BRITISH STANDARD

BS EN ISO 10993-6:2009

Biological evaluation of medical devices

Part 6: Tests for local effects after implantation (ISO 10993-6:2007)

ICS 11.100.20

BSI British Standards

National foreword

This British Standard is the UK implementation of EN ISO 10993-6:2009. It is identical to ISO 10993-6:2007. It supersedes BS EN ISO 10993-6:2007 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee CH/194, Biological evaluation of medical devices.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard cannot confer immunity from legal obligations.

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This European Standard was approved by CEN on 28 April 2009.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN Management Centre has the same status as the official versions.

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Management Centre: Avenue Marnix 17, B-1000 Brussels

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Foreword

The text of ISO 10993-6:2007 has been prepared by Technical Committee ISO/TC 194 “Biological evaluation of medical devices” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 10993-6:2009 by Technical Committee CEN/TC 206 “Biological evaluation of medical devices” the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by November 2009, and conflicting national standards shall be withdrawn at the latest by March 2010.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 10993-6:2007.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directives 93/42/EEC on Medical Devices and 90/385/EEC on Active Implantable Medical Devices.

For relationship with EU Directives, see informative Annex ZA and ZB, which is an integral part of this document.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of ISO 10993-6:2007 has been approved by CEN as a EN ISO 10993-6:2009 without any modification.

Annex ZA
(informative)

Relationship between this European Standard and the Essential Requirements of EU Directive 93/42/EEC on Medical Devices

This European Standard has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association to provide a means of conforming to Essential Requirements of the New Approach Directive 93/42/EEC on medical devices.

Once this standard is cited in the Official Journal of the European Communities under that Directive and has been implemented as a national standard in at least one Member State, compliance with the clauses of this standard given in table ZA confers, within the limits of the scope of this standard, a presumption of conformity with the corresponding Essential Requirements of that Directive and associated EFTA regulations.

Table ZA — Correspondence between this European Standard and Directive 93/42/EEC on medical devices

Clause(s)/sub-clause(s) of this EN	Essential Requirements (ERs) of Directive 93/42/EEC	Qualifying remarks/Notes
4, 5, 6 & Annexes B,C, D	Annex I: 7.1, 7.2, 7.5	

WARNING — Other requirements and other EU Directives may be applicable to the product(s) falling within the scope of this standard.

Annex ZB
(informative)

Relationship between this European Standard and the Essential Requirements of EU Directive 90/385/EEC on Active Implantable Medical Devices

This European Standard has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association to provide a means of conforming to Essential Requirements of the New Approach Directive 90/385/EEC on active implantable medical devices.

Once this standard is cited in the Official Journal of the European Communities under that Directive and has been implemented as a national standard in at least one Member State, compliance with the clauses of this standard given in table ZB confers, within the limits of the scope of this standard, a presumption of conformity with the corresponding Essential Requirements of that Directive and associated EFTA regulations.

Table ZB — Correspondence between this European Standard and Directive 90/385/EEC on active implantable medical devices

Clause(s)/sub-clause(s) of this EN	Essential Requirements (ERs) of Directive 90/385/EEC	Qualifying remarks/Notes
4, 5, 6 & Annex B, C, D	Annex I : 9	

WARNING — Other requirements and other EU Directives may be applicable to the product(s) falling within the scope of this standard.

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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.

INTERNATIONAL STANDARD ISO 10993-6:2007(E)

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.

When indicated by signs of ill health, body-mass measurements should be taken at appropriate intervals. The use of post-operative analgesics shall be in line with the requirements of ISO 10993-2.

At the end of the experimental period, euthanize the animals with an overdose of anaesthetic or by some other humane method in line with the principles set out in ISO 10993-2.

5.5 Evaluation

5.5.1 General

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

NOTE Examples of grading systems are given in Annex E and in the Bibliography.

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.

For a cylindrical specimen this 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 a sufficient number of samples shall be evaluated as defined in Annexes 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 should include assessment of the regional draining lymph nodes Tilney [32]. Use of a lens with low magnification is recommended. Record the nature and extent of any tissue reaction observed, such as haematoma, oedema, encapsulation and/or additional gross findings. Record the presence, form and location of implant including possible remnants of degradable materials. Macro photography shall be used for documentation.

