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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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Contents

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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.

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.

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

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

— *Part 1: Guidance on selection of tests*

— *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 cytotoxicity: in vitro methods*

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

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 9: Degradation of materials related to biological testing* [Technical Report]

— *Part 10: Tests for irritation and sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymers*

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

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

— *Part 16: General guidance on toxicokinetic study design for degradation products and leachables from medical devices*

— *Part 17: Glutaraldehyde and formaldehyde residues in industrially sterilized medical devices*

Future parts will deal with other relevant aspects of biological testing.

Annexes A, B and C of this part of ISO 10993 are for information only.

Introduction

This International Standard gives methods of biological testing of medical and dental materials and devices, and their evaluation in regard to their biocompatibility.

ISO 10993-1 offers a guide for selection of methods for biological testing. The intention is to reduce animal tests to the justifiable minimum (see ISO 10993-2). A search of the literature precedes any testing, as data concerning the biological safety of the candidate material could be available.

The test methods described in this part of ISO 10993 are based on established implantation tests. This part of ISO 10993 describes animal tests for the study of local effects after implantation. The use of *in vivo* implantation techniques for characterizing the biological response of tissues to materials allows for the assessment of such materials not achieved by other procedures.

These test methods may not be appropriate for all types of medical devices. The user is cautioned to consider the appropriateness of the method in view of the materials being tested, their potential applications, and the recommendations contained in ISO 10993-1.

ISO/TC 194 appreciates any information for the further development of this part of ISO 10993.

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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 of an implant material on living tissue, at both the macroscopic and microscopic level.

The test specimen is implanted into a site and tissue appropriate for evaluation of the biological safety of the material. The implant is not intended to be subjected to mechanical or functional loading. The local effects are evaluated by a comparison of the tissue response caused by a test specimen to that caused by materials used in medical devices whose clinical acceptability has been established.

The test methods for local effects after implantation are used to assess subchronic effects (short-term, up to 12 weeks), or chronic effects (long-term, longer than 12 weeks).

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 10993. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10993-1:1992, *Biological evaluation of medical devices — Part 1: Guidance on selection of tests.*

ISO 10993-2:1992, *Biological evaluation of medical devices — Part 2: Animal welfare requirements.*

3 Common provisions for implantation test methods

3.1 General

For the purposes of this part of ISO 10993, the definitions given in ISO 10993-1 and ISO 10993-2 apply.

The provisions in this clause shall apply to the test methods described in clauses 4 to 6.

It is important that the researcher plans the study in such detail that the maximum of information can be extracted from the use of each animal (see ISO 10993-2).

3.2 Preparation of specimens for implantation

3.2.1 Solid specimens (excluding powders)

Physical characteristics (that is form, density, hardness, surface finish) can influence the character of the tissue response to the test material.

Each implant shall be manufactured, processed, cleaned of contaminants and sterilized by the method intended for the final product.

After final preparation and sterilization, the implant specimens shall be handled in such a way as to en-

sure that they are not scratched, damaged or contaminated in any way prior to or during insertion.

3.2.2 Non-solid specimens (including powders)

Non-solid specimens may be liquids, pastes and particulates, as distinct from the materials covered in 3.2.1. The components may be mixed before use (e.g. bone cements, dental materials), and set after varying time periods.

The materials may be contained in tubes for the purpose of testing for local effects after implantation. Polyethylene (PE), polypropylene (PP), or polytetrafluoroethylene (PTFE) tubes are commonly used for this purpose.

Prior to test the tubes shall be rinsed with 70 % (*V/V*) ethanol and distilled water and sterilized by autoclaving or other appropriate methods relevant for clinical applications. Materials tested in their freshly mixed state shall be tested for microbiological contamination.

Prepare the test material according to the manufacturer's instructions and insert the material into the tube until level with the top. Exercise the utmost care to prevent contamination of the outer surface of the tube by the test material. 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 PE tubes may be deformed by autoclaving. It is difficult to section PTFE tubes 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.

3.2.3 Control specimens

The size, 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. When the test material is contained in a tube, the control shall be a rod of the same material as the tube and with the same diameter as the outer diameter of the tube. The control specimens shall be handled, cleaned and sterilized in such a manner as to maintain them as acceptable and well characterized controls.

Selection of control material(s) should be based on their established use in clinical applications similar to those proposed for the candidate test material and is not restricted to those indicated in annex A and C.1.

3.3 Animals, tissues, test periods, surgery, postoperative care, euthanasia

3.3.1 Animals and tissues

Animal husbandry shall be in accordance with ISO 10993-2 and/or national regulatory requirements for laboratory animals.

Select an animal species with due consideration of the size of the implant test specimens, the intended duration of the test in relation to the expected lifespan of the animals, as well as the recognized species differences in biological response in both hard and soft tissues.

