with the finished device as such. Instead, they 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 for tests when required.

Fortunately, manufacturers do not have to take these requirements directly into consideration unless they conduct testing themselves. However, they must consider the feasibility of implementation according to the test standard when selecting testing laboratories. Manufacturers must also consider ISO 10993-2, ISO 10993-12 and currently recognized best laboratory practices, i.e., GLP, ISO/IEC 17025.

Communication with testing laboratories

Manufacturers should take care to properly select the test method to obtain evidence of biocompatibility. Though ISO 10993-1 is internationally recognized, not all testing methods are — e.g., some of the ISO 10993 series in the EU or Japan. Depending on the target markets, the manufacturer should discuss with their testing laboratory a potential adaptation of the test method to comply with the recognized standards. When adaptation is impossible, individual tests — e.g., Japan's MHLW guinea pig maximization sensitization test — must be implemented in compliance with each national standard.

Additionally, various testing methods may be used to mitigate the same hazard for a biological concern or biocompatibility. For instance, maximization sensitization testing and Buehler testing both address 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 assess and evaluate multiple biological hazards or combinations of biocompatibility interactions. For instance, the risk of local effects after device implantation and the risk of systemic toxicity may sometimes be addressed through a single test compliant with both ISO 10993-6 and ISO 10993-11.

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 and reference materials

Standard: ISO 10993-12:2021

Though the testing laboratory generally prepares the samples, the manufacturer must understand the principles of sample preparation, inclusive of sample selection and extract preparation.

Test sample selection and preparation
Testing is to be performed on the final device, representative samples, materials processed in the same manner as the final device and appropriate extracts. Any materials introduced through the manufacturing process, intentional or not, should be considered in the test sample.

When the device cannot be used in its natural state, e.g., it's too large, samples must be created by cutting the original device or producing a sample representative. The sample must undergo the same manufacturing process — e.g., coating, sealing, cleaning, sterilization — to be considered equivalent to the original device. If possible, portions of the medical device without patient contact should be excluded from sample extracts.

Extracts of device
This preparation is applicable when required by the test procedure. Extraction is used to collect the residues issued from a manufacturing process, e.g., oil or grease, or released from raw materials or a medical device. Under specific extraction conditions, the medical device is submerged in an extraction vehicle that can detach the residues. The extraction conditions must be justified regarding 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 24±2 hours; 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 hour; or other conditions described and justified
- Extraction vehicles: Polar, e.g., NaCl, and nonpolar, e.g., sesame oil, at a specific justified ratio, e.g., 3 cm²/mL or 0.2 g/mL; see Table 1 of ISO 10993-12

Extraction conditions (time/temperature) and vehicle(s) should simulate exaggerated exposure when possible.

Biological endpoint testing

Cytotoxicity

Standard: ISO 10993-5:2009

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, from no reactivity to severe reactivity, respectively); quantitative evaluation (reduction of cell viability by more than 30% is considered cytotoxic)

Examples of tests: Agarose overlay, MEM elution, direct contact, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromid) cytotoxicity, colony formation cytotoxicity, neutral red uptake (NRU) cytotoxicity

Sensitization

Standard: ISO 10993-10:2021

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 wherein the extract or solution is placed in contact with the skin. The animals are observed and compared to control group(s) to score the delayed allergic response.

Results:
***In vivo* LLNA test:**
Radioactivity in mouse lymph node cells (counts/min, cpm) is converted to disintegration per minute (dpm). The stimulation index (SI) is determined by dividing mean test dpm by blank dpm; an SI of > 3.0 indicates a sensitizer.

***In vivo* Buehler, GPMT test:**
The observations are scored from 0 (no visible change) to 3 (intense erythema and swelling); Grade 1 or greater generally indicates sensitization.

Examples of tests: Closed patch (Buehler test), murine local lymph node assay (LLNA), guinea pig maximization test (GPMT, Magnusson-Kligman)

Irritation

Standard: ISO 10993-23:2021 or ISO 10993-10:2021

Type: *In vitro* or *in vivo*

Note: It is recommended to verify the recognition status of ISO 10993-23 by the relevant regulatory authorities in the markets of interest.

