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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 third edition cancels and replaces the second edition (ISO 10993-12:2002), 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*

— *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 delayed-type hypersensitivity*

— *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* [TS]

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

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

This corrected version of ISO 10993-12 contains changes to definition 3.10 on page 3 and changes to footnote references in A.3 on page 11.

Introduction

This part of ISO 10993 specifies methods of sample preparation and the selection of reference materials in the biological evaluation of medical devices.

Sample preparation methods should 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.

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 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 the ISO 10993 series. Specifically this part of ISO 10993 addresses:

- 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 materials or devices 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 10993-1:2003, *Biological evaluation of medical devices — Part 1: Evaluation and testing*

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
blank
extraction vehicle not containing the test material, retained in a vessel identical to that which holds the test sample and subjected to identical conditions to which the test sample is subjected 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.

3.2
certified reference material
(CRM)
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, definition 2.2]

3.3
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

3.4
extract
liquid that results from extraction of the test sample or control

3.5
homogeneous
property of a material and its relationship to a biological endpoint such that it is of uniform structure or composition to consistently render 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 removed.

3.6
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 may include blanks and extraction vehicles/solvents.

3.7
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

3.8
reference material
(RM)
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

[ISO Guide 30, definition 2.1]

NOTE For the purpose of this part of ISO 10993, a reference material 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.

3.9
stability
⟨of property values⟩ 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, definition 2.7.

3.10
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

3.11
simulated-use extraction
extraction 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 probable for duration of exposure and takes into consideration both tissue(s) exposed and temperature of exposure.

3.12
exaggerated extraction
any 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 Exaggerated extraction might result in a chemical change of the material or the substances being extracted.

3.13
accelerated extraction
extraction that provides a measure of the leachable 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

NOTE Examples of accelerated extraction conditions are as follows: elevated temperature, agitation, changing of the extraction vehicle, etc.

3.14
exhaustive extraction
extraction until the amount of leachable material in a subsequent extraction is less than 10 % of that detected in the initial extraction, or until there is no analytically significant increase in the cumulative leachable material levels detected

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.

4 General requirements

4.1 As described in ISO 14971, in the identification of hazard and risk estimation for 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 samples for test and preparation of extracts of those devices. Particular attention shall be given to residues, e.g., trace elements and cleaning and disinfection agents, of those manufacturing processes.

4.2 Because ISO 10993 describes many different biological assay systems, the individual standards shall be consulted to ascertain if these recommendations are appropriate for specific test systems.

4.3 Experimental controls shall be used in biological evaluations 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 as 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 may be affected by animal welfare regulations.

5 Reference materials

5.1 General

Reference materials (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 reference materials.

NOTE 1 See also ISO Guide 35.

Certified reference materials (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 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 RMs shall identify the biological characterization necessary to qualify an RM for a specific test or procedure. Commercially available materials may be used as RMs provided they are certified and qualified.

5.2.3 Certification of an 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, such as either 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 Use of an RM will facilitate the comparability of the response between laboratories and assist in assessing reproducibility of 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 as 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, or representative samples from the final products or 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 residues from the manufacturing processes shall be considered to be 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 which 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 If sterile test samples are required for the test procedure, the effect of the sterilization or resterilization process on the test sample and RMs shall be considered.

8.3 When test samples and RMs need to be cut into pieces as described in 10.3.3 b), 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 contacts 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.

10 Preparation of extracts of samples

10.1 General

If extracts of the 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 device materials, leachable substances or residues shall be considered when choosing the extraction conditions.

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

10.2 Containers for extraction

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

10.2.2 To ensure that the extraction vessels do not adulterate the extract of the test sample the extraction vessels shall be:

a) borosilicate glass tubes with caps having an inert liner [e.g., poly(tetrafluoroethylene)];

b) other inert extraction vessels as required for specific materials and/or extraction procedures.