In addition to the inspection of the implant site, whenever an animal has shown signs of ill health or reactions to the implant, a gross necropsy as appropriate shall be conducted.

5.5.3 Implant retrieval and tissue sample collection

After the animal has been humanely killed, 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 millimetres of normal tissue on all sides of the expected implant site. For non-degradable implants, draining lymph nodes should be collected as indicated by the gross pathology. For degradable implants, draining lymph nodes should be collected, when feasible, as evaluation of draining lymph nodes is of importance to demonstrate migration of degradable materials.

NOTE 1 It is recognised that it is not always possible to locate the draining lymph nodes of all specimens.

If indicated by ill health and gross pathology, or by experimental design to assess systemic toxicity, other organs shall be collected as appropriate.

Process the excised tissue samples according to appropriate procedures needed for histological evaluation, including fixation, excision, embedding, sectioning and staining. If appropriate, record the orientation of the implant, number of sections and cutting geometry.

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. Take care not to destroy the implant/tissue interface.

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 used for the preparation of histological sections. It shall be demonstrated that the technique of embedding in plastics does not markedly alter the interface tissue.

NOTE 2 For "soft" implants in soft tissues, processing of the tissue samples can be performed without removing the implant.

5.5.4 Microscopic assessment

The scoring system used for the histological evaluation shall take into account the extent of the area affected, either quantitatively (e.g. in micrometres) or semi-quantitatively (see Annex E). Record the implant orientation, number of sections and cutting geometry.

Record the section orientation in relation to the implant dimensions.

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

— the extent of fibrosis/fibrous capsule (layer in micrometres) and inflammation;

— the degeneration as determined by changes in tissue morphology;

— the number and distribution as a function of distance from the material/tissue interface of the inflammatory cell types, namely polymorph nuclear neutrophilic leucocytes, lymphocytes, plasma cells, eosinophils, macrophages and multinucleated cells;

— the presence, extent and type of necrosis;

— other tissue alterations such as vascularization, fatty infiltration, granuloma formation and bone formation;

— the material parameters such as fragmentation and/or debris presence, form and location of remnants of degraded material;

— the quality and quantity of tissue ingrowth, for porous and degradable implant materials.

Adverse histological responses shall be documented by photomicrograph.

For degradable/resorbable materials, at the intermediate or nearly complete degradation levels, some residual material of the degradable implant should be present in the tissue samples examined. In addition, for evaluation of the restoration to normal structure, representative areas of the implant site shall be evaluated, as indicated by marker or template.

For implants in 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. Record the presence of bone resorption or new bone formation if these are present.

5.5.5 Evaluation of responses

Examples of quantitative scoring systems are described in [17], [18], [25] and [26].

Examples of semi-quantitative scoring systems are shown in Annex E.

In addition, examples of other scoring systems are included in the Bibliography.

6 Test report

The test report shall have sufficient detail to allow an independent assessment of the results. The report shall include the items listed in 5.1 to 5.5. In addition, the following items shall be reported.

a) Implant specimens

— Description of test and control materials, such as identification, surface condition, and the shape, size, weight and form of the implants. The rationale for choice of control sample and the physical form of the material implanted shall be given.

b) Animals and implantation

— Species, strain, sex, age and/or weight and origin shall be reported and justified. Test conditions including housing and diet shall be reported. All observations during the study shall be recorded and documented.

— Insertion techniques including the surgical procedure, anaesthesia and post-surgical analgesia, and location and number of implants per animal shall be recorded and reported. Problems associated with implantation or explantation and all observations made during the study shall be recorded.

c) 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.

— Implant evaluation, including gross observations of implants, tissues and organs shall be recorded. Techniques employed for the fixation and preparation of the histological sections shall be described.

— Methods and results of histological evaluation of implant site and any organs showing alterations at necropsy, when indicated.

— For degradable materials, the report shall have a description of the degree of degradation, including material characteristics at explantation (free particles, fibre formation, amorphous gel, crystallinity).

d) Macroscopic and microscopic evaluation

— Macroscopic observations shall include the observations made on each implant as well as the macroscopic appearance of the tissue surrounding the implant. When applicable, this shall include observation of the draining lymph nodes.

