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Third edition
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Dentistry — Evaluation of biocompatibility of medical devices used in dentistry

Médecine bucco-dentaire — Évaluation de la biocompatibilité des dispositifs médicaux utilisés en médecine bucco-dentaire

Reference number
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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

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

For an explanation on the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 106, *Dentistry*.

This third edition of ISO 7405 cancels and replaces ISO 7405:2008 and ISO/TS 22911:2016 which have been technically revised. It also incorporates the Amendment ISO 7405:2008/Amd.1:2013.

The main changes compared to the previous edition are as follows:

— as crucial first step in the biological evaluation a material characterization is required before biological tests are conducted (see 5.4.2)

— modifications of contents of 'pulp and dentine usage test' and 'endodontic test'

— deletion of Annex C (Acute toxicity testing);

— addition of ISO/TS 22911 as new Annex C.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document describes the evaluation of the biocompatibility of medical devices used in dentistry. It is to be used in conjunction with the ISO 10993 series of standards. This document contains special tests, for which ample experience exists in dentistry and which acknowledge the special needs of dentistry.

Only test methods for which the members of the committee considered there was sufficient published data have been included. In recommending test methods, the need to minimize the number and exposure of test animals was given a high priority. It is essential that the decision to undertake tests involving animals be reached only after a full and careful review of the evidence indicating that a similar outcome cannot be achieved by other types of test. In order to keep the number of animals required for tests to an absolute minimum, consistent with achieving the objective indicated, it can be appropriate to conduct more than one type of test on the same animal at the same time, e.g. pulp and dentine usage test and pulp capping test. However, in accordance with ISO 10993-2 these tests are performed both in an efficient and humane way. On all occasions when animal testing is undertaken, such tests are conducted empathetically and according to standardized procedures as described for each test.

This document does not explicitly describe test methods for occupationally related risks.

Annex B is included to encourage the development of *in vitro* and *ex vivo* test methods which will further reduce the use of animals in the evaluation of the biocompatibility of medical devices used in dentistry. Annex C is based on and replaces ISO/TS 22911.

Dentistry — Evaluation of biocompatibility of medical devices used in dentistry

1 Scope

This document specifies test methods for the evaluation of biological effects of medical devices used in dentistry. It includes testing of pharmacological agents that are an integral part of the device under test.

This document does not cover testing of materials and devices that do not come into direct or indirect contact with the patient's body.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 1942, *Dentistry — Vocabulary*

ISO 6344-1, *Coated abrasives — Grain size analysis — Part 1: Grain size distribution test*

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

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

ISO 10993-3, *Biological evaluation of medical devices — Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

ISO 10993-5, *Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity*

ISO 10993-6, *Biological evaluation of medical devices — Part 6: Tests for local effects after implantation*

ISO 10993-10, *Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization*

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 10993-18, *Biological evaluation of medical devices — Part 18: Chemical characterization of materials*

ISO/TS 10993-19, *Biological evaluation of medical devices — Part 19: Physico-chemical, morphological and topographical characterization of materials*

ISO 14971, *Medical devices — Application of risk management to medical devices*

ISO 16443, *Dentistry — Vocabulary for dental implants systems and related procedure*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 1942, ISO 10993-1, ISO 10993-12, ISO 16443 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
dental material
material and/or substance or combination of materials and/or substances specially formulated and prepared for use in the practice of dentistry and/or associated procedures

3.2
final product
medical device or device component that includes all manufacturing processes for the "to be marketed" device including packaging and sterilization, if applicable, and that includes processes prior to intended use, such as mixing, preconditioning and preparation

3.3
positive control material
well characterized material and/or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately positive or reactive response in the test system

3.4
negative control material
well characterized material and/or substance that, when evaluated by a specific test method, demonstrates the suitability of the test system to yield a reproducible, appropriately negative, non-reactive or minimal response in the test system

Note 1 to entry: In practice, negative controls include blanks, vehicles/solvents and *reference materials* (3.5).