10.3 Extraction conditions and methods

10.3.1 Extraction conditions are based on common practice and are justified on the basis of providing a standardized approach that is, in many ways, an appropriate exaggeration of product use. Extraction shall be conducted under one of the following conditions (see also C.5):

a) (37 ± 1) °C for (72 ± 2) h;

b) (50 ± 2) °C for (72 ± 2) h;

c) (70 ± 2) °C for (24 ± 2) h;

d) (121 ± 2) °C for (1 ± 0,1) h.

NOTE Extraction at (37 ± 1) °C for (24 ± 2) h in tissue culture media might be acceptable for cytotoxicity testing. See ISO 10993-5.

Extraction conditions described above, which have been used to provide a measure of the hazard potential for risk estimation of the device or material, are based on historical precedent. Other conditions that simulate the extraction that occurs during clinical use, or provide an adequate measure of the hazard potential, may be used, but shall be described and justified.

Extraction is a complex process influenced by time, temperature, surface-area-to-volume ratio, extraction vehicle and the phase equilibrium [1)] of the material. The effects of higher temperatures or other conditions on extraction kinetics and the identity of the extraction vehicle(s) should be considered carefully if accelerated or exaggerated extraction is used.

For example, two possibilities exist when elevated temperatures are used:

1) the energy of the increased temperature may cause increased cross-linking and/or polymerization of the polymer and, therefore, decrease the amount of free monomer that is available to migrate from the polymer;

2) the increased temperature could cause degradation products to form that are not typically found in the finished device under conditions of use.

10.3.2 For materials that dissolve or resorb under conditions of use, follow the extraction conditions described in 10.3.1. Perform extraction using the appropriate extraction vehicle and time/temperature conditions to simulate exaggerated exposure wherever possible. Complete dissolution may be appropriate.

10.3.3 The standard surface area can be used to determine the volume of extraction vehicle needed. This area includes the combined area of both sides of the sample and excludes indeterminate surface irregularities. When surface area cannot be determined due to configuration of the sample, a mass/volume of extracting fluid shall be used. See Table 1.

a) Other surface area to volume extraction ratios, e.g., those related to evaluation of porous materials, can be used if they simulate the conditions during clinical use or result in a measure of the hazard potential.

b) Materials shall be cut into small pieces before extraction to enhance submersion in the extract media, except when otherwise inappropriate (for example, see 10.3.4). For example, for polymers, pieces approximately 10 mm × 50 mm or 5 mm × 25 mm are appropriate.

Table 1 — Standard surface areas and extract liquid volumes

Thickness mm	**Extraction ratio** (surface area or mass/volume) ± 10 %	**Examples of forms of materials**
< 0,5	6 cm²/ml	film, sheet, tubing wall
0,5 to 1,0	3 cm²/ml	tubing wall, slab, small moulded items
> 1,0	3 cm²/ml	larger moulded items
> 1,0	1,25 cm²/ml	elastomeric closures
Irregularly shaped solid devices	0,2 g sample/ml	powder, pellets, foam, non-absorbent moulded items
Irregularly shaped porous devices (low density materials)	0,1 g/ml	membranes

NOTE While there are no standardized methods available at present for testing absorbents and hydrocolloids, the following is a suggested protocol.

Determine the volume of extractiom vehicle that each 0,1 g or 1,0 cm^2 of material absorbs. Then, in performing the material extraction, add this additional volume to each 0,1 g or 1,0 cm^2 in an extraction mixture.

1) The phase equilibrium of a material during extraction 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 than the T_g compared with that below. The diffusion rate is lowest in the crystalline phase. The extraction conditions should not alter the phase equilibrium of the material. Phase alteration may affect the amount and type of extractables.

10.3.4 Elastomers, coated materials, composites, laminates, etc., shall be tested intact whenever possible because of potential differences in extraction characteristics between the intact and cut surfaces.

NOTE As a result of manufacturing processes, many elastomers might have surface properties that are different from those of the bulk material.

