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Part 12:
Sample preparation and reference materials

Évaluation biologique des dispositifs médicaux —

Partie 12: Préparation des échantillons et matériaux de référence

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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 194, *Biological and clinical evaluation of medical devices*.

This fifth edition cancels and replaces the fourth edition (ISO 10993-12:2012), which has been technically revised.

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

— change of scope to cover extractions only for biological evaluation tests,

— harmonization of definitions with ISO 10993-18,

— revision of 10.3.1 extraction condition table and Annex D regarding exhaustive extraction.

A list of all parts in the ISO 10993- series can be found on the ISO website.

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

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 document is based on existing national and international specifications, regulations and standards wherever possible.

Biological evaluation of medical devices —

Part 12:
Sample preparation and reference materials

1 Scope

This document 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 test systems onlyin accordance with one or more parts of ISO 10993. Specifically, this document addresses the following:

— test sample selection;

— selection of representative portions from a medical device;

— test sample preparation;

— experimental controls;

— selection of, and requirements, for reference materials;

— preparation of extracts.

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

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

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

3.1
blank
extraction vehicle not containing the test material, which is exposed to identical vessels and conditions as the test sample during extraction

Note 1 to entry: The purpose of the blank is to evaluate possible confounding effects due to the extraction vessel, extraction vehicle and extraction process.

3.2
certified reference material
CRM
reference material (RM) characterized by a metrologically valid procedure for one or more specified properties, accompanied by an RM certificate that provides the value of the specified property, its associated uncertainty, and a statement of metrological traceability

Note 1 to entry: The concept of value includes a nominal property or a qualitative attribute such as identity or sequence. Uncertainties for such attributes may be expressed as probabilities or levels of confidence.

Note 2 to entry: Metrologically valid procedures for the production and certification of RMs are given in, among others, ISO Guide 34 and ISO Guide 35.

Note 3 to entry: ISO Guide 31 gives guidance on the contents of RM certificates.

Note 4 to entry: ISO/IEC Guide 99:2007 has an analogous definition (5.14) .

[SOURCE: ISO Guide 30:2015, 2.1.2]

3.3
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 1 to entry: It is important to ensure that the exaggerated extraction does not result in a chemical change of the material.

3.4
exhaustive extraction
extraction conducted until the amount of extractable material in a subsequent extraction is less than 10 % by gravimetric analysis (or that achieved by other means) of that detected in the initial extraction

Note 1 to entry: 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.

3.5
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

3.6
extract
liquid that results from extraction of the test sample or control

3.7
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

3.8
homogeneous
property of a material and its relationship to a biological endpoint, meaning that it is of uniform composition, and chemical/physical characteristics thereby producing a consistent test result

Note 1 to entry: 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.

3.9
leachables
substances that can be released from a medical device or material during clinical use

3.10
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 1 to entry: In practice, negative controls are reference materials but can include blanks and extraction vehicles/solvents.

3.11
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

3.12
reference material
RM
material, sufficiently homogeneous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process

Note 1 to entry: RM is a generic term.

Note 2 to entry: Properties can be quantitative or qualitative, e.g. identity of substances or species.

Note 3 to entry: Uses may include the calibration of a measurement system, assessment of a measurement procedure, assigning values to other materials, and quality control.

Note 4 to entry: ISO/IEC Guide 99:2007 has an analogous definition (5.13) , but restricts the term “measurement” to apply to quantitative values. However, Note 3 of ISO/IEC Guide 99:2007, 5.13 (VIM), specifically includes qualitative properties, called “nominal properties”.

[SOURCE: ISO Guide 30:2015, 2.1.1]

3.13
simulated-use extraction
extraction performed using a method that simulates product use

Note 1 to entry: The laboratory is to demonstrate that the simulated-use extraction is carried out under conditions that provide an appropriate representation of intended use. Product-use simulation is carried out assuming the medical 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.

3.14
stability
characteristic of a material, when stored under specified conditions, to maintain a specified property value within specified limits for a specified period of time

Note 1 to entry: See also the IUPAC Compendium of Analytical Nomenclature[5].

3.15
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 evaluation testing

4 General requirements

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 material additives, unintentional base material impurities and manufacturing process residues, e.g. trace elements,and cleaning and disinfection agents.

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

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 specifications of a particular test, negative controls, blanks and/or positive controls shall be used.

NOTE The same type of control can be applicable to different tests and can 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

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 17034.

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.

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

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.

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.

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

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.

For absorbable materials that could potentially have toxic degradants and residuals, testing of intermediate products should be considered.

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

8 Test sample and RM preparation

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

NOTE For additional guidance on preparation, see Annex B.

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

— Test samples which are clean, sterile and disinfected, shall be processed by the method recommended by the manufacturer and handled aseptically, if appropriate to the test procedure.

— The influence of the cleaning process and cleaning agent shall be considered in the selection and handling of the test sample.

Test samples from medical 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.

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 medical device

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

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

— 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 medical device which comes into contact with patients.

— Documents shall be provided (e.g. schematic or photo) of the medical device components that are sampled, and those that are not sampled.

9.2 Composite materials shall be tested as finished materials.

9.3 When different materials are present in a single medical 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 medical device that are known to have potential for a biological response.

9.5 Non-contacting portions of the medical device should, if possible, be excluded either physically from test sample extracts or by exclusion of the surface area in the calculation of the extraction ratio. When this is not possible, the extraction ratio shall be justified. Care must be taken to ensure that all contacting portions are covered by the selected extraction vehicle volume.

9.6 Medical device components with different type or duration of tissue contact might need to be extracted and tested separately.

10 Preparation of extracts of samples

10.1 General

If extracts of the medical device are required for a test procedure, the extraction vehicles and conditions of extraction used shall be appropriate to the nature and use of the final product and to the purpose of the test, e.g. hazard identification, risk estimation or risk assessment. The physico-chemical properties of the medical device materials, leachable substances or residues shall be considered when choosing the extraction conditions (see ISO 10993-18 and ISO/TR 10993-19).

NOTE For additional guidance on the extraction of samples, see Annex C.

10.2 Containers for extraction

The extraction shall be performed in clean, chemically inert, closed containers with minimum dead space.

To ensure that the extraction vessels do not adulterate the extract of the test sample, the extraction vessels shall be either borosilicate glass tubes with caps having an inert liner, e.g. polytetrafluoroethylene or other inert extraction vessels, as required for specific materials and/or extraction procedures.