Description: ISO 10993-23:2021 separates irritation tests from ISO 10993-10:2021 and describes a stepwise approach to chemical characterization, literature review (ISO 10933-1), *in vitro* testing using reconstructed human epidermis (RhE), *in vivo* animal testing and human testing. Irritation tests evaluate and categorize the potential to cause irritation.

To determine the potential for skin irritation, 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 intended to contact breached or compromised surfaces, external communicating or implanted intracutaneous reactivity is measured by injecting an extract to determine the local reaction of tissues. The RhE method may be suitable instead of the irritation by skin exposure or intracutaneous test methods.

Results: *In vitro* test results with RhE are determined by measuring the reduction of cell viability via MTT; if mean tissue viability is < 50% in at least one extraction vehicle, it is considered an irritant.

In skin irritation (primary or cumulative), the test result obtained is a score between 0 and 8, calculated from the various observations. These scores indicate negligible (0-0.4), slight (0.5-1.9), moderate (2-4.9) or severe (5-8) irritation.

Intracutaneous reactivity (edema, erythema) is scored at 24, 28 and 72 hours. The test result is a score between 0 and 8, calculated from the various observations. An overall mean score of 1.0 or less is considered a non-irritant in RhE testing.

Examples of tests: RhE model, skin irritation, intracutaneous reactivity, human skin irritation, special irritation tests, e.g., ocular irritation, oral mucosa irritation, penile irritation, rectal irritation, vaginal irritation

Acute/subacute systemic toxicity

Standard: ISO 10993-11: 2017

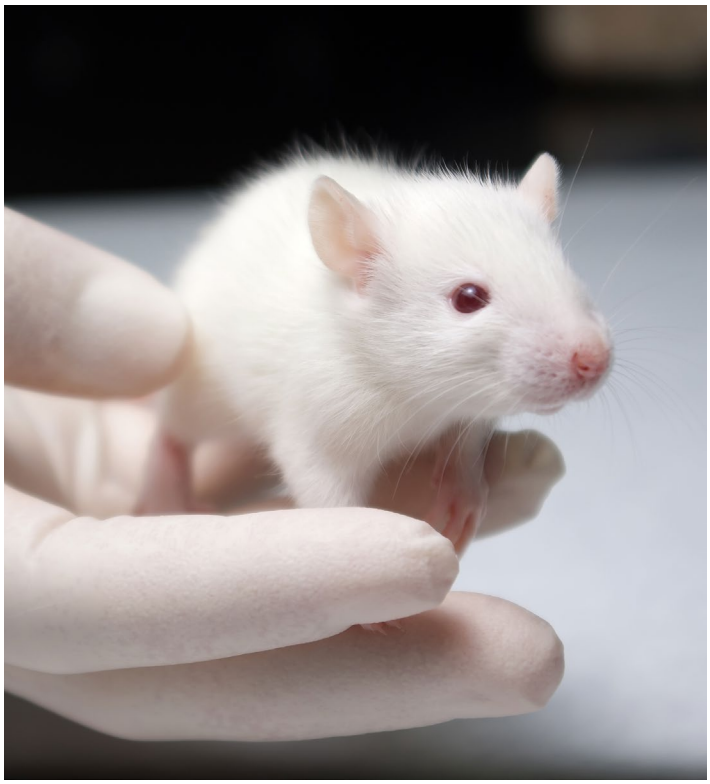
Type: *In vivo*

Description: Acute/subacute toxicity tests are conducted to provide data on the effects of exposure during a period of at least 72 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 into rodents — 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 by measuring animal weight and clinical observations, e.g., change in skin, respiration, 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 (lesions, change of body or organ weight, clinical pathology, gross pathology, histopathology) is made and recognized, and accepted statistical methods are used to come to a conclusion about the toxicity.

Examples of tests: Acute systemic toxicity, subacute toxicity



Subchronic/chronic systematic toxicity

Standard: ISO 10993-11:2017

Type: *In vivo*

Description: Continuous or repeated exposure may result in the potential accumulation of chemicals in tissues, thereby leading to adverse effects. Subchronic and/or chronic toxicity tests are conducted to assess effects occurring following repeated or continuous exposure by the intended clinical route for a period (subchronic toxicity) or major period (chronic toxicity) of time. The tests are implemented during:

- A period not exceeding 10% of the lifespan of the animal model[2] (subchronic toxicity)
- A major period of the lifespan 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 administered by the intended clinical route. Usually, the test consists of repeated injections of extracts into rodents — 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, respiration, 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.

