INTERNATIONAL STANDARD

ISO 7405

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Dentistry — Preclinical evaluation of biocompatibility of medical devices used in dentistry — Test methods for dental materials

Art dentaire — Évaluation préclinique de la biocompatibilité des dispositifs médicaux utilisés en art dentaire — Méthodes d'essai des matériaux dentaires

Reference number
ISO 7405:1997(E)

Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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

International Standard ISO 7405 was prepared by Technical Committee ISO/TC 106, *Dentistry*, in conjunction with the World Dental Federation (FDI).

This first edition cancels and replaces ISO/TR 7405:1984, which has been technically revised (see Introduction) and converted into an International Standard.

Annexes A, B and C of this International Standard are for information only.

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Introduction

This International Standard concerns the preclinical testing of dental materials in medical devices used in dentistry. It has been developed from and supersedes ISO/TR 7405:1984, *Biological evaluation of dental materials*, and its supplements, and should be read in conjunction with the ISO 10993, *Biological evaluation of medical devices*, series of standards. This International Standard differs from ISO/TR 7405 in several important ways. Firstly, it contains details of test methods applicable only to dental materials. Many test methods previously included in ISO/TR 7405 are now included in the ISO 10993 series of standards and details of them have therefore been excluded from this standard. Secondly, only test methods for which the members of the committee considered there was sufficient published data have been included. Thirdly, in recommending test methods, the need to minimize the use of animals was given a high priority.

The annexes are informative, to encourage the development of *in vitro* and *in vivo* test methods which will further reduce the use of animals in the preclinical evaluation of the biocompatibility of medical devices used in dentistry.

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Dentistry — Preclinical evaluation of biocompatibility of medical devices used in dentistry — Test methods for dental materials

1 Scope

This International Standard specifies methods for the evaluation of biological effects of dental materials. It includes testing of pharmacological agents that are an integral part of the device under test.

2 Normative references

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

ISO 1942-2:1989, *Dental vocabulary — Part 2: Dental materials*

ISO 10993-1:—[1], *Biological evaluation of medical devices — Part 1: Evaluation and testing*

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

ISO 10993-3:1992, *Biological evaluation of medical devices — Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

ISO 10993-5:1992, *Biological evaluation of medical devices — Part 5: Tests for cytotoxicity:* in vitro *methods*

ISO 10993-6:1994, *Biological evaluation of medical devices — Part 6: Tests for local effects after implantation*

ISO/TR 10993-9:1994, *Biological evaluation of medical devices — Part 9: Degradation of materials related to biological testing*

ISO 10993-10:1995, *Biological evaluation of medical devices — Part 10: Tests for irritation and sensitization*

ISO 10993-11:1993, *Biological evaluation of medical devices — Part 11: Tests for systemic toxicity*

ISO 10993-12:—[2], *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

ISO 10993-13:—[2], *Biological evaluation of medical devices — Part 13: Identification and quantification of degradation products from polymers*

ISO 10993-14:— [2], *Biological evaluation of medical devices — Part 14: Identification and quantification of degradation products from ceramics*

ISO 10993-15:— [2], *Biological evaluation of medical devices — Part 15: Identification and quantification of degradation products from coated and uncoated metals and alloys*

ISO 10993-16:— [2], *Biological evaluation of medical devices — Part 16: Toxicokinetic study design for degradation products and leachables.*

1) To be published. (Revision of ISO 10993-1:1992)

2) To be published

3 Definitions

For the purposes of this International Standard, the definitions given in ISO 10993-1, ISO 1942-2 and the following definitions apply.

3.1 medical device: Any instrument, apparatus, appliance, material or other article, including software, whether used alone or in combination, intended by the manufacturer to be used for human beings solely or principally for the purpose of

— diagnosis, prevention, monitoring, treatment or alleviation of disease, injury or handicap;

— investigation, replacement or modification of the anatomy or of a physiological process;

— control of conception;

and which does not achieve its principal intended action in or on the human body by pharmacological, immunological or metabolic means, but which may be assisted in its function by such means.

NOTE 1 Devices are different from drugs, and their biological evaluation requires a different approach.

NOTE 2 In this International Standard, the term "medical device" is understood to include dental devices and dental materials.

3.2 dental material: Substance or combination of substances specially prepared and/or presented for the use of authorized persons in the practice of dentistry and/or its associated procedures.

3.3 final product: Medical device in its "as-used" state.

NOTE — Many dental materials are used in a freshly mixed state, and evaluation of the materials in both freshly mixed and set conditions should be considered.

3.4 positive-control material: Material or substance which, when tested by the procedure described, demonstrates the suitability of the procedure to yield a reproducible, appropriate positive or reactive response in the test system.

3.5 negative-control material; reference material: Material or substance which, when tested by the procedure described, demonstrates the suitability of the procedure to yield a reproducible, appropriate negative, nonreactive or background response in the test system.