10.3.5 Extraction using both polar and non-polar extraction vehicles shall be performed. Examples of extraction vehicles are:

a) polar extraction vehicle: water, physiological saline, culture media without serum;

b) non-polar extraction vehicle: freshly refined vegetable oil (e.g., cottonseed or sesame oil) of quality defined in various pharmacopoeia;

c) additional extraction vehicles: ethanol/water, ethanol/saline, polyethylene glycol 400 (diluted to a physiological osmotic pressure), dimethyl-sulfoxide and culture media with serum.

NOTE Other extraction vehicles appropriate to the nature and use of the device or to the methods for hazard identification may also be used if their effects on the material and the biological system are known.

10.3.6 Extractions shall be performed with agitation or circulation. When extraction under static conditions is considered to be appropriate, the method shall be justified, specified and reported.

10.3.7 Liquid extracts shall, if possible, be used immediately after preparation to prevent sorption on to the extraction container or other changes in composition. If an extract is stored longer than 24 hours, then the stability and homogeneity of the extract under the storage conditions shall be verified.

10.3.8 Extract pH shall not be adjusted unless a rationale is provided.

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

10.3.10 For hazard identification, exaggerated extraction conditions shall be considered to increase the exposure dose of leachables. The extraction vehicle and conditions of extraction shall be selected on the basis of physicochemical properties of the material and/or predicted low molecular weight chemicals that might be extracted.

10.3.11 For materials or devices not expected to dissolve or resorb under conditions of use, any solvents used in the extraction of a polymeric material or device shall not cause dissolution of the polymer formulation. No more than a slight softening of the polymeric material shall occur in the presence of the volatile solvent (e.g., less than 10 % dissolution). The solvent shall be removed (prior to use in a bioassay) to the extent that any residues do not adversely affect the biological assay (e.g., cause protein denaturation or skin irritation). For materials or devices expected to dissolve or resorb under conditions of use, see 10.3.2.

10.3.12 If the device is an aqueous fluid, do not extract. Use the fluid directly in the test system.

10.3.13 Where fluids circulate through the device under normal conditions of use, e.g., extra-corporeal devices, extraction via re-circulation may be used. When possible, one or more of the conditions shall be exaggerated, e.g., temperature, time, volume, flow rate. The rationale for the extraction chosen shall be reported.

10.4 Extraction conditions for hazard identification and risk estimation in the exaggerated-use condition

10.4.1 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 samples for test and preparation of extracts of those devices, in accordance with ISO 14971. Particular attention shall be given to residues, e.g., trace elements and cleaning and disinfection agents, of those manufacturing processes.

10.4.2 Where the toxic potential is shown to be within the requirement for a product tested by exaggerated extraction, there shall be no need to further challenge the device by simulated-use extraction.

10.4.3 In the case of products that polymerize *in situ*, the samples to be tested shall represent the intended clinical conditions of use to provide information on the potential toxicity of the reacting components in the polymer during the curing process. Test extracts prepared at different times, if appropriate, shall be based on the kinetics of polymerization after mixing the components, including an extract prepared at the expected cure time. Testing of the material after curing shall be justified.

Where extracts are used in the test methods, for evaluation of materials that cure *in situ*, initiation of the extraction shall occur from the point in the cure at which the material is placed *in situ*.

For test methods that use these materials directly, e.g., direct contact or agar overlay cytotoxicity, implantation, some genotoxicity tests, and direct contact hemolysis, the material shall be used as in clinical use, with *in situ* cure in the test system.

NOTE Modification of the clinical delivery system might be appropriate so that the designated size or weight of the material is delivered for testing.

11 Records

Documentation of the sample and its preparation shall include, but not be limited to:

a) type and, if known, composition of material, source of material, device, device portion or component;

NOTE A written description, drawing, photograph or other methods can achieve all or part of this requirement.

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 extraction vehicle, extraction ratios, the conditions for extraction, means of agitation, as well as any deviations from the conditions specified in this part of ISO 10993, such as filtration of the extract or extraction media.

