DRAFT INTERNATIONAL STANDARD ISO/DIS 10993-6

ISO/TC **194** Secretariat: **DIN**

Voting begins on: **2004-12-23** Voting terminates on: **2005-05-23**

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

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

[Revision of first edition (ISO 10993-6:1994)]

ICS 11.100.20

ISO/CEN PARALLEL ENQUIRY

The CEN Secretary-General has advised the ISO Secretary-General that this ISO/DIS covers a subject of interest to European standardization. **In accordance with the ISO-lead mode of collaboration as defined in the Vienna Agreement, consultation on this ISO/DIS has the same effect for CEN members as would a CEN enquiry on a draft European Standard.** Should this draft be accepted, a final draft, established on the basis of comments received, will be submitted to a parallel two-month FDIS vote in ISO and formal vote in CEN.

In accordance with the provisions of Council Resolution 15/1993 this document is circulated in the English language only.

Conformément aux dispositions de la Résolution du Conseil 15/1993, ce document est distribué en version anglaise seulement.

To expedite distribution, this document is circulated as received from the committee secretariat. ISO Central Secretariat work of editing and text composition will be undertaken at publication stage.

Pour accélérer la distribution, le présent document est distribué tel qu'il est parvenu du secrétariat du comité. Le travail de rédaction et de composition de texte sera effectué au Secrétariat central de l'ISO au stade de publication.

THIS DOCUMENT IS A DRAFT CIRCULATED FOR COMMENT AND APPROVAL. IT IS THEREFORE SUBJECT TO CHANGE AND MAY NOT BE REFERRED TO AS AN INTERNATIONAL STANDARD UNTIL PUBLISHED AS SUCH.

IN ADDITION TO THEIR EVALUATION AS BEING ACCEPTABLE FOR INDUSTRIAL, TECHNOLOGICAL, COMMERCIAL AND USER PURPOSES, DRAFT INTERNATIONAL STANDARDS MAY ON OCCASION HAVE TO BE CONSIDERED IN THE LIGHT OF THEIR POTENTIAL TO BECOME STANDARDS TO WHICH REFERENCE MAY BE MADE IN NATIONAL REGULATIONS.

Contents

Page

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 (EN 30993-6:1994), 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*

— *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 the 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: Method for the establishment of allowable limits for leachable substances*

— *Part 18: Chemical characterization of materials*

— *Part 19: Physico-chemical, mechanical and morphological characterization*

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

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

Biological evaluation of medical devices — Part 6: Tests for local effects after implantation

1 Scope

This part of the ISO 10993 specifies test methods for the assessment of the local effects after implantation of biomaterials intended to be used in medical devices. The standard applies to solid biomaterials that are non-degradable, degradable and/or resorbable, non-solid biomaterials, such as porous materials, and liquids, pastes and particulates.

The test specimen is implanted into a site and tissue appropriate for the evaluation of the biological safety of the material. This implantation test is not intended to determine the performance of the test specimen in terms of mechanical or functional loading. This standard may also be applied to medical devices which are intended to be used on surfaces breached or intact, 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 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 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 standard 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 issues.

NOTE For the convenience of the user of this standard a bibliography is included.

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, *Biological evaluation of medical devices – Part 1: Guidance on selection of tests*

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 10993-16 and the following apply.

3.1
degradation
chemical breakdown or decomposition of material

4 Common provisions for implantation test methods

4.1 General

The provisions of clause 4 shall apply to the test methods described in Annex B, C and D.

All animal studies shall be performed in a facility approved by a nationally recognized organisation 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 in ISO 10993 -2.

It is important that the researcher plans the study in such detail 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).

4.2 Preparation of specimens for implantation.

Test sample and reference material preparation shall be in compliance with 10993-12. The implant size and shape shall be documented and justified. Test specimens for various implant sites are described in Annex B to 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.

Each implant shall be manufactured, processed, cleaned of contaminants and sterilized by the method intended for the final product and accordingly documented. After final preparation and sterilization, the implant specimens shall be handled aseptically and in such a way as to ensure that they are not scratched, 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 with cells, as the immune reaction of the laboratory animal to the cellular components of such products 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 set after varying time periods. Materials that are designed for use in devices with *in situ* polymerisation shall be introduced in a manner such that *in situ* polymerisation occurs. For certain types of studies other procedures may be used. The procedure used shall be documented.

