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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.

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. polytetrafluoroethylene;

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 is acceptable for cytotoxicity testing. For medical devices which are in short-term contact with intact skin or mucosa and which are not implanted, extraction times of less than 24 h, but not less than 4 h, are acceptable (see ISO 10993-5). Extraction temperatures greater than (37 ± 1) °C can adversely impact chemistry and/or stability of the serum and other constituents in the culture medium.

The 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 leachables occurring during clinical use, or that 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, the 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.

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, at temperatures higher than T_g, the diffusion rate is considerably higher compared with that below T_g. The diffusion rate is lowest in the crystalline phase. The extraction conditions should not alter the phase equilibrium of the material. Phase alteration can affect the amount and type of extractables.

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

— 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;

— 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 the surface area cannot be determined due to configuration of the sample, a mass/volume of extracting fluid shall be used. See Table 1.

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.

Materials shall be cut into small pieces before extraction to enhance submersion in the extract media, except when otherwise inappropriate (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 cm^2/ml	Film, sheet, tubing wall
0,5 to 1,0	3 cm^2/ml	Tubing wall, slab, small moulded items
>1,0	3 cm^2/ml	Larger moulded items
>1,0	1,25 cm^2/ml	Elastomeric closures
Irregularly shaped solid devices	0,2 g/ml	Powder, pellets, foam, non-absorbent moulded items
Irregularly shaped porous devices (low-density materials)	0,1 g/ml	Membranes, textiles

NOTE While there are no standardized methods available at present for testing absorbents and hydrocolloids, a suggested protocol is as follows:

— determine the volume of extraction 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.

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. The following are examples of extraction vehicles:

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 the quality defined in various pharmacopoeias;

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 1 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 (see Annex D).

NOTE 2 The use of a culture medium with serum is preferred for extraction in testing for cytotoxicity because of its ability to support cellular growth as well as extract both polar and non-polar substances.

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 for 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 of polymeric devices, exhaustive extraction conditions shall be considered. The extraction vehicle and conditions of extraction shall be selected on the basis of physico-chemical 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 10.3.12.

10.3.12 For solution and soluble materials, the standard extraction methods used for insoluble materials might be inappropriate. The following guidance should be considered in addition to information contained in Table 1.

a) Factors such as test system compatibility, route of administration and extent of dissolution or degradation should be considered in the final preparation for testing. Use an appropriate vehicle and conditions to simulate exaggerated exposure wherever possible. A pre-test can help to determine appropriate conditions.

b) If the material completely dissolves, in a vehicle or diluent that is compatible with the material and the test system, the resulting solution can be evaluated neat, provided the solution properties are also compatible with the test system, e.g. pH, osmolarity, solute concentrations.

c) If the material is an aqueous solution and used in this form, it shall be tested directly and not extracted, provided the solution properties are compatible with the test system [see also a) and b) above].

d) OECD Guidelines for the Testing of Chemicals, or similar chemical testing standards, can be used as guidance in determining maximum concentrations of test substances used for specific test methods.

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 (points to consider in relation to Annex D)

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 in the 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 and/or exhaustive 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 in order 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 haemolysis, 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, 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 might 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 RMs and controls

Test	Positive control	Negative control [a]	RM [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

NOTE Information on RMs and controls is supplied only for those tests in ISO 10993 which do not call for specific RMs or controls.

[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 RMs are, for example, high-density polyethylene[2)3)4)5)] low-density polyethylene[6)], silica-free polydimethylsiloxane[7)8)], polyvinylchloride[9)], polyether urethane[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[12)], segmented polyurethane rod[13)] or films[14) 15)] containing zinc diethyldithiocarbamate (SPU-ZDEC) [13) 14)] or dibutyldithiocarbamate (SPU-ZDBC)[15)], 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.

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, 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, 6230 Frankfurt 80, Germany.

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

11) PP 146 tubing: RAUMEDIC AG, Postfach 501, 95205 Münchberg, Germany. PP film is available from Hoechst AG, 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 rod — ZDEC: RM-F, Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523, Japan.

14) Polyurethane film — ZDEC: RM-A, Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523, Japan.

15) Polyurethane film — ZDBC (SPU-ZDBC): RM-B, Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523, Japan.

The information given in footnotes 2) to 15) 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 should be representative of the composition and surface characteristics of the final product and of the processes used in its manufacture (see 7.1).