Annex A
(informative)

Experimental controls

A.1 The materials listed in the following paragraphs may meet the criteria for an appropriate experimental control in selected tests. It is the responsibility of the investigator to make the appropriate choices. See Table A.1.

Table A.1 — Examples of available reference materials and controls

Information on reference materials and controls are supplied only for those tests in ISO 10993 which do not call for specific reference materials or controls.

Test	Positive control	Negative control [a]	Reference material [a]
Implantation	PVC-org. Sn	PE	
	SPU-ZDEC	Silicone	
	Natural rubber latex	Alumina	
		Stainless steel	
Cytotoxicity	PVC-org. Sn	PE	
	SPU-ZDEC		
	SPU-ZBEC		
	Natural rubber latex		
	Polyurethane		
Blood compatibility			PVC 7506 PUR 2541

[a] Abbreviations on this Table refer to specific materials available from sources designated in A.2 and A.3.

A.2 Materials that have been used as negative controls or reference materials are, for example, high density polyethylene [2),3),4),5)] low density polyethylene [6)], silica-free polydimethylsiloxane [7),8)], polyvinylchloride [9)],

2) High-density polyethylene (Negative Control Plastic RS) can be obtained from the US Pharmacopeia, Rockville, MD 20852, USA.

3) HDPE film: RM-C Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 JAPAN.

4) HDPE sheet: RM-D Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 JAPAN.

5) HDPE rod: RM-E Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 JAPAN.

6) PE 140 tubing: RAUMEDIC AG, Postfach 501, 95205 Münchberg, Germany. PE film is available from Hoechst AG, D-6230 Frankfurt 80, Germany.

7) Biomaterials Program, Devices and Technology Branch, National Heart, Lung and Blood Institute, NIH, Building, 7550 Wisconsin Ave., Bethesda, MD 20892, USA.

8) SIK 8363 tubing: RAUMEDIC AG, Postfach 501, 95205 Münchberg, Germany.

9) PVC 7506 and PVC 7536 tubing: RAUMEDIC AG, Postfach 501, 95205 Münchberg, Germany. PVC-DEHP and PVC-TEHTM film is available from Hoechst AG, D-6230 Frankfurt 80, Germany.

polyetherurethane [10], polypropylene [11], aluminium oxide ceramic rods, stainless steel and commercially pure (cp) titanium alloys.

A.3 Materials that have been used as positive controls are, for example, polyvinylchloride containing organotin additives, segmented polyurethane films containing zinc diethyl-[12),13)], or dibutyldithiocarba-mate [14], certain latex formulations, solutions of zinc salts, and copper. Substances that have been used as positive controls for extract samples are dilutions of phenol and water.

10) PUR 2541 tubing: RAUMEDIC AG, Postfach 501, 95205 Münchberg, Germany. PU film is available from Frontline Filmblasning, S-60003 Norrköping, Sweden.

11) PP 146 tubing: RAUMEDIC AG, Postfach 501, 95205 Münchberg, Germany. PP film is available from Hoechst AG, D-6230 Frankfurt 80, Germany.

12) Positive Control Material, code 499-300-000-000: Portex Limited [same as Positive control RS which can be obtained from the US Pharmacopeia, Rockville, MD 20852, USA].

13) Polyurethane film – ZDEC: RM-A; Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 JAPAN.

14) Polyurethane rod – ZDEC: RM-F; Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 JAPAN.

The information given in footnotes 2) to 14) lists examples of suitable products available commercially. This information is given for the convenience of users of this part of ISO 10993 and does not constitute an endorsement by ISO of these products.

Annex B
(informative)

General principles on and practices of test sample preparation and sample selection

The material used in the biological assay shall be representative of the composition and surface characteristics of the final product and of the processes used in its manufacture. See 7.1 and ISO 10993-1:2003, 5.1 a).

Documentation of the composition of plastic and rubber materials shall include identification of the resin, polymer and any additives. The formulation description shall specify the history of the material, e.g., information on the thermal processing, and whether it is virgin or reground and, if reground, the specification for the maximum allowable regrind.