Non-solid materials (including powders) may be contained in 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 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 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 is done by comparison of 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 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. In general, a positive control is not necessary.

5 Test methods, general aspects

5.1 Tissue and implantation site

The test specimen shall be implanted into the tissues most relevant to the intended clinical use of the material. The justification for the choice of tissue shall be documented. Test methods for various implantation sites are given in Annex B to D. If other implantation sites are chosen, the general scientific principles behind the test methods described in Annex B to D shall still be adhered to.

For degradable/resorbable materials the implantation site shall be marked in a manner suitable for identification of the site at the designated time periods. The use of a 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. A sham surgical procedure might be used to evaluate the impact of the procedure on the tissue involved.

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

5.2 Animals

Animal husbandry 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 considerations of the particular biomaterial under study.

Select an animal species with due considerations of 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.

For short-term testing in subcutaneous tissue, muscle, and bone, animals such as mice, rats, guinea pigs or 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.

NOTE Especially for biodegradable materials it is recommended that a pilot study in rodents be undertaken to determine the expected rate of degradation before embarking on studies in larger animals.

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. Whenever possible, the reference control and the test specimen should be 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. For non-degradable/resorbable

materials the short-term responses are normally assessed from 1 week up to 4 weeks and long-term tests exceeding 12 weeks. 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 time elapsed after surgery. Up to two weeks, 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 to 12 weeks. Implantation in bone tissue may need longer observation periods before a steady state is reached.

For degradable/resorbable materials the test period shall be related to the estimated degradation time of the test product. See Annex A for general considerations regarding degradable/absorbable materials. Before starting with animal studies 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 it is to be expected that long-term experiments (exceeding 12 weeks) are needed for the evaluation of degradable materials.

The milestones for evaluation of degradable biomaterials will depend on the degradation rate of the materials. The following may be used for guidance:

— Early responses may be determined up to 2 weeks after implantation;

— **Short-term:** local reaction to be evaluated at 2-12 weeks after implantation;

— **Intermediate:** when it is anticipated that degradation is taking place;

— **Long-term:** when it is estimated that degradation is nearing completion, or a steady state has been reached.

Even 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 1 In vivo degradation may need a rather long period of time for up to more then one year. Additional animals may be used to extend the observation period when the implant has not been degraded completely within the expected investigational time period.

NOTE 2 Although this standard does not address the issues of systemic toxicity (ISO 10993-11), it is recommended that the information required to meet this standard (ISO 10993-6) be obtained from any systemic toxicity studies using implantation.

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

Table 1 — Selection of test periods for implantation in subcutaneous tissue, muscle and bone

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 normally 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 Annex B, C and D, respectively.

The number of implants per animal and the number of animals per observation period are described in Annex B to 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. The 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 another mechanical means. Wash the area with an antiseptic solution. 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 should be observed at appropriate intervals during the test period, and any abnormal findings should be recorded, including local, systemic and behavioural abnormalities.

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

5.5 Evaluation

5.5.1 General

Evaluate the biological response by grading and documenting the macroscopic and histopathological responses as a function of time. Compare the responses to the test sample to the responses to the control sample or sham operated sites.

Carry out comparison of the control and 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.

NOTE 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 each of the endpoints sufficient number of samples shall be evaluated as defined in Annex B, C and D. These samples shall be obtained from at least 3 different animals.

5.5.2 Macroscopic assessment

Each implant site shall be examined for alterations of the normal structure. This shall include regional draining lymph nodes. Use of a lens with low magnification is recommended. Record the nature and extent of any tissue reaction observed such as haematoma, oedema, and/or additional gross findings. Record presence, form and location of implant including possible remnants of degradable materials. Macro photography might be considered for maintaining a permanent record.

Additionally to the inspection of the implant site, whenever an animal has show signs of ill health or reactions to the implant, a gross pathology as appropriate shall be conducted.

5.5.3 Implant retrieval

Excise the implant together with sufficient unaffected surrounding tissue to enable evaluation of the local histopathological response. If the candidate material is not evident at the site examined (degradable/resorbable materials), extend the explantation site to include several mm of normal tissue on all