— The report shall include the results obtained from each histological examination and (statistical) analysis when applied.

e) Final evaluation

— The report shall include a comparative evaluation of the local effects after implantation in terms of the biological responses to test and control materials.

NOTE When the ultimate goal of an implant is to result in tissue remodelling, evaluation of the formation of the expected normal tissue at the site rather than complete degradation might be considered.

Annex A
(informative)

General considerations regarding implantation periods and tissue responses to degradable/resorbable materials

It is anticipated that the tissue response to degradable materials will be different from the response to non-resorbable solid materials. It should be emphasized that in contrast to non-resorbable materials a steady state can only be obtained after complete degradation and adsorption of the implant. Until that stage is reached, there is a continuous interaction of the degrading material with the surrounding tissue. In general, a chronic inflammatory response may be observed during the degradation phase, but the local histology should return to normal after degradation of the implant. Therefore, the residual minimal tissue response usually equated with ”biocompatibility” may require implantation periods that are as long as the degradation profile of the material. As degradation is a continuous process, and it is possible that a “burst” release of acidic degradation products might occur, it is also necessary to evaluate the tissue response at intermediate degradation stages for the evaluation of local adverse responses to the residual implant and its degradation products.

Real-time degradation *in vivo* may need a rather long period of time before complete degradation or a steady state in tissue response is reached. Therefore, implantation of *in vitro* predegraded material (for instance up to 50 % weight loss or 50 % loss of mechanical strength) may be considered in order to simulate late-occurring events after implantation. However, these exploratory studies would not take the place of other implant studies that would be necessary to fully characterize the real-time *in vivo* degradation profile of the material.

The provisions in this Annex are also applicable to the evaluation of local effects of degradable materials used, for instance as carrier for slow drug release, degradable scaffolds for tissue-engineered medical products or as resorbable surface coatings for non-degradable implants.

Annex B
(normative)

Test methods for implantation in subcutaneous tissue

B.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.

B.2 Principle

The method compares the biological response to implants of test specimens with the biological response to implants of control specimens. The control materials are those used in medical devices of which the clinical acceptability and biocompatibility characteristics have been established.

B.3 Test specimens

Common provisions for the preparation of test and control specimens are described in 4.2. Implant sizes are based on the size of the test animal. The following dimensions shall be considered.

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

   NOTE The subcutaneous site, deep to the panniculus carnosa 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*.

b) Bulk materials shall be fabricated into specimens of 1,5 mm in diameter and 5 mm in length, and shall have rounded ends.

c) Non-solid specimens (including powders) shall be prepared in tubes of 1,5 mm in diameter and 5 mm in length (see 4.2).

B.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 in accordance with the provisions of ISO 10993-2.

Use at least three animals and sufficient sites to yield a total of 10 test and 10 control samples for each material and implantation period. When multiple tissue specimens are taken from a single implant site, sections for histology shall be at least 1 cm apart.

When using a well-known (non-degradable) control material, it may be justified to evaluate the response to the control material at one time point only. This shall be justified and documented.

B.5 Implantation procedure

Select one of the procedures described in B.5.1 and B.5.2.

B.5.1 Implantation alongside 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. Alternatively, both flanks may be used.

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

B.5.2 Implantation in the 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 through the tunnel to position it at the neck [23], [24].

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. Alternatively, both flanks and/or hind legs may be used.

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

B.6 Implantation period

To ensure a steady state of biological tissue response, the implantation period(s) shall be as specified in 5.3

B.7 Evaluation of biological response

The evaluation shall take into account the items specified in Clause 5.

B.8 Format of test report

The presentation of the test results and final test report shall include the items specified in Clause 6 and shall include justifications for the specific methods selected.

Annex C
(normative)

Test method for implantation in muscle

C.1 Field of application

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

C.2 Principle

The implant is inserted in the muscle of a test animal. The method compares the biological response to implants of test specimens with the biological response to implants of control specimens. The control materials are those used in medical devices of which the clinical acceptability and biocompatibility characteristics have been established.

C.3 Test specimens

Common provisions for preparation of test and control specimens are described in 4.2. Implant sizes are based on the size of the muscle group chosen.

For rabbit paravertebral muscles, implants of a width of 1 mm to 3 mm with a length of approximately 10 mm are typically used. Alternatively, larger samples up to 10 mm in diameter and 3 mm in thickness may be surgically implanted.