Examples of tests: Subchronic toxicity, chronic toxicity

Pyrogenicity

Standard: ISO 10993-11:2017

Type: *In vivo* and *in vitro*

Description: Pyrogenicity testing per ISO 10993-11 is conducted to mitigate the risk of a material-mediated[4] pyrogenic response. *In vivo* material-mediated pyrogenicity should be evaluated when the device is composed of material(s) known to previously induce a pyrogenic response or the pyrogenic potential is unknown.

Methods for the *in vivo* rabbit pyrogen test are available in the US, European and Japanese Pharmacopeias. Pyrogenicity tests in rabbits are often conducted according to USP Chapter <151> and consist of an injection of extract into the ear vein of three rabbits to observe temperature rise.

When the risk assessment indicates the potential presence of endotoxin-mediated pyrogenicity, using the *in vitro* Limulus Amebocyte Lysate (LAL) test may be more appropriate. Such testing may be conducted according to USP Chapter <85> and/or AAMI ST72 with LAL that can coagulate when in contact with bacterial endotoxin. It should be noted that commonly sterile products are expected to complete bacterial endotoxin testing periodically to minimize potential risks associated with endotoxin exposure and localized responses. A plan for how often to conduct this testing should be included in the technical documentation.

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 nonpyrogenic if not more than three rabbits (out of eight) increase their temperatures beyond 0.5°C and if the sum of temperature rises does not exceed 3.3°C.

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

Examples of tests: Material-mediated pyrogenicity (rabbit pyrogen test), LAL test

Implantation

Standard: ISO 10993-6:2016

Type: *In vivo*

Description: Assessments are made to evaluate the risk of local intolerance after a medical device is implanted. This consists of implantation by surgical procedure in an appropriate number of animals. The animal model is selected based on the implant type and size, the intended duration of the test and biological animal responses.

Degradation products require special consideration 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 or sham-operated sites. When systemic toxicity tests are performed by implantation, with local and systemic effects evaluated, the data can potentially address both biological endpoints.

Results: Various scoring systems may be used and are proposed in Annex E of ISO 10993-6 or in the literature. Results are often reported applying a semiquantitative scoring system like that described in Annex E.2, with results considered as non-irritant (0.0 to 2.9), slight irritant (3.0 to 8.9), moderate irritant (9.0 to 15.0) or severe irritant (>15.0).

Examples of tests: Intramuscular implantation, subcutaneous tissue implantation, bone implantation

Hemocompatibility

Standard: ISO 10993-4:2017

Type: *In vitro*, *in vivo*, or *ex vivo*

Description: Hemocompatibility 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. *Ex vivo* testing is appropriate for devices intended for use *ex vivo*, e.g., external communicating, or potentially *in vivo*, e.g., assess acute response to implant.

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. For instance, a mechanical cardiac valve should be tested for thrombosis, e.g., occlusion percentage test, and hematology, e.g., hemolysis test. As applicable, the appropriate animal model must be chosen and justified according to the type of test conducted.

Note: For a device made of materials already known for the intended use, the US FDA recommends considering hemolysis, complement activation and thrombogenicity tests for direct blood-contacting devices, and hemolysis tests only for indirect blood-contacting devices.[5]

Examples of tests: Hemolysis (ASTM hemolysis), partial thromboplastin time (PTT), complement activation, thrombogenicity

Genotoxicity

Standard: ISO 10993-3:2014

Type: *In vitro* or *in vivo*

Description: Genotoxicity assessments are conducted to evaluate the risk of gene mutations, chromosome structure and other DNA or gene toxicities caused by medical devices. *In vitro* tests are preferred according to various methods, e.g., Organisation for Economic Co-operation and Development (OECD) guidelines. *In vivo* tests may be considered when the genotoxic activity of a compound may be influenced, such as pharmacokinetics or genotoxic mechanism.