4 Categorization of medical devices used in dentistry

4.1 Categorization by nature of contact

For the purposes of this International Standard, 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.

4.1.1 Noncontact devices

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

4.1.2 Surface-contacting devices

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

NOTE — Dentine and cementum are considered as surfaces; e.g. after gingival recession.

4.1.3 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.

4.1.4 Implant devices (see ISO 10993-1)

These devices include dental devices and implants that are partially or fully embedded within the soft tissue, bone or pulpodentinal system of the tooth, or any combination of these, and are not exposed to the oral environment.

4.2 Categorization by duration of contact

For the purposes of this International Standard, medical devices used in dentistry are classified by duration of contact as described in ISO 10993-1, i.e.:

4.2.1 Limited exposure devices

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

4.2.2 Prolonged exposure devices

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

4.2.3 Permanent contact devices

Devices whose single, multiple or long-term use or contact exceeds 30 days.

5 Selection of biological evaluation test

5.1 The general guidance for the selection of biological evaluation tests stated in ISO 10993-1 shall apply. Tests should be selected from the methods described in the ISO 10993 series of standards or in this International Standard or in both. If tests not included in these International Standards are selected, a statement shall be made that indicates that the tests described in the International Standards have been considered and shall include a justification for the selection of other tests.

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

5.3 The selection of test methods shall be based upon consideration of:

a) the intended use of the material;

b) the tissue(s) which the material may contact;

c) the duration of the contact.

5.4 According to the categorization of the device, tests shall be considered for use as summarized in table 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 1 shall be justified in the test report on each device. The types of test listed are regarded as a framework for the preclinical evaluation of the biocompatibility of dental materials. 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 may be more appropriate.

Table 1 — Types of test for preclinical evaluation of biocompatibility of dental materials

		Group I			Group II									Group III		
Nature of contact	**Duration of contact**	**Cytotoxicity tests** ISO 7405, 6.1 and 6.2	**Cytotoxicity tests** ISO 10993-5	**Cytotoxicity tests** ISO 7405, annex A	**Acute systemic toxicity — Oral application** ISO 10993-11, 6.5.1	**Acute systemic toxicity — Oral application** ISO 7405, annex B	**Acute systemic toxicity — Application by inhalation** ISO 10993-11, 6.5.3	**Subchronic systemic toxicity — Oral application** ISO 10993-11, 6.7.1	**Skin irritation and intra-cutaneous reactivity** ISO 10993-10, 5.2 and 5.4	**Sensitization** ISO 10993-10, 6.2 and 6.3	**Subchronic systemic toxicity — Application by inhalation** ISO 10993-11, 6.7.3	**Genotoxicity** ISO 10993-3, clause 4	**Local effects after implantation** ISO 10993-6, clauses 4, 5 and 6	**Pulp and dentine usage test** ISO 7405, 6.3	**Pulp capping test** ISO 7405, 6.4	**Endo-dontic usage test** ISO 7405, 6.5
Surface-contacting devices	⩽ 24 h	X	X				X		X	X						
	> 24 h to 30 days	X	X				X	X	X	X	X					
	> 30 days	X	X				X	X	X	X	X	X				
External communi-cating devices	⩽ 24 h	X	X	X	X	X	X		X	X				X		
	> 24 h to 30 days	X	X	X			X	X	X	X	X	X	X	X		
	> 30 days	X	X	X			X	X	X	X	X	X	X	X		
Implant devices	⩽ 24 h	X	X						X	X					X	X
	> 24 h to 30 days	X	X					X	X	X		X	X		X	X
	> 30 days	X	X					X	X	X		X	X		X	X

NOTE — X indicates test shall be considered for use.

A justification for the choice of all methods shall be included in the test report of each device. This is of particular importance when methods not included in this International Standard are used.

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

a) 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 diffusion and filter diffusion methods, appropriate to dental materials, are included in this International Standard. The *in vitro* cytotoxicity methods include:

1) agar diffusion test (6.1);

2) filter diffusion test (6.2);

3) direct contact or extract tests in accordance with ISO 10993-5;

4) dentine barrier tests (annex A).

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

NOTE 2 The use of dentine barrier tests is encouraged and reference to these is presented in annex A.

b) Group II

This group comprises tests in accordance with ISO 10993 and particular tests, where appropriate, are identified:

1) acute systemic toxicity — oral application (ISO 10993-11, 6.5.1 and annex B);

2) acute systemic toxicity — application by inhalation (ISO 10993-11, 6.5.3);

3) subchronic systemic toxicity — oral application (ISO 10993-11, 6.7.1);

4) skin irritation and intracutaneous reactivity (ISO 10993-10, 5.2 and 5.4);

5) sensitization (ISO 10993-10, 6.2 and 6.3);

6) subchronic systemic toxicity — application by inhalation (ISO 10993-11, 6.7.3);

7) genotoxicity (ISO 10993-3, clause 4);

8) local effects after implantation (ISO 10993-6, clauses 4, 5 and 6).