3.5
reference material
material with one or more property values that are sufficiently reproducible and well established to enable use of the material or substance for the calibration of an apparatus, the assessment of a measurement method or for the assignment of values to materials

Note 1 to entry: For the purpose of this document, a reference material is any well characterized material and/or substance that, when tested by the procedure described, demonstrates the suitability of the procedure to yield a reproducible, predictable response. The response may be negative or positive.

3.6
in vitro pulp chamber
device that holds a thin slice of dentine between two chambers and allows fluid and molecules to filter or to diffuse across the "dentine barrier"

3.7
diffusion
establishment of passive movement of solutes (solubilized constituents) by means of a diffusion gradient through the "dentine barrier"

4 Categorization of medical devices

4.1 Categorization by nature of contact

4.1.1 General

For the purposes of this document, the classification of medical devices used in dentistry is derived from ISO 10993-1. If a device or material can be placed in more than one category, the more rigorous testing requirements shall apply. With multiple exposures the decision into which category a device is

placed shall take into account the potential cumulative effect, bearing in mind the period of time over which these exposures occur.

NOTE In this context the term dentistry includes the oromaxillofacial environment.

4.1.2 Non-contact devices

These devices do not contact the patient's body directly or indirectly, and are not included in ISO 10993-1.

4.1.3 Surface-contacting devices

These devices include those that contact the surface of intact or breached or otherwise compromised skin, the surface of intact or breached or otherwise compromised oral mucosa, and those that contact the external surfaces of dental hard tissue, including enamel, dentine and cementum.

NOTE In some circumstances, dentine and cementum are considered as surfaces, e.g. after gingival recession.

4.1.4 External communicating devices

These devices include dental devices that penetrate and are in contact with oral mucosa, dental hard tissues, dental pulp tissue or bone, or any combination of these, and are exposed to the oral environment.

NOTE This group also includes any kind of lining or base material to be used under a restoration.

4.1.5 Implant devices used in dentistry

These devices include dental implants and other dental devices that are partially or fully embedded in one or more of the following:

a) soft tissue, e.g. subperiosteal implants and subdermal implants;

b) bone, e.g. endosteal implants and bone substitutes;

c) pulpodentinal system of the tooth, e.g. endodontic materials;

d) any combination of these, e.g. transosteal implants.

4.2 Categorization by duration of contact

4.2.1 General

For the purposes of this document, medical devices used in dentistry are classified by duration of contact as described in ISO 10993-1 and listed in 4.2.2 to 4.2.4.

4.2.2 Limited exposure devices

Devices whose cumulative single or multiple use or contact is likely to be up to 24 h.

4.2.3 Prolonged exposure devices

Devices whose cumulative single, multiple or long-term use or contact is likely to exceed 24 h but not 30 d.

4.2.4 Permanent contact devices

Devices whose cumulative single, multiple or long-term use or contact exceeds 30 d. With multiple exposures to the device, the decision into which category a device is placed should take into account the potential cumulative effect, bearing in mind the period of time over which these exposures occur.

NOTE The definition of the term "permanent" is meant to be applied solely for the use of this document. It is consistent with the definition given in ISO 10993-1.

5 Biological evaluation process

5.1 General

Each medical device used in dentistry shall be subjected to a structured biological evaluation programme within a risk management process (see ISO 10993-1). Guidance on the implementation of this programme in ISO 14971 and ISO 10993-1 shall be used.

The biological evaluation programme shall include the review of data sets concerning the biological properties of each medical device used in dentistry. When this part of the biological evaluation programme indicates that one or more data sets are incomplete and that further testing is necessary, the tests shall be selected from the methods described in the ISO 10993 series of standards or in this document, or in both. If tests that are not included in these International Standards are selected, a statement shall be made that indicates that the tests described in these International Standards have been considered and shall include a justification for the selection of other tests.

For combination products the final product shall be evaluated according to this document in conjunction with any applicable standards.

NOTE 1 In this context, combination products are dental devices of any kind that incorporate, or are intended to incorporate, as an integral part, a substance that:

a) if used separately, would be a medicine or a biological product;

b) is liable to affect the patient's body by an ancillary action.

An example would be a bone filling/augmentation device containing a growth factor (i.e. a biological product).