The specimens shall have rounded edges and the ends finished to a full radius.

C.4 Test animals and implant sites

Under anaesthesia insert the implants in the muscle of the animals. Ensure that the muscles are of sufficient size to accommodate the implant specimens. Use only one species per test.

NOTE The paravertebral muscles of rabbits are the preferred implant sites. Alternatively, for smaller samples, the gluteal muscles of rats or the thigh muscles of rabbits may be used.

Use at least three animals and sufficient implant sites to yield a total of 10 test specimens and 10 control specimens for each implantation period.

In cases where a comparative control material is expected to elicit more than a minimal response, use an additional control material known to evoke a minimal tissue reaction in a location opposite the test materials.

When using a well-known (non-degradable) control material, it may be justified to evaluate the response to the control material at one time point only. This shall be justified and documented.

C.5 Implantation procedure

Implantation shall be by hypodermic needle or trocar. For larger implants other appropriate surgical implantation techniques may be used.

Implant test specimens into the body of the muscle with the long axis parallel to the muscle fibres.

For rabbit paravertebral muscles, implant sufficient specimens of the test materials along one side of the spine, 25 mm to 50 mm from the midline and parallel to the spinal column, and about 25 mm apart from each other. In similar fashion implant sufficient specimens of the control material in the contralateral muscle of each animal.

C.6 Implantation period

To ensure a steady state of biological tissue response, the implantation period(s) shall be as specified in 5.3.

C.7 Evaluation of biological response

The evaluation shall take into account the requirements specified in Clause 5.

C.8 Format of test report

The presentation of the test results and final test report shall include the requirements specified in Clause 6.

Annex D
(normative)

Test method for implantation in bone

D.1 Field of application

This test method is used for assessing the biological response of bone tissue to an implanted material. The implantation site in cancellous ("spongy") or dense compact bone should be selected according to the end use of the 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.

D.2 Principle

The implant is inserted into the bone tissue of test animals. The method compares the biological response to implants of test specimens with the biological response to implants of control specimens. The control materials are those used in medical devices of which the clinical acceptability and biocompatibility characteristics have been established.

D.3 Test specimens

D.3.1 General

Common provisions for preparation of test and control specimens are described in 4.2.

D.3.2 Shape of implant specimens

Solid samples may be screw-shaped or threaded to provide initial stability of the implants in the bone. If preparation of a screw shape is impractical, a cylinder shape may be used.

Other forms of sample (e.g. cylinders, rods, pastes) may be used depending on the nature of the materials and study objective.

D.3.3 Size of test specimens

Implant sizes are based on the size of the test animal and bone chosen. The following typical dimensions shall be considered for implants in mid-shaft cortical bone:

— rabbits – cylindrical implants 2 mm in diameter and 6 mm in length;

— dogs, sheep and goats – cylindrical implants of 4 mm in diameter and 12 mm in length;

— rabbits, dogs, sheep, goats and pigs – 2 mm to 4,5 mm orthopaedic bone screw-type implants.

D.4 Test animals and implant sites

D.4.1 Test animals

The implants shall be inserted into the bone of rodents, dogs, sheep, goats, pigs or rabbits. Select one species among these in line with the principles set out in ISO 10993-2. Species' differences are important in bone physiology and should be assessed before implantation procedures are initiated. In addition, bone quality may vary between non-purpose-bred animals of the same species, and bone densitometry may be required to identify suitable test animals and to interpret the test results. Selection shall be justified and documented.

D.4.2 Implant sites

Equivalent anatomical sites shall be used for test and control specimens. The test implants shall be contralateral to the control implants. Select the implant site to minimize the risk of mobility of the implant. At least 10 test specimens and 10 control specimens shall be evaluated for each implantation period. Non-degradable control samples may be evaluated at one time point only. This shall be justified and documented.

NOTE The femur and tibia are suitable. Other sites may be considered.

The number of implant sites shall be as follows:

a) in each rabbit there shall be a maximum of six implant sites: three for test specimens and three for control specimens;

b) in each dog, sheep, goat or pig, there shall be a maximum of twelve implant sites: six for test specimens and six for control specimens.

Do not insert more than twelve specimens in any one animal.

