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**Biološko ovrednotenje medicinskih pripomočkov - 6. del: Preskusi, povezani z lokalnimi učinki po implantaciji (ISO/DIS 10993-6:2024)**

Biological evaluation of medical devices - Part 6: Tests for local effects after implantation (ISO/DIS 10993-6:2024)

Biologische Beurteilung von Medizinprodukten - Teil 6: Prüfungen auf lokale Effekte nach Implantationen (ISO/DIS 10993-6:2024)

Évaluation biologique des dispositifs médicaux - Partie 6: Essais concernant les effets locaux après implantation (ISO/DIS 10993-6:2024)

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# DRAFT International Standard

## ISO/DIS 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*

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## ISO/DIS 10993-6:2024(en)

### Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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 documents 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](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

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, *Biocompatibility of medical and dental materials and 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:

- Informative elements of the scope have been moved to a new introduction clause;
- New definitions for “comparative material”, “coupon”, “euthanasia”, “local effect”, “location marker” and “steady-state” have been added to [clause 3](#);
- New paragraph on the use of smaller compositionally representative samples or coupons has been added to [4.2.2](#);
- New [4.3](#) “Selection of control materials” has been added;
- [Clause 4](#) and [Clause 5](#) have been revised;
- 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;
- The old [Annex E](#) was moved to [Annex F](#);
- Updated entries in the bibliography;
- Document has been revised editorially.

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](http://www.iso.org/members.html).

## ISO/DIS 10993-6:2024(en)

### Introduction

The objective of the implantation test methods is to characterize the local tissue response after implantation of a medical device/material including final 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 material local effects are evaluated by a comparison of the tissue response caused by a test sample to that caused by control materials used in medical devices whose clinical acceptability and biocompatibility characteristics have been established.

Long-term animal studies designed to evaluate local biological effects might provide insight into some effects (such as systemic toxicity, carcinogenicity, teratogenicity or mutagenicity). Careful study design can include other relevant endpoints to reduce the number of animals used while accomplishing all study objectives. For example, a systemic toxicity study that is designed to incorporate the methods and outcomes of implantation testing might satisfy the requirements of this document and ISO 10993-11, when the requirements of both standards are fulfilled.

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# 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 materials intended for use in medical devices. In order to evaluate local tissue responses from medical devices that are intended to be used where skin or mucosal tissue is breached, this document is applicable when required.

This document applies to medical device/materials that require implantation evaluation and can be solid or non-solid (such as porous materials, liquids, gels, pastes, powders, and particulates), absorbable/degradable or 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 biocompatibility 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: Evaluation and testing 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 10993-12:2021, *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 10993-12, ISO 10993-16 and the following apply.

ISO and IEC maintain terminological 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/>

## ISO/DIS 10993-6:2024(en)

### 3.1

#### **absorb**

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

### 3.2

#### **comparative control**

medical device with a history of safe clinical use that may be used as a point of comparison for a new medical device seeking regulatory approval

### 3.3

#### **coupon**

smaller compositionally representative sample of a more complex and/or larger device that contains all the materials, surface finishes, and processing as the final, finished device

### 3.4

#### **degradation**

decomposition of a material

[SOURCE: ISO 10993-9:2019, 3.1]

### 3.5

#### **degradation product**

intermediate or final substance which results from the physical, metabolic, and/or chemical decomposition of a material or substance

[SOURCE: ISO/TS 37137-1:2021, 3.2, modified – agent has been replaced by substance.]

### 3.6

#### **degrade**

physically, metabolically, and/or chemically decompose a material or substance

[SOURCE: ISO/TS 37137-1:2021, 3.3]

### 3.7

#### **euthanasia**

humane killing of an animal by a method causing a minimum of physical and mental suffering

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

### 3.8

#### **local effects**

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

### 3.9

#### **location marker**

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

### 3.10

#### **steady-state**

biologically stable condition wherein change in the test system's cellular activity/morphology/features is not detected over a period of time

## 4 Common provisions for implantation test methods

### 4.1 General

Prior to choosing to pursue implantation studies, consideration to the appropriate method and approach to implantation studies should be given to ensure that the data collected covers the concerns for biological reactivity. Evaluation of the type of data needed and the best way of collecting it to comply with the ISO 10993

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series general framework of not doing animal testing unless this data cannot be obtained by other means, should be performed. 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 (see ISO 10993-2, ISO 10993-11 and ISO 10993-16).

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 comply with the requirements of ISO 10993-2. These studies shall be performed under good laboratory practices or other recognized, quality assurance systems.

The provisions of this clause shall apply to the test methods specified in [Annex A](#), [Annex B](#), [Annex C](#), [Annex D](#), and [Annex E](#).

### 4.2 Preparation of samples for implantation

**4.2.1** Test sample and control article should be in a final finished state as described in ISO 10993-12. 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](#), [Annex D](#) and [Annex E](#). 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. Control articles should be matched as closely as reasonably possible for physical properties and geometrical characteristics. Otherwise, a well characterized material with a well characterized response can be used, such as High Density Polyethylene (HDPE).