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, ISO 10993-3 adds requirements regarding sample preparation with three proposed methods: a direct method for dissolution or suspension (Method A), exaggerated extract (Method B) or a simulated use extraction method like ISO 10993-12 (Method C). The appropriate method should be selected according 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, which essentially depends on their recognition by the countries where the device will be marketed. For instance, the US FDA recommends two *in vitro* tests — e.g., bacterial gene mutation assay OECD 471 and one *in vitro* mammalian genotoxicity assay such as mouse lymphoma gene mutation assay OECD 476, *in vitro* chromosomal aberration assay OECD 473 or *in vitro* micronucleus assay OECD 487 — and an optional third *in vivo* test — e.g., bone marrow micronucleus assay OECD 474, bone marrow chromosomal aberration assay OECD 475 or peripheral blood micronucleus assay OECD 474.[5] In comparison, the standard recommends no further testing if the results of two *in vitro* tests are negative.

Examples of tests: Ames mutagenicity, chromosomal aberration, mouse lymphoma, mouse micronucleus

Other

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

Biological endpoint testing — timelines

The following table details estimated timelines by contact duration of the device and the biological evaluation test. The range of estimation depends on the choice of testing laboratory, type of device, selection of appropriate testing (according to test method, contact duration, etc.) and laboratory practices used, e.g., ISO 17025, GLP.

Biological evaluation	Estimated timeline	Comments
By contact duration		
Limited contact (≤ 24 h)	2-3 months	–
Prolonged contact (> 24 h to 30 d)	6+ months	Depends significantly on novelty of materials and additional testing such as degradation and reproductive/developmental toxicity
Long-term (> 30 d)	6+ months	
By test		
Chemical characterization (ISO 10993-18)	3-6 weeks	–
Cytotoxicity (ISO 10993-5)	1-2 weeks	–
Sensitization (ISO 10993-10)	6-8 weeks	–
Irritation or intracutaneous reactivity (ISO 10993-23)	2-4 weeks	–
Material-mediated pyrogen (ISO 10993-11)	1-2 weeks	–
Acute systemic toxicity (ISO 10993-11)	8-10 weeks	–
Subacute/subchronic toxicity (ISO 10993-11)	3-4 months	–
Chronic toxicity (ISO 10993-11)	Variable	Varies with study duration; generally, 6-12 months
Implantation (ISO 10993-6)	Variable	Varies based on absorbable (degradation time) and nonabsorbable materials; generally, 13-104 weeks, depending on animal model
Hemocompatibility (ISO 10993-4)	6-8 weeks	–
Genotoxicity (ISO 10993-3)	2-4 weeks	–
Carcinogenicity (ISO 10993-3)	18-24 months	Test indicated by assessed risk of carcinogenesis; varies based on animal model
Reproductive and developmental toxicity (ISO 10993-3)	Variable	Intended for devices with potential impact on reproductive potential, medical devices or materials used during pregnancy; varies based on necessity for additional testing
Degradation (ISO 10993-9)	Variable	Consider for absorbable medical devices or indications that the finished medical device may release toxic degradation products during body contact

Table 5: Estimated Timelines

Note: Timelines and fees vary by testing laboratory. It is recommended to request estimates from multiple testing laboratories.

Summary and conclusion

Evaluating biological safety is a significant process that begins during the device design stage as part of risk management.

Biological safety generally includes a review of scientific literature, applicable standards, a biological evaluation plan and a biological report. These should document the material selected for the intended use and the implementation of testing programs.

Manufacturers often rely on the expertise and experience of testing laboratories to recommend an appropriate strategy, conduct testing and interpret data regarding biological safety. However, manufacturers must have sufficient knowledge of the requirements to review the testing laboratories' recommendations, test results and conclusions since they are accountable for the biological evaluation program.

Biological evaluation is not a one-time action but must be revisited regularly for suitability when a change is implemented, a standard is revised or when the results of post-market surveillance indicate a potential adverse biological effect with clinical use.

End Notes

1. ISO 14971:2019, Medical Devices – Application of Risk Management to Medical Devices
2. Subchronic toxicity studies are generally 90 days in rodents.
3. Chronic toxicity studies are generally 6-12 months in rodents.
4. A 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 undergoes limulus amebocyte lysate (LAL) testing (AAMI/ST72).
5. Use of International Standard ISO 10993-1, "Biological Evaluation of Medical Devices – Part 1: Evaluation and Testing Within a Risk Management Process" Guidance for Industry and Food and Drug Administration Staff, issued Sept. 4, 2020

About the author

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