For combination products, where the device and pharmacological components are packaged separately, it may be informative to test the device components alone.

All tests shall be conducted according to recognized current/valid best laboratory/quality practices, where applicable.

NOTE 2 Examples of relevant guidance include GLP (Good Laboratory Practice) or ISO/IEC 17025.

5.2 Selection of tests and overall assessment

The selection of tests and the overall assessment of the results shall be carried out by an expert who has the appropriate chemical, physical and biological data concerning the device and who is aware of the intended conditions of use.

5.3 Selection of test methods

The selection of test methods shall be based upon consideration of

a) the intended use of the medical device,

b) the tissue(s) which the medical device may contact, and

c) the duration of the contact.

If a test selected is not included in the International Standards, a justification for the choice of the methods shall be included in the test report for each device. If more than one test method in the same category is recommended by the standards, the selection of one test over the others shall be justified.

5.4 Types of test

5.4.1 General

According to the categorization of the device, tests shall be considered for use as summarized in Table A.1. This table indicates which types of test method shall be considered, but not that they are necessarily required to be carried out. A decision not to carry out a type of test identified in Table A.1 shall be justified in the test report on each device. The types of test listed are regarded as a framework for the evaluation of the biocompatibility of medical devices used in dentistry. For most types of test, particular methods are identified, although for some devices it is recognized that alternative methods not included in the International Standards listed can be more appropriate.

5.4.2 Physical and chemical characterization

Material characterization of the medical device or component (see Table A.1) is a crucial first step in the biological evaluation. Material characterization, if performed, shall be conducted in accordance with ISO 10993-18 and ISO/TS 10993-19. For nanomaterials, see ISO/TR 10993-22.

For convenience, the types of biological tests have been listed in three groups.

5.4.3 Group I

This group comprises *in vitro* tests of cytotoxicity. General guidance for *in vitro* cytotoxicity tests is presented in ISO 10993-5 and shall be followed. Detailed test protocols for the agar or agarose diffusion and filter diffusion methods, appropriate to dental materials, are included in this document. The *in vitro* cytotoxicity methods include

a) agar diffusion test (see 6.2),

b) filter diffusion test (see 6.3),

c) direct contact or extract tests in accordance with ISO 10993-5, and

d) dentine barrier cytotoxicity test (see Annex B).

NOTE 1 The order of listing does not indicate any preference for one method over another.

NOTE 2 This list does not indicate that all cytotoxicity tests mentioned have to be performed for each medical device under consideration.

NOTE 3 The use of the dentine barrier cytotoxicity test is encouraged and a description of the test is presented in Annex B. References to this test are presented in the Bibliography.

5.4.4 Group II

This group comprises tests in accordance with the ISO 10993 series of standards and particular tests, where appropriate:

a) acute systemic toxicity — oral application — in accordance with ISO 10993-11;

b) acute systemic toxicity — application by inhalation — in accordance with ISO 10993-11;

c) subacute and subchronic systemic toxicity — oral application — in accordance with ISO 10993-11;

d) skin irritation and intracutaneous reactivity in accordance with ISO 10993-10;

e) delayed-type hypersensitivity in accordance with ISO 10993-10;

f) genotoxicity in accordance with ISO 10993-3;

g) local effects after implantation in accordance with ISO 10993-6.

NOTE 1 In order to allow use of the latest edition of the referenced document only, an undated cross-reference is possible. An indication of the appropriate clause and subclause is only possible for dated references. Therefore, the user of this document is requested to check the referenced documents for the appropriate clause numbers.

In the evaluation of materials following local implantation involving mineralized tissues in accordance with ISO 10993-6, examination of undemineralized sections, in addition to routine demineralized sections, is recommended.

NOTE 2 If appropriate, the local effects after implantation are evaluated in accordance with dental implant usage test instead of ISO 10993-6 [see 5.4.5, d)].

5.4.5 Group III

This group comprises tests, specific for medical devices used in dentistry, not referred to in the ISO 10993 series of standards:

a) pulp and dentine usage test (see 6.4);

b) pulp capping test (see 6.5);

c) endodontic usage test (see 6.6);

d) endosseous dental implant usage test (see Annex C).

