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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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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 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).

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).

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

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 194, *Biological and clinical evaluation of medical devices*.

This third edition cancels and replaces the second edition (ISO 10993-6:2007), which has been technically revised with the following changes:

a) addition of guidance on biological evaluation of absorbable medical devices;

b) new Annex D.

ISO 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

— *Part 1: Evaluation and testing within a risk management process*

— *Part 2: Animal welfare requirements*

— *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

— *Part 4: Selection of tests for interactions with blood*

— *Part 6: Tests for local effects after implantation*

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 9: Framework for identification and quantification of potential degradation products*

— *Part 10: Tests for irritation and skin sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymeric medical devices*

— *Part 14: Identification and quantification of degradation products from ceramics*

— *Part 15: Identification and quantification of degradation products from metals and alloys*

— *Part 16: Toxicokinetic study design for degradation products and leachables*

— *Part 17: Establishment of allowable limits for leachable substances*

— *Part 18: Chemical characterization of materials*

— *Part 19: Physico-chemical, morphological and topographical characterization of materials* [Technical specification]

— *Part 20: Principles and methods for immunotoxicology testing of medical devices* [Technical specification]

— *Part 33: Guidance on tests to evaluate genotoxicity — Supplement to ISO 10993-3* [Technical Report]

The following parts are under preparation:

— *Part 5: Tests for in vitro cytotoxicity*

Biological evaluation of medical devices —

Part 6: Tests for local effects after implantation

1 Scope

This part of ISO 10993 specifies test methods for the assessment of the local effects after implantation of biomaterials intended for use in medical devices.

This part of ISO 10993 applies to materials that are

— solid and non-absorbable,

— non-solid, such as porous materials, liquids, gels, pastes, and particulates, and

— degradable and/or absorbable, which may be solid or non-solid.

The test sample is implanted into a site and animal species appropriate for the evaluation of the biological safety of the material. These implantation tests are not intended to evaluate or determine the performance of the test sample in terms of mechanical or functional loading. This part of ISO 10993 can also be applied to medical devices that are intended to be used topically in clinical indications where the surface or lining might have been breached, in order to evaluate local tissue responses.

The 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. The objective of the test methods is to characterize the history and evolution of the tissue response after implantation of a medical device/biomaterial including final integration or absorption/degradation of the material. In particular for degradable/absorbable materials, the degradation characteristics of the material and the resulting tissue response should be determined.

This part of ISO 10993 does not deal with systemic toxicity, carcinogenicity, teratogenicity or mutagenicity. However, the long-term implantation studies intended for evaluation of local biological effects might provide insight into some of these properties. Systemic toxicity studies conducted by implantation might satisfy the requirements of this part of ISO 10993. When conducting combined studies for evaluating local effects and systemic effects, the requirements of both standards is to be fulfilled.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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, *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.

3.1
absorb/absorption
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
degradation
decomposition of a material

[SOURCE: ISO 10993-9:2009, 3.1]

3.3
degradation product
any intermediate or final by-product which results from the physical, metabolic, and/or chemical decomposition of a material or substance

[SOURCE: ISO/TR 37137:2014, 2.2, modified]

3.4
degrade
to physically, metabolically, and/or chemically decompose a material or substance

[SOURCE: ISO/TR 37137:2014, 2.3]

3.5
biomaterial
material or substance intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body.

[SOURCE: European Society Biomaterials Conference II]

4 Common provisions for implantation test methods

4.1 General

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, and Annex D.

4.2 Preparation of samples for implantation

4.2.1 Test sample and reference or control material preparation shall be in accordance with ISO 10993-12. The implant size and shape shall be documented and justified. Test samples for various implant sites are described in Annex A, Annex B, Annex C, and Annex D. Physical characteristics (such as form, density,

hardness, surface) 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 characteristics.

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.

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 the 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.

4.2.4 For composite materials (e.g. bone cements, dental materials), the components may be mixed before use and allowed to set before implantation. For multicomponent materials designed to be cured prior to placement, the components may 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) shall be introduced in a manner such that *in situ* polymerization occurs. The procedure used shall be documented and justified

4.2.5 Non-solid materials (including powders) may be contained in open-ended cylindrical tubes for the purpose of testing for local effects after implantation (see ISO 10993-12 for the selection of materials for tubes). Prepare the test material according to the manufacturer's instructions and insert the material into the tube until level with the end, taking care not to contaminate the outer surface of the tube with the test material. If contamination occurs, the sample shall not be implanted. Avoid entrapment of air in the tube and ensure that the end surfaces of the inserted material in the tube and the tube ends are smooth.

Polyethylene (PE), polypropylene (PP), or polytetrafluoroethylene (PTFE) tubes are commonly used for this purpose. PE tubes can be deformed by autoclaving.

