International Standard

ISO 10993-6

Biological evaluation of medical devices —

Part 6: Tests for local effects after implantation

Évaluation biologique des dispositifs médicaux —

Partie 6: Essais concernant les effets locaux après implantation

Fourth edition
2026-04

Reference number
ISO 10993-6:2026(en)

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Published in Switzerland

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 194, *Biological and clinical evaluation of medical devices,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 206, *Biological and clinical evaluation of medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

This fourth edition cancels and replaces the third edition (ISO 10993-6:2016), which has been technically revised.

The main changes are as follows:

— new definitions for “comparative control”, “coupon”, “euthanasia”, “local effect”, “location marker”, “steady-state” and “reference control” have been added to Clause 3;

— a new paragraph on the use of smaller compositionally representative samples or coupons has been added to 4.2.2;

— a new subclause 4.3 “Selection of control materials” has been added;

— the discussion of assessment of lymph nodes for certain materials has been expanded;

— a new Annex E “Test methods for devices contacting peripheral nerve tissue” and Annex G “Microscopic evaluation of tissue responses to implanted materials” have been added;

— tissue and pathological terminology has been updated throughout this document;

— bibliographical entries have been updated.

A list of all parts in the ISO 10993 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The objective of the implantation test methods is to characterize the local tissue response after implantation of a medical device or material (test sample) including integration, degradation, or absorption in an appropriate animal model.

The test sample is implanted into an anatomical site appropriate for the evaluation of the local effects of the medical device (or portion of) in an animal.

The medical device or material local effects are evaluated by a comparison of the tissue response caused by a test sample to that caused by comparative or reference control samples used in medical devices whose clinical acceptability and biocompatibility characteristics have been established.

Careful study design can include other relevant biological effects to reduce the number of animals used to evaluate safety and efficacy while accomplishing all study objectives. Additionally, a long-term systemic toxicity study that is designed to incorporate the methods, biological effects, additional timepoints and outcomes of implantation testing can satisfy the requirements of this document and ISO 10993-11.

Biological evaluation of medical devices —

Part 6:
Tests for local effects after implantation

1 Scope

This document specifies requirements for implantation test methods for preclinical assessment of the local effects after implantation of medical devices or materials intended for use in medical devices. This document is applicable to the evaluation of local tissue responses from medical devices that are intended to be used where skin or mucosal tissue is breached, when required.

This document is applicable to medical device or materials that require implantation evaluation and can be solid or non-solid (such as porous materials, liquids, gels, pastes, powders, and particulates), absorbable, degradable, non- absorbable, or can be tissue-engineered medical products (TEMPs).

These implantation tests are not intended to evaluate or determine the performance of the test sample in terms of mechanical loading or functional performance. This document also does not provide guidance on methods and study design to satisfy requirements for systemic toxicity, carcinogenicity, teratogenicity or mutagenicity. However, the study designs can be modified to also assess other biological effects.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Requirements and general principles for the evaluation of biological safety within a risk management process*

ISO 10993-2, *Biological evaluation of medical devices — Part 2: Animal welfare requirements*

ISO 10993-4, *Biological evaluation of medical devices — Part 4: Selection of tests for interactions with blood*

ISO 10993-9, *Biological evaluation of medical devices — Part 9: Framework for identification and quantification of potential degradation products*

ISO 10993-12:2021, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

ISO 10993-16, *Biological evaluation of medical devices — Part 16: Toxicokinetic study design for degradation products and leachables*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10993-1, ISO 10993-2, ISO 10993-4, ISO 10993-9, ISO 10993-12, ISO 10993-16 and the following apply.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
absorb
action of a non-endogenous (foreign) material or substance, or its decomposition products passing through or being assimilated by cells and tissue over time

3.2
comparative control
medical device or material with a history of safe clinical use that is used as a point of comparison for a new medical device seeking regulatory approval

3.3
coupon
multiple smaller compositionally representative portions of a more complex or larger device that together contain all materials, surface finishes, and processing as the final, finished device

3.4
degradation product
intermediate or final substance which results from the physical, metabolic, or chemical decomposition of a material or substance

[SOURCE: ISO/TS 37137-1:2021, 3.2, modified — "and/or" has been changed to "or" and "agent" has been replaced with "substance".]

3.5
degrade
physically, metabolically or chemically decompose a material or substance

[SOURCE: ISO/TS 37137-1:2021, 3.3, modified — "and/or" has been changed to "or".]

