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Biological evaluation of medical devices —

Part 12:
Sample preparation and reference materials

Évaluation biologique des dispositifs médicaux —

Partie 12: Préparation des échantillons et matériaux de référence

Reference number
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Contents

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Foreword

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

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

International Standard ISO 10993-12 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

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

— *Part 1: Evaluation and testing*

— *Part 2: Animal welfare requirements*

— *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

— *Part 4: Selection of tests for interactions with blood*

— *Part 5: Tests for cytotoxicity: in vitro methods*

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

— *Part 7: Ethylene oxide sterilization residuals*

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

— *Part 10: Tests for irritation and sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

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

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

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

— *Part 16: Toxicokinetic study design for degradation products and leachables*

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

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

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

Introduction

This part of ISO 10993 gives guidance on methods of sample preparation and on the use of reference materials for use in biological evaluation. Because of the many different biological assay systems described in ISO 10993, the individual standards should be consulted to ascertain the appropriateness of these recommendations for a specific test system.

Sample preparation methods should consider both the biological evaluation methods and the materials being evaluated. Each biological test restricts selection of solid samples and extraction solvents or conditions by its own methodology.

This part of ISO 10993 is based on existing national and international specifications, regulations and standards wherever possible. It is open to regular review whenever new research work is presented to improve the state of scientific knowledge.

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Biological evaluation of medical devices —

Part 12:
Sample preparation and reference materials

1 Scope

This part of ISO 10993 specifies requirements and gives guidance on procedures to be followed in the preparation of samples of medical devices for testing in biological systems in accordance with one or more other parts of ISO 10993. These include:

a) test material selection;

b) selection of representative portions from a device;

c) test sample preparation;

d) the selection of reference materials to demonstrate the suitability of the test system and/or to enable relative comparison of the biological activity of the test sample; and,

e) preparation of extracts.

2 Normative references

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

ISO 9000-1:1994, *Quality management and quality assurance standards — Part 1: Guidelines for selection and use.*

ISO 9000-2:1993, *Quality management and quality assurance standards — Part 2: Generic guidelines for the application of ISO 9001, ISO 9002 and ISO 9003.*

ISO 9000-3:1991, *Quality management and quality assurance standards — Part 3: Guidelines for the application of ISO 9001 to the development, supply and maintenance of software.*

ISO 9000-4:1993, *Quality management and quality assurance standards — Part 4: Guide to dependability programme management.*

ISO Guide 25:1990, *General requirements for competence of calibration and testing laboratories.*

ISO Guide 30:1992, *Terms and definitions used in connection with reference materials.*

3 Definitions

For the purposes of this part of ISO 10993, the definitions given in ISO Guide 30 and the following definitions apply.

3.1 blank liquid: Liquid treated in the same manner as that for preparing extract liquid but without test material, and used for comparison with extract liquid.

3.2 extract liquid: Liquid resulting from the extraction of the test material.

3.3 negative control: 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.

3.4 positive control: 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 reference material: Material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials. [ISO Guide 30]

NOTE 1 When tested by the procedure described, the reference material demonstrates the suitability of the procedure to yield a reproducible, predictable response. The response may be negative or positive.

3.6 certified reference material: Reference material, accompanied by a certificate, one or more of whose property values are certified by a procedure which establishes its traceability to an accurate realization of the unit in which the property values are expressed, and for which each certified value is accompanied by an uncertainty at a stated level of confidence. [ISO Guide 30]

3.7 test material: Material, device, device portion or component thereof that is sampled for biological or chemical testing.

3.8 test sample: Extract or portion of the test material that is subjected to biological or chemical testing.

4 Experimental controls

4.1 Experimental controls shall be used in biological evaluations to validate a test procedure. Depending on the biological test being used, negative and positive controls and blanks shall be used as appropriate. The same control may be applicable to different tests to allow cross-reference to established materials and test methods. Additional guidance on the selection of experimental controls is given in annex A.

NOTE 2 A comparable clinically accepted device may satisfy this requirement.

4.2 Reference materials used as experimental controls shall meet the established quality assurance procedures of the manufacturer and test laboratory consistent with ISO Guide 25 and ISO 9000-1, ISO 9000-2, ISO 9000-3 and ISO 9000-4. Reference materials shall be identified as to source, manufacturer, type, grade and lot number, as appropriate.