Endosseous dental implant usage test is not required, but if applicable, is recommended.

5.5 Re-evaluation of biocompatibility

In accordance with ISO 10993-1, a device shall be considered for re-evaluation of its biocompatibility as described in 5.4 when revisions or modifications to the formula, quality and/or performance specifications are made.

NOTE See also ISO 10993-1:2018, B.4.5.1 which provides indications on when to commence a re-evaluation.

6 Test procedures specific to dental materials

6.1 Recommendations for sample preparation

6.1.1 General

These recommendations have been designed for *in vitro* testing, but can also be used for other purposes, if suitable.

6.1.2 General recommendations for sample preparation

For the preparation of test samples, consult the respective product standards and/or the manufacturer's instructions, and follow those descriptions as closely as possible. Justify any deviation from the manufacturer's instructions. A detailed description of the sample preparation shall be included in the test report. Take the following (e.g. environmental) factors into account, considering the final use of the device:

a) temperature;

b) humidity;

c) light exposure: samples of photosensitive materials shall be produced under the condition that ambient light does not activate them;

d) material of sample mould: ensure that the material of the sample mould and eventual lubricant used do not interfere with the setting process of the material;

NOTE Suitable sample mould materials can be semitranslucent or white plastic materials such as polyethylene or polytetrafluoroethylene (PTFE).

e) oxygen exposure: for materials that produce an oxygen inhibition layer during hardening ensure that the sample mould is properly sealed during hardening;

f) sterilization: samples shall either be produced under aseptic conditions or be sterilized by the method appropriate to the material, if necessary and possible; ensure that sterilization does not affect the material (e.g. sterilization shall not elute substances from material);

g) ratio of sample surface area versus cell layer surface or cell culture medium: document the ratio of sample surface area versus cell layer surface or cell culture medium; justify the selection of shape and sample surface area and the applied ratio of sample surface area versus cell layer surface or cell culture medium;

h) extracts: if extracts are required for a test procedure, prepare extract samples in accordance with ISO 10993-12:2012, Clause 10.

6.1.3 Specific recommendations for light curing materials

Take the following factors into account, considering the final use of the light curing material:

a) **material of sample mould**: the reflection coefficient of materials used for sample moulds should be as close as possible to that of dentine in order to simulate the clinical situation;

NOTE Suitable sample mould materials can be semitranslucent or white plastic materials such as polyethylene or PTFE.

b) **light exposure**: light curing shall be done to simulate clinical usage as closely as possible. The manufacturer's instructions for use shall be followed to provide the same level of curing as would be the case in actual usage. This will often require curing from one side only but will sometimes entail a two-sided cure. The cure method is material and/or process specific. Where fully cured test samples are required for testing, it is important to ensure that the test samples are homogeneous after removal from the mould. In the case of one-component materials, there shall be no voids, clefts or air-bubbles present when viewed without magnification. Reference shall be made to the light source used (light intensity, curing time, spectral distribution of curing light and type of curing light shall be documented). Care shall be taken to ensure that the light source is recommended for the materials to be tested and that it is in a satisfactory operating condition;

c) **oxygen exposure**: for materials that produce an oxygen inhibition layer during light curing, both ends of the mould shall be covered with transparent oxygen barrier materials (e.g. a polyester film) during light curing. If the material is recommended by the manufacturer for surface finishing after curing, the sample surfaces shall be ground and polished using the recommended clinical procedures. If there are no such instructions and if required for testing, the samples shall be ground on both ends, with a P2 000 paper in accordance with ISO 6344-1, after first being set against the transparent oxygen barrier material.

6.1.4 Specific recommendations for chemically setting materials

Take the following factors into account, considering the final use of the chemically setting materials:

a) **mixing**: mix sufficient material to ensure that the preparation of each test sample is completed from one batch. Prepare a fresh mix for each test sample. The mixing shall be performed in accordance with the respective product standards, if applicable;

b) **oxygen exposure**: for materials that produce an oxygen inhibition layer during chemical curing, both ends of the mould shall be covered with oxygen barrier materials (e.g. a polyester film) during curing. If the material is recommended by the manufacturer for surface finishing after curing, the sample surfaces shall be ground and polished using the recommended clinical procedures. If there are no such instructions and if required for testing, the samples shall be ground on both ends, with a P2 000 paper in accordance with ISO 6344-1, after first being set against the oxygen barrier material.