3.6
euthanasia
humane killing of an animal by a method causing minimal physical and mental suffering

[SOURCE: ISO 10993-2:2022, 3.5]

3.7
local effect
tissue response to an implanted test sample that can be seen with gross pathology and histopathology at the site of test sample administration or implantation

3.8
location marker
inert, non-absorbable material or process used to mark the location in tissue of an implanted material

3.9
reference control
material with known properties considered to be generally inert in terms of biological local tissue effects and has been established in a historical basis within a pharmacopeia or similar standard

3.10
steady-state
biologically stable condition wherein change in the test system's cellular activity, morphology or features is no longer detected over a period of time

4 Common provisions for implantation test methods

4.1 General

The ISO 10993 series requires animal testing to be avoided unless the required data cannot be obtained by other means. Prior to choosing to pursue implantation studies, consideration to the type of data required,

the appropriate method and approach to implantation studies should be given to ensure that the data collected covers the concerns for biological reactivity. It is important that the study be planned in sufficient detail such that all relevant information can be extracted from the use of each animal and each study plan or protocol according to ISO 10993-2, ISO 10993-11 and ISO 10993-16. The implantation tests shall be planned and documented in the study documentation, including the information on the selected test methods, test samples, test animals, implantation sites, test duration, measurement frequencies, assessment methods and evaluation. When relevant vertical standards exist, refer to those as guidance for biological specific evaluation (e.g. ISO 15798, ISO 11979-5, ISO 7405, ISO 14708 series, ISO 5840 series).

All animal studies shall be performed in a facility approved by a nationally recognized organization and in accordance with all appropriate regulations dealing with laboratory animal welfare to conform with the requirements of ISO 10993-2. These studies shall be performed under Good Laboratory Practices or other recognized, quality assurance systems. While pilot implant studies can or cannot be conducted under Good Laboratory Practices, the test facility should have an adequate quality system.

The provisions of this clause shall apply to the test methods specified in Annex A, Annex B, Annex C and Annex D.

4.2 Preparation of samples for implantation

4.2.1 Test and control samples shall be in a final finished state as described in ISO 10993-12 (unless used in a pilot study). The implant size and shape shall be documented and justified. For certain devices, clinically relevant dosing can be considered. A justification should be provided to demonstrate mimicking a clinically relevant dose in the implantation study.

Test samples for various implant sites are described in Annex A, Annex B, Annex C and Annex D. Physical properties (such as form, density, hardness, surface porosity and texture), geometrical characteristics (form, shape and size) and composition can influence the character of the tissue response to the test material and shall be recorded and taken into account when the response is characterized. Comparative control samples should be matched as closely as reasonably possible for physical properties and geometrical (form and shape) characteristics. Otherwise, a reference control (well characterized material with a well characterized response) can be used, such as high density polyethylene (HDPE).

Additional control samples may be included in the study to help interpret tissue reactions [e.g. in case of combination drugs, an additional control such as a placebo device (without drug) can be considered].

4.2.2 Each implant shall be manufactured, processed, cleaned of contaminants and sterilized by the method intended for the final product and this shall be confirmed in the study documentation. After final preparation and sterilization, the implant samples shall be handled aseptically and in such a way as to ensure that they are not damaged or contaminated in any way prior to or during implantation.

The use of smaller test samples or test coupons that are consistent with the requirements as described in ISO 10993-12 can be relevant if the medical device to be tested cannot be tested as is, due to size or complex geometry. The test samples or test coupons shall contain all tissue-contacting materials and surface finishes as in the final, finished medical device. For medical devices comprising two or more different materials, implanted test samples should be of similar composition, surface finish and include each individual material to the final product. With multiple materials, it can be necessary to implant several coupons together, each containing a subset of device materials. The potential for synergies and interactions of different materials in the final product should be considered in the choice of test sample.

NOTE Refer to the respective annexes for guidance on the number of test and control samples for each material and implantation period.

4.2.3 For materials used as scaffolds for tissue-engineered medical products, it can be appropriate not to use the final preparation pre-populated with cells or proteins, as the immune reaction of the animal to human cellular or protein components of such products and the reaction of the cells to the animal can interfere with the resulting local tissue response, making it difficult to interpret.

NOTE 1 Implantation in an appropriate immune-deficient animal can be an option to avoid the xenograft responses, if justified.

NOTE 2 Products with living cells are not considered medical devices in all jurisdictions.

4.2.4 For multicomponent material device designed to be cured prior to placement, the components shall be mixed before use and allowed to set before implantation. However, materials that are designed to polymerize in situ (e.g. bone cements, many dental materials, tissue sealants and glues) shall be introduced in a manner such that in situ polymerization occurs. The procedure used shall be justified and documented.