4.2.1 Reference materials and certified reference materials should be in the same material class as the test sample, e.g. polymer, ceramic, metal, colloid, etc.

4.2.2 Reference materials are established by individual laboratories. The extent of chemical, physical and biological characterization is determined by the individual laboratory. Commercially available articles may be used as reference materials (see note 2, in 4.1).

4.2.3 Certified reference materials are selected for their high purity, critical characteristics, suitability for the intended purpose and general availability. The critical chemical, physical and biological characteristics shall be determined by collaborative testing in three or more laboratories, and made available to the investigator by the distributor.

5 Test material selection

5.1 It is preferable to test medical devices in their final product form and condition; this should be the first choice whenever practical. The second choice is to test representative portions of the device (see 5.2). When neither of these is possible or practical, representative specimens of the formulated material that have been preconditioned by the same processing as the final product should be tested.

5.2 If a device cannot be tested as a whole and contains several materials, each individual material which can come into contact with body tissues in the final product shall be represented proportionally in the test sample, except as described in 5.3.

5.2.1 The test sample of devices with surface coatings shall include both coating material and substrate.

5.2.2 The test sample shall include a representative portion of the joint and/or seal if adhesives, radio-frequency (RF) seals, or solvent seals are used.

5.2.3 Composite materials shall be tested as finished materials.

5.2.4 Materials which cure *in situ*, e.g. cements, adhesives and monomers, shall be tested after the specified minimum cure which may occur during clinical use.

5.3 There may be exceptions to the proportionality of materials in the test sample.

5.3.1 Selected tests (e.g. implantation) may require that individual materials be evaluated.

5.3.2 The test sample may be chosen to maximize the exposure of the test system to any material of a device that is known to have a potential for a biological response.

5.3.3 For specific tests, the influence of the geometric shape of the test sample may be stronger than the influence of the type of material. The geometric shape should prevail above the proportional composition regarding the different materials in the selection of a representative portion of the device as test sample to be used in these tests.

5.3.4 When different materials are present in a single device, the potential for synergies or interactions shall be considered in the choice of the test sample.

5.4 The same test material selection procedures apply when an extract of that material is required.

6 Test sample and reference material preparation

6.1 Test samples and reference materials shall be handled so as to prevent contamination. Residues from the processes of manufacturing, fabrication, cleaning, sterilization, etc., shall be considered to be integral to the device, device portion or component. Additional guidance on the preparation of test samples and reference materials is given in annex B.

6.1.1 Test samples from sterilized devices and reference materials shall be handled aseptically if appropriate to the test procedure.

6.1.2 Test samples from devices which are normally supplied nonsterile but are sterilized before use shall be sterilized by the method recommended by the manufacturer and handled aseptically if appropriate to the test procedure.

6.2 If sterilized test samples are prepared for the test procedure, the effect of the sterilization and any resterilization process on the test sample and reference materials shall be considered, including the effect of allowed multiple sterilization of the final product.

6.3 When test samples and reference materials need to be cut into pieces, the influence of previously unexposed surfaces, e.g. lumens or cut surfaces, shall be considered. Techniques used for cutting medical devices into representative portions for testing should be as clean as practical to prevent contamination.

7 Preparation of extracts of test materials

7.1 General

If extracts of the device are required for a test protocol, the extraction liquid and conditions of extraction used shall be appropriate to the nature and use of the final product. Additional guidance on the extraction of samples is given in annex C. Special requirements for preparing extracts of samples are sometimes necessary for special biological tests described in other parts of ISO 10993. In these cases, such requirements described in the corresponding parts of ISO 10993 shall take precedence over those in this part.

7.2 Containers for extraction

The extraction shall be performed in clean, chemically inert closed containers with minimum headspace consistant with safety. Extractions shall be performed under conditions which prevent contamination of the sample.