6.1.5 Positive control material

For *in vitro* tests and certain *in vivo* tests (e.g. pulp and dentine usage test), it is advisable to include a standard positive control material, which is handled and processed like the test materials (i.e. being plastic after mixing and then setting) and which is based on freely available chemicals or materials.

Such a positive control material for *in vitro* testing of plastic filling materials is described in Annex B, Table B.1. The use of this specific positive control material is optional and other materials with a validated history and other well characterized positive control materials with reproducible data on toxicity can be used instead.

6.2 Agar diffusion test

6.2.1 Objective

This test is designed to demonstrate the nonspecific cytotoxicity of test materials after diffusion through agar or agarose. This test method is not appropriate for leachables that do not diffuse through agar or agarose.

6.2.2 Cell line

Use an established fibroblast or epithelial cell line, which is readily available [e.g. from the American Type Culture Collection (ATCC), see https://www.atcc.org][1)]. Specify in the report the identification number of the cell line, if applicable, the description and designation of the cell line used and a justification for its selection.

6.2.3 Culture medium, reagents and equipment

Use the culture medium specified for the selected cell line. Sterilize by filtration. For the preparation of the agar, prepare a double-concentration of the culture medium. Sterilize by filtration. Prepare either 3 % agar or 3 % agarose. Sterilize by autoclaving.

Prepare the vital stain by diluting a stock solution of 1 % aqueous neutral red solution (record source) 1:100 with 0,01 mol/l phosphate-buffered saline solutions [e.g. Dulbecco's phosphate-buffered saline solution[2)]] immediately before use. Store neutral red solutions protected from the light. Use 6-well

1) This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

2) Dulbecco is a trade name. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

tissue culture plates (35 mm in diameter) or Petri dishes of 50 mm to 100 mm in nominal diameter suitable for tissue culture.

6.2.4 Sample preparation

Prepare the samples in accordance with 6.1. The test shall be performed on either an extract of the material and/or the material itself, according to the guidance in ISO 10993-5.

a) For solid materials, prepare circular test samples of approximately 5 mm diameter, with a flat surface to ensure adequate contact with the agar overlay.

b) For setting materials, insert the freshly mixed material into rings of internal diameter 5 mm and height 2 mm. The material of the ring shall be stated in the test report. When testing materials in the freshly mixed state, place the rings on the agar prior to inserting the material. When testing after various setting periods, fill the rings so that the material is flush with the rim and allow it to set at (37 ± 2) °C and a relative humidity of (90 ± 10) % until ready for testing.

c) For fluid test samples or extracts, imbibe 0,01 ml of the fluid on a borosilicate microglass filter disc of 5 mm diameter, placed on the agar.

NOTE 1 Suitable inert materials are glass or PTFE.

NOTE 2 Suitable discs can be prepared from prefilters.

6.2.5 Controls

Use positive controls, negative controls and reference materials.

6.2.6 Test procedure

Culture the cells until they reach the end of the log growth phase. Pipette the proper volume (e.g. 10 ml for a 100 mm Petri dish) of cell suspension ($2{,}5 \times 10^5$ cells/ml) into a sufficient number of Petri dishes and incubate at (37 ± 2) °C in a water-saturated atmosphere with 5 % (volume fraction) carbon dioxide for 24 h. If different cell culturing conditions are used, justification shall be provided.

Heat the sterile agar or agarose to 100 °C in a water bath and allow it to cool to 48 °C. Mix one part of agar or agarose with one part of double-concentrated, freshly prepared culture medium and heat to 48 °C. Aspirate the liquid culture medium from each Petri dish and replace with 10 ml of freshly prepared agar or agarose/culture medium mixture.