For short-term testing in subcutaneous tissue and muscle, animals such as mice, rats, guinea-pigs and rabbits are commonly used. Select one species among these.

For long-term testing in subcutaneous tissue, muscle and bone, animals such as rats, guinea-pigs, rabbits, dogs, sheep, goats, pigs and other animals with a relatively long life expectancy are suitable. Select one species among these.

The specimens of test and control materials shall be implanted under the same conditions in the same species 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.

3.3.2 Test periods

The local tissue response to implanted materials is assessed in short-term tests up to 12 weeks and in long-term tests exceeding 12 weeks.

Test periods are chosen to ascertain that a steady state has been reached with respect to biological response. The local biological response to implanted materials depends both on the properties of the materials and on the trauma of surgery. The tissue configuration found in the vicinity of an implant changes with the time elapsed after surgery. Usually, at one week observation periods, a high cell activity is found, followed by a transitional stage. In muscle and connective tissue, depending on the species, a steady state is seen in the cell population after 9 to 12 weeks. Implantation in bone tissues may need longer observation periods.

Test periods shall be selected from those specified in table 1 for short-term implantation, or from table 2 for long-term implantation.

Table 1 — Selection of test periods for short-term implantation in subcutaneous tissue and muscle

Species	Implantation period weeks				
	1	3	4	9	12
Mice	x	x		x	
Rats	x		x		x
Guinea-pigs	x		x		x
Rabbits	x		x		x

Table 2 — Selection of test periods for long-term implantation in subcutaneous tissue, muscle and bone

Species	Implantation period weeks				
	12	26	52	78	(104)
Rats	x	x	x		
Guinea-pigs	x	x	x		
Rabbits	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

Depending on the intended use of the test material, not all implantation periods may be necessary (see ISO 10993-1). An observation period of 104 weeks may be of interest in selected instances.

The number of implants per animal and the number of animals per observation period are described in clauses 4 to 6. A sufficient number of implants shall be inserted to ensure that the final number of specimens to be evaluated will give valid results.

3.3.3 Surgery

Anaesthetize the animals. Remove hair from the surgical area by clipping, shaving or other mechanical means. Wash the area with an antiseptic solution. Ensure that hair does not come in contact with the implants or the wound surfaces. The specific insertion or implantation procedures are described in clauses 4 to 6.

The surgical technique may profoundly influence the result of any implantation procedure. The surgery shall be carried out under aseptic conditions and in a manner that minimizes trauma at the implant site.

After surgery close the wound, using either wound clips or sutures, taking precautions to maintain aseptic conditions.

3.3.4 Post-operative assessment

Observe each animal at appropriate intervals during the test period and record any abnormal findings, including local, systemic and behavioural abnormalities.

3.3.5 Euthanasia

At the termination of the experimental period, euthanize the animals with an overdose of anaesthetic or by some other acceptable humane method (see ISO 10993-2).

3.4 Evaluation of biological response

Evaluate the biological response by grading and documenting the macroscopic and histopathological test responses as a function of time. Compare the responses to the test material and control material.

Carry out comparison of the control and the test implants at equivalent locations relative to each implant so that the effect of relative motion between the tissue and implant is at a minimum (see note 2).

NOTE 2 For a cylindrical specimen the region is midway between its ends. With grooved cylindrical implants the centre portions between the grooves as well as the flat top end surfaces of the implant are suitable for evaluation.

For a non-solid or particulate material incorporated into a tube, the area at the end of the tube is the only available area for evaluation.

3.4.1 Macroscopic assessment

Examine each implant site with the aid of a low magnification lens. Record the nature and extent of any tissue reaction observed.

3.4.2 Preparation for histology — Implant retrieval and specimen preparation

Excise the implant together with sufficient unaffected surrounding tissue to enable evaluation of the local biological response. Process the excised tissue blocks containing test or control implants for histopathological and other studies as appropriate.

When conventional techniques are used, the tissue envelope may be opened before or after exposure to

a fixative and the condition of the implant surface and tissue bed shall be reported. However, with this technique the tissue layers closest to the implant are usually destroyed.

When the implant/tissue interface is to be studied, embedding of the intact tissue envelope with the implant *in situ* using hard plastics is preferred. Appropriate sectioning or grinding techniques are employed for the preparation of histological sections. It shall be demonstrated that the technique of embedding in plastics does not markedly alter the interface tissue.

3.4.3 Histological assessment

The extent of response may be determined by measurement of the distance from the implant/tissue interface to unaffected areas with the characteristics of normal tissue and of normal vascularity. Record the section orientation in relation to the implant dimensions. Record the implant orientation, number of sections and cutting geometry.

