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

This fourth edition cancels and replaces the third edition (ISO 10993-12:2007), 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 process*

— *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 skin sensitization*

— *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* [Technical Specification]

— *Part 20: Principles and methods for immunotoxicology testing of medical devices* [Technical Specification]

Introduction

This part of ISO 10993 specifies methods of sample preparation and provides requirements and guidance for the selection of reference materials for the biological evaluation of medical devices.

It is important that sample preparation methods be appropriate for both the biological evaluation methods and the materials being evaluated. Each biological test method requires the selection of materials, extraction solvents and conditions.

This part of ISO 10993 is based on existing national and international specifications, regulations and standards wherever possible. It is periodically reviewed and revised.

1 Scope

This part of ISO 10993 specifies requirements and gives guidance on the procedures to be followed in the preparation of samples and the selection of reference materials for medical device testing in biological systems in accordance with one or more parts of ISO 10993. Specifically, this part of ISO 10993 addresses the following:

— test sample selection;

— selection of representative portions from a device;

— test sample preparation;

— experimental controls;

— selection of, and requirements, for reference materials;

— preparation of extracts.

This part of ISO 10993 is not applicable to live cells, but can be relevant to the material or device components of combination products containing live cells.

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 (all parts), *Biological evaluation of medical devices*

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

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
accelerated extraction
extraction that provides a measure of the leachable or extractable materials of the device or material, using conditions that shorten the time for leaching of the substances into the extraction vehicle but do not result in a chemical change of the substances being extracted

EXAMPLE Elevated temperature, agitation, changing of the extraction vehicle.

3.2
blank
extraction vehicle not containing the test material, which is retained in a vessel identical to that holding the test sample and subjected to conditions identical to the ones the test sample is subjected to during its extraction

NOTE The purpose of the blank is to evaluate possible confounding effects due to the extraction vessel, extraction vehicle and extraction process.

3.3
CRM
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 30:1992, definition 2.2]

3.4
exaggerated extraction
extraction that is intended to result in a greater amount of a chemical constituent being released as compared to the amount generated under the simulated conditions of use

NOTE It is important to ensure that the exaggerated extraction does not result in a chemical change of the material.

3.5
exhaustive extraction
extraction conducted until the amount of extractable material in a subsequent extraction is less than 10 % by gravimetric analysis of that detected in the initial extraction

NOTE As it is not possible to demonstrate the exhaustive nature of residual recovery, the definition of exhaustive extraction adopted is as above. See also Annex C.

3.6
experimental control
substance with well-characterized responses, which is used in a specific test system to assist in evaluating if the test system has responded in a reproducible and appropriate manner

3.7
extract
liquid that results from extraction of the test sample or control

3.8
extractables
substances that can be released from a medical device or material using extraction solvents and/or extraction conditions that are expected to be at least as aggressive as the conditions of clinical use

3.9
homogeneous
property of a material and its relationship to a biological endpoint, meaning that it is of uniform structure or composition, thereby consistently rendering, or not, a specific biological response

NOTE A reference material is said to be homogeneous if the biological response to a specific test is found to lie within the specified uncertainty limits of the test, irrespective of the batch or lot of material from which the test sample is extracted.

3.10
leachables
substances that can be released from a medical device or material during clinical use

3.11
negative control
any well-characterized material and/or substance, which, when tested by a specific procedure, demonstrates the suitability of the procedure to yield a reproducible, appropriately negative, non-reactive or minimal response in the test system

NOTE In practice, negative controls are reference materials but can include blanks and extraction vehicles/solvents.

3.12
positive control
any well-characterized material and/or substance, which, 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

3.13
RM
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 Adapted from ISO Guide 30:1992, definition 2.1.

NOTE 2 For the purpose of this part of ISO 10993, an RM is any well-characterized material or substance, which, 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.

3.14
simulated-use extraction
extraction conducted to demonstrate compliance with the requirements of this part of ISO 10993 by evaluating leachable material levels available to the patient or user from devices during the routine use of a device, using an extraction method that simulates product use

NOTE The burden of validation on the analytical laboratory is to demonstrate that the simulated-use extraction is carried out under conditions that provide the greatest challenge to the intended use. Product-use simulation is carried out assuming the device is assigned to the most stringent category possible for the duration of exposure and takes into consideration both the tissue(s) exposed and the temperature of exposure.

3.15
stability
ability of a material, when stored under specified conditions, to maintain a specific stated biological response, within specified limits, for a specific period of time

NOTE Adapted from ISO Guide 30:1992, definition 2.7.

3.16
test sample
medical device, component or material (or a representative sample thereof, manufactured and processed by equivalent methods), or an extract or portion thereof that is subjected to biological or chemical testing or evaluation

4 General requirements

4.1 When identifying hazards and estimating risk in relation to medical devices, hazards that arise from changes in the manufacturing process, or insufficient control of the manufacturing process, shall be considered in the design and preparation of test samples, as described in ISO 14971. Particular attention shall be given to residues, e.g. trace elements and cleaning and disinfection agents, of manufacturing processes.