4.3 Selection of control samples

The process of implanting any material in tissue induces some degree of cellular response. In the evaluation of material local effects after implantation, the choice of control samples is important in determining the acceptability of the observed tissue reaction to the test sample. Evaluation should be performed by comparing the tissue reaction to that of a similar material (comparative control) whose clinical acceptability and biocompatibility characteristics have been established. The geometrical and physical characteristics such as shape, size, curvature and especially the surface condition (including porosity and texture) of the control article(s), should be as similar to that of the implant test samples as is practical. Any deviations shall be explained, justified and recorded. If no appropriate comparative control is available, a reference control can be used. For further guidance, see ISO 10993-12. For example, reference materials as specified in ISO 10993-12:2021, Table A.1 negative control column can provide a good control for solid, smooth, non-absorbable materials. Conversely, the reaction can likely be different if it is used to compare against a non-absorbable or degradable hernia mesh due to the differing physical form characteristics. Ideally, a commercially available material should be used as a comparative control so the tissue reaction is similar to what has been clinically proven. For non-absorbable medical devices or materials, a comparative control shall be used made of a stable non-absorbable comparative material (see Annex A, Annex B, Annex C and Annex D).

Since absorbable materials encounter changing tissue responses as degradation proceeds at rates that differ based on composition, processing and sterilization techniques, similar absorbable comparative medical device or material controls should be considered.

The absorption rate of the control material or medical device should be similar to the test material or medical device (for more information, see ISO/TS 37137-1). Alternatively, a non-absorbable comparative control can be used, if justified (e.g. there are no clinically relevant absorbable controls). If a comparative control is used, typically reference materials as specified in ISO 10993-12:2021, Annex A are also implanted into the animal for comparison and serving as a procedural control yielding a total of three separate articles included in the study. For novel materials or medical devices, if a commercially available comparative material or medical device does not exist, the choice of a control that is as close as possible is preferred. The nature of any adverse inflammatory response or high reactivity rating or similar histopathological evaluation conclusion should be discussed in the context of the control article chosen. The choice of control shall be documented and justified. If no control or a sham control is used, this also shall be documented and justified.

5 General aspects and requirements for implantation test

5.1 Tissue and implantation site

5.1.1 The test sample shall be implanted into or onto the tissues most relevant to the intended clinical use of the material. The justification for the choice of sample numbers, tissue, implantation sites and test period shall be documented. Test methods for various implantation sites are given in Annex A, Annex B, Annex C and Annex D. If other implantation sites are chosen, the general scientific principles behind the test methods described in Annex A, Annex B, Annex C and Annex D shall still be adhered to and a justification be provided for selecting an anatomical location that does not align with the use of the device.

NOTE For some devices, there are vertical standards prescribing specific implant studies to evaluate local tissue responses, e.g. for intraocular lens implant (see ISO 11979-5) and dental devices (see ISO 7405). The studies described in the standards can be used to satisfy the requirements in ISO 10993-6.

5.1.2 For absorbable materials, the implantation site shall be marked in a manner suitable for identification of the site at the end of the designated time periods, taking into consideration the growth and aging of the implanted animal(s). The use of a non-invasive permanent skin marker or a template marking the placement of the sample is recommended as a single application for short-term study intervals only. This method of marking can come off as skin exfoliates and requires remarking as often as needed to maintain the marked site. Additionally, in many laboratory animals, the loose highly mobile skin limits the precision of marking sites that are deeper than skin. In most circumstances, a location marker comprising an appropriate non-absorbable inert or biocompatible material (e.g. HDPE 1 mm by 2 mm by 5 mm, polypropylene suture, gold band, clips) may be used to identify the location of the implant site. These location markers should be far enough away from each implant site to not induce changes in the local tissue reaction to the implanted material. If this is not possible, i.e. the marker of the implanted material is adjacent to or on the edge of the implanted material, then the tissue reaction to this marker should not be included in the evaluation of the tissue reaction to the implanted material.

5.1.3 A sham surgical procedure can be used to evaluate the impact of the procedure on the tissue involved; in these cases, the specific justification shall be provided. If a sham surgical procedure is performed, the same implantation procedure that is used for the test or control should be used for the sham procedure.

5.2 Animal model

5.2.1 All aspects of animal care and accommodation shall be performed in accordance with ISO 10993-2.

5.2.2 Animals are used to evaluate local effects following implantation and are described as animal models in the context of this document. In general, small laboratory animals such as mice, rats, guinea-pigs or rabbits are preferred. The use of larger animals such as dogs, sheep, goats or pigs may be justified based upon special scientific considerations of the test sample under study, length of study in relation to animal life expectancy, or if needed to accommodate implant size, with whole device testing or applicable sham defect size.