Allow the agar or agarose/culture medium mixture to solidify at room temperature (approximately 30 min). Add 10 ml neutral red solution and keep dark for 15 min to 20 min. Aspirate excess neutral red solution.

Protect the culture from light in the presence of neutral red, as the cells can be damaged.

Apply to each dish an appropriate number of samples of test material and controls, with an adequate distance (>20 mm) between adjacent samples, if applicable. Incubate at (37 ± 2) °C in a water-saturated atmosphere with 5 % (volume fraction) carbon dioxide for 24 h. Examine each test material at least in quadruplicate (i.e. two dishes per test material).

6.2.7 Parameters of assessment

Assess the decolorization zone around the test materials and controls using an inverted microscope with a calibrated screen, and determine a decolorization index and a lysis index for each test sample in accordance with the criteria specified in Tables 1 and 2.

6.2.9 Test report

Submit the results in a test report that includes a complete record of all procedures followed, all results obtained and any other data necessary for the assessment of results. Include details of the preparation and methods of application of the test material, together with the lot number of the material when appropriate.

6.3 Filter diffusion test

6.3.1 Objective

This test is designed to demonstrate the nonspecific cytotoxicity of test materials after diffusion through a cellulose acetate filter.

6.3.2 Cell line

Use an established fibroblast or epithelial cell line, which is readily available [e.g. from the American Type Culture Collection (ATCC), see https://www.atcc.org]. Specify in the report the identification number of the cell line, if applicable, the description and designation of the cell line used, and a justification for its selection.

6.3.3 Culture medium, reagents and equipment

Prepare culture medium and agar or agarose for use as an overlay as described in 6.2.3. Prepare solutions either for succinate dehydrogenase staining or for nonspecific hydrolase staining.

For succinate dehydrogenase staining, prepare the following stock solutions:

a) **succinate solution**, 13,6 g sodium succinate in 100 ml of 0,2 mol/l phosphate buffer, pH 7,6;

b) **nitro blue tetrazolium chloride solution**, 100 mg nitro blue tetrazolium chloride in 100 ml of 0,2 mol/l phosphate buffer, pH 7,6;

c) **phenazine methosulfate solution**, 4 mg phenazine methosulfate in 10 ml fresh demineralized water.

Prepare a staining solution of 1 ml succinate solution, 9 ml nitro blue tetrazolium chloride solution and 1 ml phenazine methosulfate solution.

For nonspecific hydrolase staining, prepare a stock solution of fluorescein diacetate consisting of 5 mg fluorescein diacetate in 1 ml acetone. For use, add 20 µl of stock solution to 100 ml phosphate-buffered saline solution (e.g. Dulbecco's phosphate-buffered saline solution). Use Petri dishes of 60 mm nominal diameter, suitable for tissue culture.

Use filters, composed of a mixture of cellulose acetate and cellulose nitrate, 47 mm diameter, 0,45 µm pore size[3)].

6.3.4 Sample preparation

Prepare the samples in accordance with 6.1. The test shall be performed on either an extract of the material or the material itself, according to the guidance in ISO 10993-5.

a) For solid materials, prepare circular test samples of approximately 5 mm diameter, with a flat surface to ensure adequate contact with the filter. The mass of the test samples shall not exceed 3,5 g.

b) For setting materials, insert the freshly mixed material into rings of internal diameter 5 mm and height 2 mm. When testing materials in the freshly mixed state, place the rings on the filter prior to inserting the material. When testing after various setting periods, fill the rings so that the material

3) Millipore HATF 04700 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

Annex C
(informative)

Endosseous dental implant usage test

C.1 General

This annex concerns animal tests relevant to the functional assessment of dental endosseous implant systems, using both macroscopic and microscopic parameters. It is intended for use only when risk analysis indicates a need for additional information that only animal testing can provide.

This animal usage test may also be helpful to evaluate the influence of functional stress upon the host responses to a dental implant system.

This annex is not intended to provide information on the mechanical strength of implantable materials themselves, but rather a qualitative evaluation of the implant-bone interface.

NOTE Mechanical properties of dental implant systems are described in ISO 14801.