4.2 ISO 10993 describes many different biological assay systems. Therefore, the individual parts shall be consulted to ascertain whether these are appropriate for specific test systems.

4.3 Experimental controls shall be used in biological evaluations carried out in order to validate a test procedure and/or to compare the results between materials. Depending on the biological test, negative controls, blanks and/or positive controls shall be used, depending on what is appropriate to the test.

NOTE The same type of control can be applicable to different tests and may allow cross-reference to other established materials and test methods. Additional guidance on the selection of experimental controls is given in Annex A. Use of positive controls for *in vivo* testing might be affected by animal welfare regulations.

5 Reference materials (RMs)

5.1 General

RMs 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 RM.

NOTE 1 See also ISO Guide 35.

CRMs 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.

NOTE 2 It is desirable for users to obtain a commitment from suppliers of RMs or CRMs stating that these materials will be available to the user for at least five years. A second but less desirable option is for the source of the RM or CRM to publish an “open formulation” for the material, i.e. publication of the source materials and details of the processing needed to ensure uniform batches of the RM.

5.2 Certification of RMs for biological safety testing

5.2.1 Qualification of an RM is a procedure that establishes the numerical or qualitative value of the biological response of the material under specified test conditions, ensuring reproducibility of the response within and/or between laboratories. The range of biological responses associated with the material shall be established through laboratory tests.

NOTE See also ISO Guide 34.

5.2.2 Suppliers of RMs shall certify the materials. The supplier determines the extent of chemical and physical characterization that is performed. The individual laboratories that use the RM shall identify the biological characterization necessary to qualify a RM for a specific test or procedure. Commercially available materials may be used as RM, provided they are certified and qualified.

5.2.3 Certification of a RM is a procedure that establishes the numerical or qualitative value of the biological response of the material under the specified test conditions. This process serves to validate the testing of the material for that particular response and results in the issuance of a certificate. The biological response of the material shall be established through interlaboratory tests.

6 Use of RMs as experimental controls

6.1 RMs or CRMs shall be used in biological tests as control materials to demonstrate the suitability of a procedure to yield a reproducible response, i.e. positive and/or negative. Any material used in this way shall be characterized with each biological test procedure for which the use of the material is desired. A material characterized and then certified for one reference test method or response, e.g. delayed-type hypersensitivity, shall not be used as an RM for another, e.g. cytotoxicity, without additional validation.

NOTE The use of an RM will facilitate the comparability of the response between laboratories and help assess reproducibility of the test performance within individual laboratories. For comparison of the biological response, it is desirable to use RMs having a range of responses, e.g. minimum, intermediate or severe.

6.2 RMs used as experimental controls shall meet the established quality assurance procedures of the manufacturer and test laboratory. They shall be identified in relation to source, manufacturer, grade and type. RMs are processed as described in Clause 8.

6.3 When RMs are used as experimental controls, they shall be in the same material class as the test sample, i.e. polymer, ceramic, metal, colloid, etc. However, pure chemicals may be used as experimental controls for mechanistically-based test procedures, e.g. genotoxicity and immune delayed-type hypersensitivity assays.

7 Test sample selection

7.1 Testing shall be performed on the final product, representative samples from the final product, materials processed in the same manner as the final product (see ISO 10993-1), or on appropriate extracts of any of these. The choice of test sample shall be justified.

NOTE In the case of materials that cure *in situ*, different test samples representative of the cured material versus the uncured state of the material might be needed.

7.2 The same test sample selection procedure applies when an extract is required.

8 Test sample and RM preparation

8.1 Test samples and RMs shall be handled with care to prevent contamination. Any residue from the manufacturing processes shall be considered integral to the device, device portion or component.

NOTE For additional guidance on preparation, see Annex B.

a) Test samples from sterilized devices and RMs shall be handled aseptically, if appropriate to the test procedure.

b) Test samples from a device which is normally supplied non-sterile, but requires sterilization prior to use, shall be sterilized by the method recommended by the manufacturer and handled aseptically, if appropriate to the test procedure.

c) If test samples are cleaned prior to sterilization, the influence of the cleaning process and cleaning agent shall be considered in the selection and handling of the test sample.

8.2 Test samples from devices not required to be sterile in use shall be used as supplied and handled aseptically throughout the test sample preparation. If sterile test samples are required for a test procedure, e.g. for cytotoxicity testing, the effect of the sterilization or resterilization process on the test sample and RM shall be considered.

8.3 When test samples and RMs need to be cut into pieces, as described in 10.3.3, the influence of previously unexposed surfaces, e.g. lumens or cut surfaces, shall be considered. Tools used for cutting medical devices into representative portions for testing shall be cleaned between uses to prevent contamination.

9 Selection of representative portions from a device

9.1 If a device cannot be tested as a whole, each individual material in the final product shall be represented proportionally in the test sample.

a) The test sample of devices with surface coatings shall include both coating material and the substrate, even if the substrate has no tissue contact.

b) 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 in the manufacture of a portion of the device which comes into contact with patients.

9.2 Composite materials shall be tested as finished materials.

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

9.4 The test sample shall be chosen to maximize the exposure of the test system to the components of a device that are known to have potential for a biological response.