Materials that may be re-sterilized by the same or alternative methods shall be tested after treatment by the multiple sterilizations.

For example, a material that is sterilized by radiation and re-sterilized by ethylene oxide shall be tested after

a) irradiation

and

b) irradiation plus ethylene oxide.

If a “worst case” exposure can be identified with appropriate justification, testing may be performed after exposure to this treatment.

Ideally, all biological tests which use a material cut from a device, a device component itself as the test material, or extract prepared from either, shall be performed with the surface of the material exposed to the test systems’ cellular/biological environment. An alternative method to cutting the surface is fabrication of miniatures of the device using the same process (extrusion, dipping, etc.), temperatures, time, atmosphere, release agents, annealing, curing, cleaning, sterilization, etc., processes used in the manufacture of the device. This assists in evaluating any effects related to surface area, surface characteristics, concentration of leachables and the material’s surface and shape.

Metals used in biological tests shall be from the same stock material used to fabricate the device and using the same machining, grinding, polishing, cleaning, passivation, surface treatment and sterilization used in the manufacture of the final product.

Ceramic materials used in biological tests shall be manufactured from the same powder stock using the same casting, investing, moulding, sintering, surface finishing and sterilization processes used to manufacture the device.

Medical devices utilizing animal tissues or their derivatives and that are treated with a fixative shall be tested after they have been preserved under the manufacturer's maximum and minimum allowable fixation times to allow for varying penetration of the fixative.

Instead of extraction of metallic materials followed by application of the extract to the test systems, testing the solutions of various concentrations of the appropriate salt of the specific metal(s) identified in the device shall be considered for identifying the hazard of the specific metal ion(s) and to know its highest non-effect level(s).

NOTE This principle is also applicable for organic materials when chemicals in the device are identified.

Extraction conditions for implant materials that may cause particle generation *in-vivo* during clinical use shall be considered in the design of tests on the materials. The effect of extraction procedures shall be considered in the design of tests of materials if particulates are generated by the extraction conditions.

The amount of material and surface area thereof shall be appropriate to the biological and physical constraints of the test system. In practice, the use of a standard sample size for a specific assay is recommended.

The attention of the users of this part of ISO 10993 is directed to the discussion of “proper use” and “misuse” of Certified Reference Materials in the introduction to ISO Guide 33. This discussion points out areas of both potential under and over utilization of Reference Materials and Certified Reference Materials. Users of this part of ISO 10993 shall also note that the use of calibration materials to evaluate the biological response of materials under investigation within a single laboratory is acceptable.

Annex C
(informative)

Principles of test sample extraction

C.1 The purpose of extraction of a medical device is to provide a suitable test sample for determining the biological reactivity of any leachables in the biological system, to demonstrate the hazard potential (hazard identification) of the leachable and for use in conducting human health risk assessments of the leachable. If extracts of the device are prepared, the extraction vehicle and conditions of extraction used shall be appropriate to the nature and use of the final product as well as to the predictability (such as test purpose, rationale, sensitivity, etc) of the test method. Extraction conditions and application of the extract to test systems, therefore, ideally shall reflect not only actual conditions of use of the products but also the purpose and predictability of the tests.

Under normal conditions of use where fluids circulate through the device, e.g., extra corporeal devices, the vertical standard should be consulted for the appropriate extraction techniques.

Biological tests are carried out for identifying hazards and estimating risks of the hazards in exaggerated-use and/or in actual conditions of use. Extractions differ for various test purposes.

a) Exaggerated extraction is appropriate for hazard identification.

b) Simulated-use extraction is applicable for generation of a safety factor for use in human health risk assessments.

c) Exhaustive extraction is applicable for the assessment of the safety of an implant device and to estimate the upper limits of the chemicals that could be released to the patient.

C.2 This part of ISO 10993 assumes that the amount of extractable substance(s) is related to the period of extraction, the temperature, the ratio of surface-area-of-material to volume-of-extraction vehicle and the nature of the extraction vehicle.