The size, mass and age of the animal and the implant site chosen should ensure that the implant placement does not cause significant risk of pathological fracture of the test site. In younger animals it is especially important to ensure that the implants avoid the epiphyseal area or other immature bone.

D.5 Implantation procedure

Perform bone preparation using low drilling speed and intermittent drilling using profuse irrigation with physiological saline solution and suction, because overheating will result in local tissue necrosis.

It is important that the diameter of the implant and the implant bed in the bone match well enough to avoid ingrowth of fibrous tissue.

Expose the cortex of each femur or tibia and drill the appropriate number of holes to receive implants. For rabbits, prepare up to three holes; for larger animals prepare up to six holes. Ream to final diameter or tap screw thread before insertion. Insert cylinders by finger pressure to allow press fit. Tighten screw-shaped implants in place with an instrument capable of delivering a predetermined torque. Record the torque.

D.6 Implantation period

To ensure a steady state of biological tissue response, the implantation period(s) shall be as specified in 5.3.

D.7 Evaluation of biological response

The evaluation shall take into account the requirements specified in Clause 5.

D.8 Format of test report

The presentation of the test results and final test report shall include the requirements specified in Clause 6.

Annex E
(informative)

Examples of evaluation of local biological effects after implantation

Examples of quantitative scoring systems are given in the Bibliography (see References [16], [17], [25] and [26].

For each histological characteristic evaluated, such as capsule formation, inflammation, presence of polymorphonuclear cells, giant cells, plasma cells and/or degradation of material, the semi-quantitative scoring system used should be described in the evaluation report.

Some examples of such semi-quantitative scoring systems are described below and in the Bibliography (see References [25], [26], [31], [40] and [41]). The evaluation system as described in Tables E.1 and E.2, may be converted to an implant evaluation system as described in Table E.3.

For examples of scoring systems used for biological evaluation of degradable materials, see Reference [19].

Table E.1 — Examples of a histological evaluation system — Cell type/response

Cell type/response	Score				
	0	1	2	3	4
Polymorphonuclear cells	0	Rare,1-5/phf [a]	5-10/phf	Heavy infiltrate	Packed
Lymphocytes	0	Rare,1-5/phf	5-10/phf	Heavy infiltrate	Packed
Plasma cells	0	Rare,1-5/phf	5-10/phf	Heavy infiltrate	Packed
Macrophages	0	Rare,1-5/phf	5-10/phf	Heavy infiltrate	Packed
Giant cells	0	Rare,1-2/phf	3-5/phf	Heavy infiltrate	Sheets
Necrosis	0	Minimal	Mild	Moderate	Severe

[a] phf = per high powered (400 ×) field.

Table E.2 — Examples of a histological evaluation system — Response

Response	Score				
	0	1	2	3	4
Neovascularisation	0	Minimal capillary proliferation, focal, 1-3 buds	Groups of 4-7 capillaries with supporting fibroblastic structures	Broad band of capillaries with supporting structures	Extensive band of capillaries with supporting fibroblastic structures
Fibrosis	0	Narrow band	Moderately thick band	Thick band	Extensive band
Fatty infiltrate	0	Minimal amount of fat associated with fibrosis	Several layers of fat and fibrosis	Elongated and broad accumulation of fat cells about the implant site	Extensive fat completely surrounding the implant

Table E.3 — Example of a semi-quantitative evaluation system

Test sample: **Implantation interval:**

	Test sample			**Control sample**		
Animal number:						
Inflammation Polymorphonuclear						
Lymphocytes						
Plasma cells						
Macrophages						
Giant cells						
Necrosis						
SUB-TOTAL (× 2)						
Neovascularisation						
Fibrosis						
Fatty infiltrate						
SUB-TOTAL						
TOTAL						
GROUP TOTAL						
AVERAGE [a]	TEST (–) CONTROL =					
Traumatic necrosis						
Foreign debris						
No. sites examined						

[a] Used to determine irritant ranking shown below as the conclusion. A negative difference is recorded as zero.

Conclusion: Under the conditions of this study, the test sample was considered a

— non-irritant (0,0 up to 2,9)

— slight irritant (3,0 up to 8,9)

— moderate irritant (9,0 up to 15,0)

— severe irritant (> 15)

to the tissue as compared to the negative control sample.

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