NOTE 1 Alternatives to the LD_{50} tests are encouraged for acute toxicity testing, and information regarding this point is presented in annex B.

NOTE 2 In the evaluation of materials following local implantation in accordance with ISO 10993-6, examination of undemineralized sections, in addition to routine demineralized sections, is recommended.

c) Group III

This group comprises tests, specific for dental materials, not referred to in ISO 10993:

1) pulp and dentine usage test (6.3);

2) pulp capping test (6.4);

3) endodontic usage test (6.5).

5.5 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.

6 Test procedures specific to dental materials

6.1 Agar diffusion test

6.1.1 Objective

The test is designed to demonstrate the nonspecific cytotoxicity of test materials after diffusion through agar or agarose.

6.1.2 Cell line

American Type Culture Collection CCL 1 fibroblasts (NCTC clone 929) shall be used; other cell lines may be used if reproducibility and accuracy of the response can be demonstrated.

NOTE — Alternative cell lines are presented in annex C.

6.1.3 Culture medium, reagents and equipment

Use Eagle's Basal Medium containing 2,2 g/l sodium bicarbonate, 3,0 g/l HEPES and 50 ml/l new-born calf serum. Prepare a double concentration of Eagle's Basal Medium omitting HEPES and reducing sodium bicarbonate to 1 g/l. Prepare either 3 % agar or 3 % agarose in distilled water.

Sterilize agar by autoclaving and medium by filtration.

Prepare the vital stain by diluting a stock solution of 1 % aqueous neutral red solution (record source) 1/100 with 0,01 ml/l phosphate-buffered saline solutions immediately before use. Store neutral red solutions protected from the light. Use Petri dishes of diameter 50 mm to 100 mm suitable for tissue culture.

6.1.4 Sample preparation

For the preparation of test materials, the manufacturer's recommended instructions shall be followed. The test shall be performed on either an extract of the material or the material itself, according to the guidance in ISO 10993-5, clause 4.

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

For setting materials, insert the freshly mixed material into glass or polytetrafluoroethylene rings of internal diameter 5 mm and height 5 mm. 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.

For fluid specimens or extracts, imbibe 0,1 ml of the fluid on a borosilicate microglass filter disc of 5 mm diameter, placed on the agar.

NOTE — Suitable discs can be prepared from prefilters[3].

6.1.5 Control specimens

Use control specimens as defined in ISO 10993-5, clause 3.

6.1.6 Test procedure

Culture the cells until they reach the end of the log growth phase. Pipette 10 ml 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 % (*V*/*V*) carbon dioxide for 24 h. Heat the sterile agar to 100 °C in a water bath and allow it to cool to 48 °C. Mix 1 part of agar with 1 part of double-concentration, freshly prepared nutrient medium and heat to 48 °C. Aspirate the liquid nutrient medium from each Petri dish and replace with 10 ml of freshly prepared agar/nutrient medium mixture.

3) Millipore prefilter AP2502200 is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

Allow the agar nutrient medium 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 two samples of test material, one positive control, one negative control and one extraction medium control if the last was used. Keep the specimens as far as possible from each other and from the wall of the Petri dish. Incubate at (37 ± 2) °C in a water-saturated atmosphere with 5 % (*V*/*V*) carbon dioxide for 24 h. Examine each test material at least in quadruplicate (i.e. two dishes per test material).

6.1.7 Parameters of assessment

The decolorization zone around the test materials and controls shall be assessed using an inverted microscope with a calibrated screen, and a Decolorization Index and a Lysis Index determined for each specimen in accordance with the following criteria:

a) **Decolorization Index**	**Description**
0	No decolorization detectable
1	Decolorization only under the test substance
2	Decolorization zone not greater than 5,0 mm from the test substance
3	Decolorization zone not greater than 10,0 mm from the test substance
4	Decolorization zone greater than 10,0 mm from the test substance
5	The total culture is decolorized

b) **Lysis Index**	**Description**
0	No cell lysis detectable
1	Less than 20 % cell lysis
2	20 % to 40 % cell lysis
3	> 40 % to < 60 % cell lysis
4	60 % to 80 % cell lysis
5	More than 80 % cell lysis

Calculate the median Decolorization Index and Lysis Index for each test material and present the cell response as follows:

Cell response = Decolorization Index/Lysis Index

If the index values for the four replicates of the test substance differ by more than 2 units in the range 0 to 3, repeat the test. With indices of 4 and 5, no repetition is necessary. When extracts are tested, subtract the median index of the extraction medium alone from the median index of the extraction medium containing test substance to obtain the index for the test substance alone. If the median index for the extraction medium serving as a control is greater than 1, repeat the test using a different extraction medium.

NOTE — For a valid test, an intact cell layer should be found under the negative control.

6.1.8 Assessment of results

All information gathered in the test shall be taken into account in assessing the test results, particularly any differences in results between the experimental and control groups. The cell response is based on the median