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

NOTE For further guidance, see ISO 10993-12.

4.2.7 The physical characteristics such as shape, and especially the surface condition of the control(s), shall be as similar to that of the implant test samples as is practical, with any deviations being explained and justified. When the test material is contained in a tube, the control shall be of the same material as the tube and have the same diameter as the outer diameter of the tube. The choice of the control rod or tube shall be documented and justified.

4.2.8 For implantation studies, the amount or size of the test and control article shall be documented.

4.3 Study design

For devices comprising/composed of two or more different materials, the test articles should be of similar composition or multiple implants may be needed, e.g. if a device is made of HDPE and titanium then the test article should be made of HDPE and titanium.

5 Test methods, general aspects

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 and implantation sites 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 the justification provided.

NOTE For some devices, there are vertical standards prescribing specific implant studies to evaluate local tissue responses, e.g. intraocular lens implant[47] and dental usage tests[12]. These studies 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. 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. In most circumstances, a location marker comprised of an appropriate non-absorbable negative control (e.g. HDPE 1 mm by 2 mm by 5 mm, PP 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.

Exceptionally, 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.

5.2 Animals

5.2.1 All aspects of animal care and accommodation shall be in accordance with ISO 10993-2. In general, small laboratory animals such as mice, rats, hamsters, or rabbits are preferred.

5.2.2 The use of larger animals may be justified based upon special scientific considerations of the particular biomaterial 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.

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 absorbable materials, a pilot study in rodents may be considered to determine the expected rate of degradation before embarking on studies on larger animals.

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 reference control and the test samples should be implanted into the same animal.

5.2.7 However, when a neuroimplantation study (see Annex D) is conducted or when the local effects after implantation are investigated as part of a systemic toxicity study by implantation, control and test samples shall not be placed in the same animal.

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 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. 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. In muscle and connective tissue, depending on the species, and the severity of the surgical trauma, a steady-state is seen in the cell population 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 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 2 weeks post-implantation should be used to assess the early tissue response.

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

   — the absorbable material and/or its degradation products are in a state of limited visually-identifiable presence.

NOTE *In vivo* degradation can occur over a long period of time, sometimes more than one year. Additional animals to extend the observation period (intervals "to-be-determined" group) can be beneficial if the implant has not been completely absorbed within the expected investigational time period and cannot be observed microscopically.

In those situations when the material is not fully absorbed within the late time frame, an appropriate scientific justification can be included for ending the study and the estimated percentage (%) of remaining absorbable material should be reported.

Long term studies that span a significant portion of the degradation time frame for the implant are recommended. Implantation of *in vitro* pre-degraded material (for instance, up to 50 % weight loss or 50 % loss of mechanical strength) may be considered on a case-by-case basis in order to more rapidly observe late stage events after implantation. However, these studies do not replace studies that characterize the real-time *in vivo* degradation profile of the absorbable device.

5.3.4 Characterization of an absorbable device's degradation process may not be applicable to the evaluation of the local effects of the same absorbable material when used in combination: with a drug as carrier for drug release, a scaffold for tissue-engineered medical products, or a surface coating for non-absorbable implants. Since combinations of devices with drugs and/or cells can introduce new issues, the appropriate regulatory authorities should be consulted regarding study designs for absorbable combination products.

5.3.5 Although this part of ISO 10993 does not address the issues of systemic toxicity given in ISO 10993-11, it is recommended that the information required to meet this part of ISO 10993 be obtained from any systemic toxicity studies using implantation.

5.3.6 For long-term studies, generally accepted observation periods for non-absorbable biomaterials are given in Table 1. Animals should be humanely sacrificed at each time point, in line with ISO 10993-2. Serial harvest under general anaesthesia with recovery may be acceptable under special circumstances, which shall be documented and justified.

Table 1 — Possible test periods for long-term implantation of biomaterials

Species	Implantation period in weeks[a]				
	13	26	52	78	104
Mice	X	X	X	—	—
Rats	X	X	X	—	—
Guinea-pigs	X	X	X	—	—
Rabbits	X	X	X	X	X
Dogs	X	X	X	X	X
Sheep	X	X	X	X	X
Goats	X	X	X	X	X
Pigs	X	X	X	X	X

[a] These implantation periods are commonly used; however, other periods may be applicable based on the specific characteristics of the test material. Depending on the intended use of the test material, not all implantation periods may be necessary.

5.4 Surgery and testing conditions

5.4.1 Surgery shall be performed under general anaesthesia. If another type of anaesthesia is used, this shall be justified and shall be in compliance with ISO 10993-2. The specific insertion or implantation procedures for subcutaneous, intramuscular, bone or neural implantation are described in Annex A, Annex B, Annex C, and Annex D, respectively.