C.2 Test method

C.2.1 Test protocol

Before testing each particular dental implant system, the manufacturer or sponsor is responsible for preparing a detailed test protocol, which shall include, at least, full details of the following:

a) the aim of the study;

b) the rationale and justification for an animal test and such other information as may be required to satisfy ISO 10993-2;

c) the dental implant system to be tested, including its chemical composition and physical structure (including surface modification, if present), its recommended mode of clinical insertion and use, and the controls to be used;

d) the animal species chosen, their husbandry and a justification for the choice;

e) the test protocol to be followed, including the number of animals and test specimens, the time intervals chosen and a justification for their use;

f) the methods of assessment to be employed, both clinically and in the laboratory, and a justification for their use;

g) the methods of analysis of the clinical and laboratory data to be employed and the criteria to be used in determining the outcome of the study;

h) the information to be included in the test report.

Because there is wide variation in the design and clinical procedures associated with the use of different dental implant systems, it is not possible to formulate a single detailed test protocol. However, this annex provides guidance for some of the fundamental features of a test method, which should be common to all protocols. The references for test protocols in non-human species are presented in the Bibliography.

C.2.2 Animals and animal welfare

C.2.2.1 Animal welfare

Conduct animal welfare in accordance with 6.4.2.

C.2.2.2 Test animals

No particular animal model of a usage test for dental implant systems has yet been validated as relevant to the human situation. It is recommended, therefore, that an animal species be chosen which meets the following criteria:

a) oral hygiene can be maintained, either naturally or artificially;

b) the jaws are of sufficient size to allow normal surgical access and to accommodate the dental implant system in its form intended for use in humans;

c) the site into which the dental implant system is to be placed shall have opposing teeth;

d) the animals shall be skeletally mature if appropriate for its intended use;

e) animals having an omnivorous pattern of masticatory jaw movement are preferable.

C.2.2.3 Number of animals

The number of animals shall be justified and shall be the minimum necessary for determining the stated objectives of the study.

C.2.3 Test procedure

C.2.3.1 Test specimens

Use supplied dental implant systems as intended for human clinical use. If dental implants that differ in any way from those intended for clinical use are included in the study, a justification for this decision shall be included.

C.2.3.2 Control specimens

Use a suitable control unless data from comparable studies are available. It is possible that data from a previously published study may be acceptable if the experimental conditions in the two studies are strictly comparable. If a dental implant system control is needed, either a dental implant system for which peer-reviewed clinical data are available or a dental implant similar to the test implant, but unloaded, may be appropriate.

C.2.3.3 Surgical preparation of sites for the placement of the dental implant and control

Where necessary, create an area of edentulous jaw prior to the insertion of the dental implant.

When this is necessary, the animals shall be anaesthetised as determined and directed by appropriate laboratory practice conditions, using a recognized anaesthesia technique prior to any surgical procedures. All surgery shall be carried out under aseptic conditions. Extract the number of teeth required to provide the site(s) needed for the placement of the dental implant(s), using appropriate methods. The animals may require appropriate medical treatment to prevent infections under clinical conditions to best simulate human conditions. Further, the animals should be placed on an appropriate soft diet for a period postoperatively to minimize the risk of damage to the healing tissues. If the test implant is not intended for immediate implantation or delayed immediate implantation but for delayed implantation, the implant shall be placed after an appropriate healing period.

C.2.3.4 Placement of dental implant systems

Carry out the surgical procedures for the placement of dental implants and associated implant component(s) aseptically and follow precisely the test protocol. Procedures shall be carried out under appropriate anaesthesia. The dental prosthesis shall be completed according to the test protocol. Post-operative care regimens shall reflect the purpose of the study and recognized procedures of aftercare.

C.2.3.5 Test periods

Assess the dental implant system and the host responses to it after time intervals that are appropriate to the objectives of the study. Where the objective of the study is to assess the appropriateness of using the dental implant system clinically, then at least three test periods, including baseline and appropriate follow-up periods after loading, are recommended. The start point for these follow-up periods shall reflect any postoperative intervals during which the animal was not on a normal diet. Unless otherwise required, only the last period would emphasize surgical removal and microscopic analyses. If evaluation of bone resorption and/or implant loosening is an objective, an appropriate long-term period should be assessed.