7.3 Extraction conditions

Extraction is a complex process influenced by time, temperature, surface-area-to-volume ratio, extraction medium and the phase equilibrium[1] of the material. The effects of higher temperatures or other conditions on extraction kinetics and the identity of the extractant(s) shall be considered carefully if accelerated or exaggerated extraction is used. Based on current practices, standard conditions that have been used to provide a measure of the hazard potential of the device or material are described below. Other

1) The phase equilibrium of a solid material at a given temperature controls the relative amounts of amorphous and crystalline phases present. For the amorphous phase, the glass transition temperature, T_g, dictates the polymer chain mobility and the diffusion rate in the phase. Usually, the diffusion rate is considerably higher at temperatures above the T_g compared with those below. The diffusion rate is lowest in the crystalline phase. The extraction conditions should be chosen to minimize alteration of phase equilibria of the material beyond that imparted by the physiological medium. Phase alteration may affect the amount and type of extractable substances. Changes in phase equilibria may be ascertained by differential scanning calorimetry analysis.

conditions that simulate the extraction that occurs during clinical use or provide a measure of the hazard potential may be used; if so, they shall be described and justification provided.

7.3.1 Standard extraction temperatures and times are as follows:

a) 37 °C ± 1 °C for 24 h ± 2 h;

b) 37 °C ± 1 °C for 72 h ± 2 h;

c) 50 °C ± 2 °C for 72 h ± 2 h;

d) 70 °C ± 2 °C for 24 h ± 2 h;

e) 121 °C ± 2 °C for 1 h ± 0,2 h.

Extraction conditions that simulate clinical conditions are preferred.

7.3.2 Standard surface areas, i.e. projected area excluding indeterminate surface irregularity, per millilitre of extractant volume are given in table 1.

Mass-to-volume and surface-area-to-volume extraction ratios, e.g. those related to evaluation of powders, foams, porous surfaces, etc., other than those given in table 1 may be used provided that they simulate the conditions during clinical use or result in a relevant measure of the hazard potential.

7.3.3 Elastomers, coated or surface-treated materials, composites, laminates, etc., should be tested intact whenever possible. Other materials should be cut into small pieces before extraction to enhance submersion in the extract liquid.

7.3.4 Examples of extraction media are:

a) polar liquid: water; physiological saline; liquid culture media without serum;

b) nonpolar liquid: freshly refined vegetable oil (e.g. cottonseed or sesame oil, oleum naturale);

c) additional extraction liquids: ethanol/water (5 % volume fraction); ethanol/physiological saline (5 % volume fraction); polyethylene glycol 400 (diluted to a physiological osmotic pressure); dimethylsulfoxide; liquid culture media with serum;

d) other liquids appropriate to the nature and use of the device, if their effects are known.

Table 1

Thickness mm	Extraction ratio[1)] ± 10 %	Example of material
⩽ 0,5	6 cm^2/ml	Metal; synthetic polymer; ceramic; composite film, sheet and tubing wall
> 0,5	3 cm^2/ml	Metal; synthetic polymer; ceramic; composite tubing wall; slab; moulded items
⩽1,0	3 cm^2/ml	Natural elastomer
> 1,0	1,25 cm^2/ml	Natural elastomer
Irregular	0,1 g/ml to 0,2 g/ml; 6 cm^2/ml	Pellets
NOTE — While there are no standardized methods available at present for testing absorbents and hydrocolloids, the following is a suggested protocol: Determine the "absorption capacity" of the material, i.e. the amount of extractant absorbed by of the material. The test sample should be 2 g of the material. The extract volume should be 20 ml more than the "absorption capacity" of the 2-g sample.		
1) Expressed as the ratio of the surface area or mass of the test piece to the volume of extractant used.		

7.3.5 Extractions may be performed under static or agitation conditions. When agitation is considered to be appropriate, the method should be specified and reported.

7.3.6 Liquid extracts shall, when possible, be used immediately after preparation to prevent sorption onto the extraction container or other changes in composition. If an extract is stored longer than 24 h, then the stability of the extract under the conditions of storage should be verified.

7.3.7 The extract should not routinely be processed by filtration, centrifugation or other methods to remove suspended particulates. However, if such processing is necessary, the rationale shall be presented.

8 Test report

The test report shall include the following documentation of sample preparation:

a) source of material, device, device portion or component;

b) lot or batch number, where appropriate;

c) description of processing, cleaning or sterilization treatments, if appropriate;

d) extraction techniques, as appropriate, including documentation of the conditions for extraction.