C.3 The period of extraction shall be sufficient to maximize the amount of material extracted. In practice, use of these standard conditions of time and temperature for extraction are recommended in lieu of other unvalidated or non-standard conditions.

C.4 An alternative practice is repeated extraction followed by concentration to obtain sufficient extractable substance(s). This practice is applicable for the purposes of hazard identification.

C.5 Extraction temperatures may vary for the different materials to be tested. Extraction shall not initiate significant degradation of the material, except if the material is intended to dissolve or be resorbed during use (see 10.3.2). The extraction temperature is dependent upon the physicochemical characteristics of the device material(s). The extraction temperature chosen for polymers, for example, shall be below the glass transition temperature. If the glass transition temperature is below the use temperature, the extraction temperature shall be below the melting temperature. Recommended conditions are given in 10.3.1.

The following examples are presented to illustrate the interpretation of 10.3.1.

a) Materials that have a melting or softening point less than (121 ± 2) °C will be extracted at a standard temperature less than the melting point (e.g., very low density polyethylene).

b) Materials that undergo hydrolysis will be extracted at a temperature that minimizes the amount of hydrolysis [e.g., polyamides are extracted at (50 ± 2) °C].

c) Materials that are processed by steam sterilization and contain a liquid during storage will be extracted at (121 ± 2) °C (e.g., pre-filled dialysers).

d) Materials that are used only at body temperature will be extracted at temperatures which provide the maximum leachables without material degradation [e.g., fixed tissues shall be extracted at (37 ± 1) °C, whereas ceramic implants may be extracted at (121 ± 2) °C].

WARNING — The application of ISO 10993-12 test methods to device materials comprising proteins shall be made with great care to ensure that the extraction procedure has not altered the biological properties of the materials being extracted.

C.6 The ratio of the surface area of the device to the volume of extraction vehicle or solvent shall be sufficient to:

a) attain the maximum amount of extractable substance(s) in an appropriate dosage volume for biological testing (i.e., dosage volume within physiological limits) or chemical analysis;

b) demonstrate the hazard potential for the use of the device in humans;

c) cover the material in the solvent volume.

In practice, the use of a standard area and solvent volume is recommended as described in 10.3.3 in lieu of device specific parameters. Some test methods require concentration of extracts to increase the sensitivity of the test.

NOTE Concentration of extracts might result in loss of volatile materials such as ethylene oxide.

C.7 The solvent(s) selected as the extraction vehicle shall:

a) be suitable for use in the specific biological test systems;

b) simulate the extraction which occurs during clinical use of the device;

c) maximize the amount of extractives.

In practice, the use of standard polar and non-polar solvents is recommended. Subclause 10.3.5 recommends these in lieu of device specific solvents.

NOTE Standardization of the parameters given in Clauses C.5 and C.6 permit the use of data obtained from biological tests of medical devices for other types of application, e.g., for the estimation of risk and to develop standardized databases.

C.8 For materials that dissolve or resorb in the body:

a) follow the conditions of Table 1;

b) follow the temperature/times of 10.3.1;

c) follow 10.3.9 regarding filtration or centrifugation.

C.9 Sample preparation of products assembled *in situ*

A standard prescription cannot be constructed to address the specialized needs of preparing extracts of polymerized *in situ* products. The individual components, time to polymerization, intended use and the extraction vehicles need to be taken into consideration to develop a relevant extract. Language should include that the polymerization kinetics need to be used in the design of the correct methodology to develop a relevant extract for testing. The uncured components need to be considered when selecting an appropriate solvent for extracting the sample.

C.10 Comments on exhaustive extraction

The duration of an exhaustive extraction is determined by performing a series of consecutive extractions at a prescribed temperature and periods of time, analysing the extraction vehicle for extracts, replacing with fresh extraction vehicle, again exposing the sample for a period of time, analysing, and repeating the process. When the level of extracted chemicals is one-tenth (0,1) of the level of the initial extraction step, the process is deemed complete so that a 10 % correction to the total extracted material can be applied.

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