NOTE For time periods of implants in tissue, see also ISO 10993-6.

C.2.3.6 Dental plaque control

If necessary, give the animals regular dental plaque control procedures, and include full details in the test protocol.

C.2.3.7 Clinical and radiographic examination

Record the status of the health of the gingival and periodontal tissues at appropriate intervals. In addition to a visual examination of the gingival tissues, it is recommended that, whenever possible, the status of oral hygiene, dental plaque and gingival inflammation are recorded, using recognized clinical indices. Make particular note of the stability or degree of mobility of the device, the presence of inflammation in the surrounding tissues and any evidence of local infection.

Take standardized radiographs of the dental implant site, the adjacent and occluding teeth and the supporting bone on prescheduled occasions when the animals are anaesthetised, so that a series of preoperative and postoperative images are obtained up to the time of termination of the study.

C.2.3.8 Termination of the test period

Carry out animal euthanasia, if necessary, at the termination of the test period, according to the guidelines in ISO 10993-2.

C.2.4 Evaluation

Evaluate the tissue responses by clinical, radiographic, histopathological, statistical and other methods of analysis as may be necessary for the study. Unless otherwise required for the purposes of the study, surgical removal of the dental implant and histopathological evaluation of the tissue responses around it should be restricted to only the longest or last follow-up period.

C.2.4.1 Clinical evaluation

Provide relevant details of the general health of the animals during the study, including body weight. The health of the soft tissues around the test and control dental implant systems and around the adjacent and occluding teeth and of both the whole jaws and associated muscular tissue shall be evaluated to determine if these structures have changed over the period of the study. Record any changes to implant superstructure including associated artificial teeth.

NOTE Any failed implants and mode of failure.

C.2.4.2 Radiographic assessment

Evaluate the radiographic appearance of the test and control dental implant systems, including the bone surrounding them and the adjacent and occluding teeth, to determine if those osseous structures have changed over the period of the study.

C.2.4.3 Specimen retrieval

Retrieve, if necessary, following the termination of the test, blocks of tissue representative of the whole jaw and associated muscular tissue and also specifically blocks of tissue containing the test dental implant systems and the control, together with the adjacent teeth, bone and oral soft tissues *in situ*, for histopathological and/or other examinations. Depending upon the particular aims of the study, it might be necessary to retrieve other blocks of tissue and/or to examine them by other techniques.

C.2.4.4 Specimen preparation for histopathological examination

If blocks of tissue have been retrieved, process them for histopathological examination as appropriate. According to the particular parameters of histopathological assessment to be undertaken, both undemineralized and demineralized sections may be necessary. Microscopic examination of hard, plastic-embedded, undemineralized specimens is recommended for the evaluation of the implant/tissue and implant/oral cavity interfaces.

C.2.4.5 Microscopic assessment

Examine a sufficient number of sections to assess the nature of the interaction between the surrounding hard and soft tissues and the dental implant system or the control. Particular attention shall be paid to quality and quantity, of, at least, the following:

a) areas of direct bone/implant contact;

b) new bone and/or fibrous tissue;

c) bone resorption;

d) inflammation, infection, abscess formation and necrosis;

e) any changes in the jaws and associated muscular tissue mass;

f) where possible, any changes to the dental implant.

A sufficient number of sections from the blocks of the adjacent and occluding teeth shall be examined to determine their status and that of their surrounding tissues.

C.2.4.6 Statistical analysis

There are many International Standards covering the field of statistical analysis. Cite the relevant International Standard, if used, or if not, specify the analytical techniques to be employed.

C.2.5 Test report

Prepare a test report containing, at least, the following:

a) all details of the study protocol as indicated in C.2.1 and any deviations from the original protocol;

b) all details of the clinical and laboratory observations and measurements and the appropriate analyses, as indicated in C.2.3;

c) an overall analysis of the data obtained from the study, including a comparison to controls, with conclusions relating to the extent to which the aim(s) of the study have been met, in particular concerning the biocompatibility of the dental implant system tested.

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