5.2.3 Select an animal species in line with the principles set out in ISO 10993-2, giving due consideration to the size of the implant test samples, the number of implants per animal, the intended duration of the test in relation to the expected lifespan of the animals, as well as potential species' differences regarding biological responses. The number of animals and implant sites within should be the minimum to account for site to site and animal to animal variability.

5.2.4 For short-term testing, animals such as rodents or rabbits are commonly used. For long-term testing, animals such as rodents, rabbits, dogs, sheep, goats, pigs and other animals with a relatively long life expectancy are suitable.

5.2.5 Before starting an animal study with degradable materials, relevant information from in vitro degradation studies should be considered for estimating relevant retrieval timing. For absorbable materials, a pilot study in rodents can be considered to determine the expected rate of degradation and the implantation duration needed to reach the steady-state before embarking on studies in larger animals.

NOTE Guidance regarding in vitro degradation characterization can be found in ISO 13781 and ASTM F1635[23] for hydrolysable polymeric constructs and in ASTM F3268[25] for absorbable metallic constructs. General guidance regarding the linkage of in vitro observations with the determination of relevant in vivo retrieval intervals can be found in ISO/TS 17137. Additional guidance on selection of implantation time points for absorbable materials can be found in ISO/TS 37137-1.

5.2.6 The samples of test and control materials shall be implanted under the same conditions in animals of the same species and of the same age, sex and strain in corresponding anatomical sites. The number and size of implants inserted into an animal depends on the size of the species and the anatomical location. Whenever possible, the control and the test samples should be implanted into the same animal unless systemic toxicity effects are also evaluated in the study. In cases where both implantation and systemic toxicity effects are assessed, control and test samples shall be implanted into separate animals. Guidance on evaluation for systemic toxicity can be found in ISO 10993-11.

5.2.7 When a neuroimplantation study (see Annex D) is conducted, control and test samples shall not be placed in the same animal. Histological assessments should follow best practices for processing and analysis of central nervous system tissues (see Annex D and Reference [70]) or peripheral nervous tissues (see Annex E and Reference [70]).

5.3 Test periods

5.3.1 The test period shall be determined by the likely clinical exposure time or be continued until or beyond when a steady-state with respect to the biological response has been reached. The time points selected, along with the explanation and justification for their selection, shall be recorded.

5.3.2 The local biological response to implanted materials depends both on the properties of the materials and on the response to the associated trauma of surgery. The tissue configuration in the vicinity of an implant changes with the time elapsed after surgery. During the first two weeks after implantation, the reaction due to the surgical procedure itself can be difficult to distinguish from the tissue reaction evoked by the implant. The time to reach a steady-state can be tissue- and device design-dependent. A justification for the time point selected for assessment in a specific tissue (i.e. muscle, bone) shall be documented. In muscle and connective tissue, depending on the species, the design of the device and the severity of the surgical trauma, a steady-state can be seen in the tissue-implant interface (including the microscopic maturation of immature fibrous tissue and associated remodelling) that can take 9 weeks to 12 weeks but can occur earlier. Implantation in bone tissue can need longer observation periods before a steady-state is reached.

5.3.3 For non-absorbable materials, the short-term responses are normally assessed from 1 week to 4 weeks and the long-term responses in tests exceeding 12 weeks. Additional intervals can be needed to characterize the response relative to the clinical use of the device.

5.3.4 For absorbable materials, the test period shall be related to the estimated degradation time of the test product at a clinically relevant implantation site. When determining the time points for sample evaluation, an estimation of the degradation time shall be made. This can be accomplished in vitro by real-time or accelerated degradation studies or in certain circumstances by mathematical modelling. In general, study duration should extend up to or beyond the point of complete absorption. The evaluation period for absorbable materials will depend in part on the degradation rate of the materials. Study intervals should span a significant portion of the degradation time frame for the implant, and shall include, as a minimum, the following time points:

a) Early time frame (where there is no or minimal degradation): For absorbable materials, usually a study interval of between 1 week to 4 weeks post-implantation should be used to assess the early tissue response.

   If a device completely absorbs within four weeks of implantation, a short-term implant study may be considered as an evaluation point; additional durations may be omitted from the implantation studies.

b) Mid time frame (when degradation is taking place): Subsequent study intervals for absorbable devices should be guided by the degradation profile of the specific absorbable material. The target interval should allow assessment of histological response when the tissue response is expected to be most pronounced (e.g. substantial structural disruption or fragmentation of the device is most likely to occur). Implants with longer-term degradation profiles can require multiple assessment time points, with intervals targeted in accordance with the expected pattern of degradation.

   When a device with multiple materials with differing absorption rates is implanted, implant intervals reflecting the degradation profile of those components should be included.