The biological response parameters which shall be assessed and recorded include:

a) extent of fibrosis/fibrous capsule and inflammation;

b) degeneration as determined by changes in tissue morphology;

c) number and distribution as a function of distance from the material/tissue interface of the inflammatory cell types, namely polymorphonuclear leucocytes, lymphocytes, plasma cells, eosinophils, macrophages and multinucleated cells;

d) presence of necrosis as determined by nuclear debris and/or capillary wall breakdown;

e) other parameters such as material debris, fatty infiltration, granuloma;

f) for porous implant materials, the quality and quantity of tissue ingrowth.

In the case of bone, the interface between the tissue and the material is of special interest. Evaluate the area of bone contact and the amount of bone in the vicinity of the implant as well as the presence of intervening non-calcified tissues. Note the presence of bone resorption and bone formation.

3.5 Test report

3.5.1 Content of test report

The test report shall have sufficient detail to allow independent assessment of the results. The report shall include the items listed in 3.5.2 to 3.5.6.

3.5.2 Implant specimens

Description of test and control materials, material condition, fabrication, surface condition, and the shape and size of implants.

Report the rationale for selection of control material(s).

The surface preparation of the specimens can affect the tissue reaction. Therefore, the preparation procedure should be noted in the report.

Report cleaning, handling and sterilization techniques employed. If not done in-house, this information should be supplied by the manufacturer before the investigation commences.

3.5.3 Animals and implantation

Report origin, age, sex and strain of animals. Report housing conditions, diet and mass of animals during the study period. The health of the animals shall be evaluated during the study. All observations, including unexpected death, shall be reported.

Report insertion techniques. Report number of implants inserted per animal, per site and per observation period.

3.5.4 Retrieval and histological procedure

The report shall include a description of the retrieval technique. The number of implants retrieved per animal and per observation period shall be recorded. All specimens shall be accounted for and considered as part of the test. The techniques employed for the fixation and preparation of the histological sections shall be described.

3.5.5 Evaluation

Macroscopic observations shall include the observations made on each implant as well as the macroscopic appearance of the tissue surrounding the implant. The report shall include the results obtained from each histological examination.

3.5.6 Final evaluation

The report shall include a comparative evaluation of the biological responses to test and control materials, as well as a descriptive narrative of the biological response.

4 Test method for implantation in subcutaneous tissue

4.1 Field of application

This test method is used for assessing the biological response of subcutaneous tissue to an implanted material.

The study may be used to compare the effect of different surface textures or conditions of the same material, or to assess the effect of various treatments or modifications of a material.

4.2 Principle

Insertion of the implants in the subcutaneous tissue of test animals. The method compares the biological response to implants of test specimens with the biological response to implants of control specimens made of materials which are established in clinical use (see 3.2.3).

4.3 Test specimens

Common provisions for preparation of test and control specimens are described in 3.2. Implant sizes are based on the size of the test animal.

4.3.1 Specimens made of sheet material shall be 10 mm to 12 mm in diameter and from 0,3 mm to 1 mm in thickness.

NOTE 3 The subcutaneous site, deep to the panniculus carnosus muscle, is particularly suitable for the evaluation of polymeric sheet material. In an intramuscular site, sheet material may become folded, which makes it difficult to assess the effect of the material *per se*.

4.3.2 Bulk materials shall be fabricated into specimens 1,5 mm in diameter and 5 mm in length, and have radiused ends.

4.3.3 Grooved specimens shall be 4 mm in diameter and 7 mm in length (see annex B).

NOTE 4 Tissue ingrowth into the grooves minimizes tissue irritation caused by interface motion.

4.3.4 Non-solid specimens (including powders) shall be prepared in tubes 1,5 mm in diameter and 5 mm in length (see 3.2.2).

4.4 Test animals and implant sites

The implants shall be inserted in the dorsal subcutaneous tissue of adult mice, rats, guinea-pigs or rabbits. Select one species among these.

Use at least three animals and sufficient sites to yield 10 specimens for each material and implantation period.

4.5 Implantation procedure

Select one of the procedures described in 4.5.1 and 4.5.2.

4.5.1 Implantation along dorsal midline

Make an incision of the skin and make one or more subcutaneous pockets by blunt dissection. The base of the pocket shall be more than 10 mm from the line of incision. Place one implant in each pocket. The implants shall not be able to touch one another.

NOTE 5 Alternatively, the implants may be delivered by a trocar to the desired site.

4.5.2 Implantation in neck

In mice, make a 10 mm long incision above the sacrum and prepare a subcutaneous tunnel by blunt dissection towards the neck. Push one implant (for design see annex B) through the tunnel to position it at the neck.

In rats, insert one implant of each of the control and candidate materials separately on each side of the neck. The implants shall not be able to touch one another.

At some distance from the implant, close the tunnel with stitches of appropriate suture material to prevent the implant from moving.

4.6 Implantation period

To ensure a steady state of biological tissue response the implantation period(s) shall be as specified in 3.3.2.

4.7 Evaluation of biological response

The evaluation shall take into account the items specified in 3.4.