INTERNATIONAL STANDARD

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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

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 10993-6 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This second edition cancels and replaces the first edition (ISO 10993-6:1994) which has been technically revised.

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 system*

— *Part 2: Animal welfare requirements*

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

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

— *Part 5: Tests for in vitro cytotoxicity*

— *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 delayed-type hypersensitivity*

— *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*

— *Part 20: Principles and methods for immunotoxicology testing of medical devices*

For the purposes of this part of ISO 10993 the CEN annex regarding fulfilment of European Council Directives will be removed at publication stage.

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-biodegradable;

— degradable and/or resorbable;

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

The test specimen 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 specimen in terms of mechanical or functional loading. This part of ISO 10993 may also be applied to medical devices that are intended to be used topically in clinical indications where the surface or lining may 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 specimen to that caused by control materials used in medical devices of which the 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 resorption/degradation of the material. In particular for degradable/resorbable 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 may provide insight into some of these properties. Systemic toxicity studies conducted by implantation may satisfy the requirements of this part of ISO 10993.

2 Normative references

The following referenced documents are indispensable for the application 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 10993-1:2003, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management system*

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

ISO 10993-11, *Biological evaluation of medical devices — Part 11: Tests for systemic toxicity*

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
degradation
decomposition of a material

[ISO 10993-9:1999, definition 3.1]

3.2
degradation product
product of a material which is generated by the chemical breakdown or decomposition of the material

[ISO 10993-16:1997, definition 3.1]

3.3
biomaterial
material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body

[Taken from 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 recognised organization and in accordance with all appropriate regulations dealing with laboratory animal welfare. These studies shall be performed under good laboratory practices or other recognized quality assurance systems, and comply with the requirements of ISO 10993-2.

The provisions of this clause shall apply to the test methods described in Annexes B, C and D.

4.2 Preparation of specimens for implantation

Test sample and reference or control material preparation shall be in compliance with ISO 10993-12. The implant size and shape shall be documented and justified. Test specimens for various implant sites are described in Annexes B, C and 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.

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 specimens 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.

For materials used as scaffolds for tissue-engineered medical products, it may be appropriate not to use the final preparation pre-populated with cells, as the immune reaction of the animal to the cellular components of such products and the reaction of the cells to the animal, may interfere with the resulting local tissue response.

For composite materials (e.g. bone cements, dental materials), the components may be mixed before use and allowed to set before implantation. However, materials that are designed for use in devices with *in situ* polymerization shall be introduced in a manner such that *in situ* polymerization occurs. For certain types of study other procedures may be used. The procedure used shall be documented and justified.

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.

NOTE 1 Polyethylene (PE), polypropylene (PP) or polytetrafluoroethylene (PTFE) tubes are commonly used for this purpose. PE tubes may be deformed by autoclaving. PTFE tubes are difficult to section in the microtome, and substitution by PE or PP tubes of the same dimensions may be preferable when the tubes are to remain in the tissue blocks during sectioning.

Evaluation shall be undertaken by comparing to the tissue reaction to that of a similar specimen/material of which the clinical acceptability and biocompatibility characteristics have been established.

NOTE 2 For further guidance, see ISO 10993-12.

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 specimens as is practically possible, 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.

5 Test methods, general aspects

5.1 Tissue and implantation site

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 Annexes B, C and D. If other implantation sites are chosen, the general scientific principles behind the test methods described in Annexes B, C and D shall still be adhered to and the justification provided.

NOTE 1 For special dental usage test, see ISO 7405.

For degradable/resorbable 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 specimen is recommended. In certain circumstances an appropriate negative control may be used as a marker for the location of the implant site. 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.

NOTE 2 Markers for identification of the implant site of resorbable test specimens may be non-absorbable sutures or skin paints.

5.2 Animals

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.

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

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 specimens, 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 (see Annex B).

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.

Before starting an animal study with degradable materials, relevant information from *in vitro* degradation studies should be considered. For biodegradable materials a pilot study in rodents should be undertaken to determine the expected rate of degradation before embarking on studies on larger animals.

The specimens 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 in an animal depends on the size of the species and the anatomical location. Whenever possible, the reference control and the test specimens should be implanted into the same animal.

However, when the local effects after implantation are investigated as part of a systemic toxicity study by implantation, control and test samples should not be placed in the same animal.

5.3 Test periods

The test period shall be determined by the likely clinical exposure time or be continued until or beyond a steady state has been reached with respect to the biological response. The time points selected shall be explained and justified.

For non-degradable and non-resorbable 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. In general, it is expected that experiments that go up to or beyond the point of absorption are needed for the evaluation of degradable materials.

For degradable/resorbable materials the test period shall be related to the estimated degradation time of the test product. Annex A gives general considerations regarding degradable/absorbable materials. Before starting with animal studies and determining the time points for sample evaluation, an estimation of the degradation time shall be made. This can be done *in vitro* by real-time or accelerated degradation studies or in certain circumstances by mathematical modelling. In general, experiments that extend up to or beyond the point of absorption are needed for the evaluation of degradable materials. The evaluation of degradable materials will depend in part on the degradation rate of the materials.

Local tissue responses shall be evaluated relative to the degradation process of the implant at various time points:

— where there is no or minimal degradation, usually to be evaluated at 1 week to 12 weeks after implantation;

— when degradation is taking place;

— when a steady state has been reached resulting in tissue restoration or degradation nearing completion.

In the absence of complete degradation, absorption, or restoration to normal tissue structure and function, the overall data collected may be sufficient to allow characterization of the local effects after implantation.

NOTE *In vivo* degradation may need a rather long period of time, sometimes more than one year. Additional animals may be beneficial to extend the observation period when the implant has not been degraded completely within the expected investigational time period.

Although this part of ISO 10993 does not address the issues of systemic toxicity as 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.

For long-term implantation studies, generally accepted observation periods are given in Table 1.

Animals should be killed 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 — Selection of test periods for long-term implantation

Species	Implantation period in weeks				
	12	26	52	78	(104) [a]
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] Depending on the intended use of the test material, not all implantation periods may be necessary (see ISO 10993-12). An observation period of 104 weeks may be of interest in selected instances.

5.4 Surgery and testing conditions

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 or bone implantation are described in Annexes B, C and D, respectively.

The number of implants per animal and the number of animals per observation period are described in Annexes B, C and D. A sufficient number of implants shall be inserted to ensure that the final number of specimens to be evaluated will give valid results.

The surgical technique may profoundly influence the result of any implantation procedure. Surgery shall be carried out under aseptic conditions and in a manner that minimizes trauma at the implant site. Remove the hair from the surgical area by clipping, shaving or other mechanical means. Disinfect the exposed area of skin with an appropriate disinfectant. Ensure that the implants or wound surfaces do not come in contact with the hair. After surgery close the wound, using either sutures or wound clips, taking precautions to maintain aseptic conditions.

The health of the animals shall be observed and recorded at regular intervals during the study. Following surgery, each animal shall be observed at appropriate intervals during the test period, and any abnormal findings shall be recorded, including local, systemic and behavioural abnormalities, and their potential influence on the results obtained described in the test reports.