Additional control articles 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) should 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 sample or test coupons that are consistent with the requirements as described in ISO 10993-12 may 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 articles should be of similar composition, surface finish, and include each individual material to the final product. Alternatively, with multiple materials it may be necessary to implant each material and its relevant surface finish as separate coupons. The potential for synergies and interactions of different materials in the final product shall 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 may be appropriate not to use the final preparation pre-populated with cells and/or proteins, as the immune reaction of the animal to human cellular/protein components of such products and the reaction of the cells to the animal may interfere with the resulting local tissue response, making it difficult to interpret.

**Note** Implantation in an immune-deficient animal, e.g., athymic rodent, can be an option to avoid the xenograft responses, if justified.

**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

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in a manner such that *in situ* polymerization occurs. The procedure used shall be justified and documented. When relevant vertical standards exist, refer to those as guidance for biological specific evaluation (e.g., ISO 15798, ISO 11979-5, ISO 7405).

**4.2.6** Evaluation shall be performed by comparing the tissue reaction to that of a similar material whose clinical acceptability and biocompatibility characteristics have been established.

NOTE For further guidance, see ISO 10993-12.

**4.2.7** The geometrical and physical characteristics such as shape, and especially the surface condition (including porosity and texture) of the article control(s), should be as similar to that of the implant test samples as is practical, with any deviations being explained and justified.

### 4.3 Selection of control materials

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 comparative control samples is important in determining the acceptability of the observed tissue reaction to the test sample. The comparative control chosen should be similar in form and shape characteristics, which can greatly affect its tissue reaction. For example, reference materials as specified in ISO 10993-12:2021, Annex A can provide a good control for solid, smooth, non-absorbable materials. Conversely, the reaction could be likely different if it is used to compare against a non-absorbable/degradable hernia mesh due to the differing physical form characteristics. Ideally, a commercially available material should be used as a control so the tissue reaction is similar to what has been clinically proven. For non-absorbable medical devices/materials, a stable non-absorbable comparative material shall be used as a comparative control (see [Annex A](#), [Annex B](#), [Annex C](#), [Annex D](#), and [Annex E](#)).

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

For absorbable medical devices/materials, an absorbable medical device/material should be used as a comparative control. The absorption rate of the control material/medical device should be similar to the test material/medical device (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 3 separate articles included in the study. For novel materials/medical devices, if a commercially available comparator material/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 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](#), [Annex D](#), and [Annex E](#). If other implantation sites are chosen, the general scientific principles behind the test methods described in [Annex A](#), [Annex B](#), [Annex C](#), [Annex D](#), and [Annex E](#) 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., intraocular lens implant (see ISO 11979-5) and dental usage tests (see ISO 7405). These studies can be used to satisfy the requirements in ISO 10993-6.

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**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. The use of a non-invasive permanent skin marker and/or a template marking the placement of the sample is recommended for short-term study intervals only. This method of marking is of limited utility since topical marker can come off as skin exfoliates. Additionally, in many laboratory animals, the loose highly mobile skin limits the precision of marking site deeper than skin. In most circumstances, a location marker comprised of an appropriate non-absorbable control article (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 can be removed without inducing artefacts to the test article-tissue interface prior to histology processing.

**5.1.3** A sham surgical procedure might 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 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 material under study, or if needed to accommodate implant size, with whole device testing.

**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 response. 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 may be considered to determine the expected rate of degradation before embarking on studies in larger animals.

**NOTE** Guidance regarding *in vitro* degradation characterization can be found in ISO 13781 and ASTM F1635 for hydrolysable polymeric constructs<sup>[23]</sup> and in ASTM F3268 for absorbable metallic constructs.<sup>[25]</sup> General guidance regarding the linkage of *in vitro* observations with the determination of relevant *in vivo* retrieval intervals can be found in ISO/TS 17137:2021 (see Figure 2 and 5.1, 5.3, 5.4, and 5.6). Additional, guidance on selection of implantation time points for absorbable materials can be found in ISO/TS 37137-1:2021, 8.7 and ASTM F1983, 8.3.1<sup>[24]</sup>.

**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 endpoints are also evaluated in the study. In cases where both implant and systemic toxicity endpoints are assessed, control and test samples should be implanted into separate animals. Guidance on evaluation for systemic toxicity can be found in ISO 10993-11.

**5.2.7** However, when a neuroimplantation study (see [Annex D](#) and [Annex E](#)) is conducted or when the local effects after implantation are investigated as part of a systemic toxicity study by implantation, control and



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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](#)) or peripheral nervous tissues (see [Annex E](#) and [59]).

### 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 shall be explained and justified.

**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 may be difficult to distinguish from the tissue reaction evoked by the implant. The time to reach a steady-state may 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-device/material interface (including the microscopic maturation of immature fibrous tissue and associated remodeling) after 9 weeks to 12 weeks. Implantation in bone tissue may 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 up to 4 weeks and the long-term responses in tests exceeding 12 weeks.

**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 and 4 weeks post-implantation should be used to assess the early tissue response.

If a device completely absorbs within 4 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 and/or fragmentation of the device is most likely to occur). Implants with longer-term degradation profiles may 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.

- c) Late time frame (when the implant is essentially absorbed) — This interval is targeted to observe when minimal amounts of the absorbable component remain at the implant site.

Gross and microscopic evaluation after complete implant absorption is highly desirable. However, in the absence of complete absorption, the overall data collected should be sufficient to allow characterization of the local effects after implantation if:

- the affected tissue's response, structure, and function have achieved an acceptable steady-state condition, and