Documentation of the composition of plastic and rubber materials should include identification of the resin, polymer and any additives. The formulation description should 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 should be tested after treatment by the multiple sterilizations.

For example, a material that is sterilized by radiation and re-sterilized by ethylene oxide should 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, the device component itself as the test material, or an extract prepared from either, should be performed with the surface of the material exposed to the test system’s cellular/biological environment. An alternative method to cutting the surface is the fabrication of miniatures of the device, using the same processes (extrusion, dipping, etc.), temperatures, time, atmosphere, release agents, and processes such as annealing, curing, cleaning and sterilization that were used to manufacture 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 should be from the same stock material used to fabricate the device and prepared using the same machining, grinding, polishing, cleaning, passivation, surface treatment and sterilization as used in the manufacture of the final product.

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

Medical devices utilizing animal tissues or their derivatives, and which are treated with a fixative, should 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 extracting metallic materials and then applying the extract to the test systems, testing the solutions at various concentrations of the appropriate salt for the specific metal(s) identified in the device should be considered in order to identify the hazard of the specific metal ion(s) and to determine its highest non-effect level(s).

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

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

The amount of material, and surface area thereof, should 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.

Users of this part of ISO 10993 are directed to the discussion of “proper use” and “misuse” of CRMs in the introduction to ISO Guide 33. This discussion points out areas of both potential under- and over-utilization of RMs and CRMs. Users of this part of ISO 10993 should 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 Extraction of a medical device can be carried out for the following purposes:

— 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 should 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 should therefore ideally 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), if a vertical standard exists, it should be consulted for the appropriate extraction techniques.

Biological tests are carried out to identify hazards and estimate the risks of the hazards occuring in exaggerated use and/or in actual conditions of use. Extractions differ for various test purposes:

a) exaggerated and exhaustive extraction is appropriate for hazard identification;

b) simulated-use extraction is applicable for the generation of a safety factor for use in human health risk assessments;

c) exhaustive extraction is applicable for the assessment of the safety of polymeric devices for long-term use in order 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 (are) related to the period of extraction, the temperature, the ratio of the surface area of the material to the volume of the extraction vehicle, and the nature of the extraction vehicle.

C.3 The period of extraction should 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 in order to obtain sufficient extractable substance(s). This practice is applicable to hazard identification (see Annex D).

C.5 Extraction temperatures may vary for the different materials to be tested. Extraction should not cause significant degradation of the material, unless the material is intended to dissolve or be resorbed during use (see 10.3.2). The extraction temperature is dependent upon the physico-chemical characteristics of the device material(s). The extraction temperature chosen for polymers, for example, should be below the glass transition temperature. If the glass transition temperature is below the use temperature, the extraction temperature should 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 of less than (121 ± 2) °C can be extracted at a standard temperature below the melting point (e.g. very-low-density polyethylene).

b) Materials that undergo hydrolysis can 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 can be extracted at (121 ± 2) °C (e.g. pre-filled dialysers).

d) Material should be extracted at temperatures which provide the maximum extractables without material degradation [e.g. fixed tissues can be extracted at (37 ± 1) °C whereas ceramic implants may be extracted at (121 ± 2) °C].

WARNING — Application of the test methods of this part of ISO 10993 to device materials comprising proteins should 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 should 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 of using 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 (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 the loss of volatile materials such as ethylene oxide.

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

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 By standardizing the parameters given in C.5 and C.6, 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, can be used.

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

a) follow the conditions given in Table 1;

b) follow the temperature/times given in 10.3.1;

c) follow 10.3.9 regarding filtration or centrifugation.

C.9 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 should be taken into consideration when developing a relevant extract. Language should include the recommendation that the polymerization kinetics be used in the design of the correct methodology to develop a relevant extract for testing. The uncured components should be considered when selecting an appropriate solvent for extracting the sample.

Annex D
(informative)

Exhaustive extraction of polymeric materials for biological evaluation

D.1 General

Polymeric materials often contain a small amount of low-molecular-weight chemical substances (LMWCs) such as catalysts, processing aids, or other additives[19], residual monomers, oligomers, etc. A major toxicological concern during the biological evaluation of polymeric materials is the toxicity of any leachables that can migrate from the polymer to the human body during device use. This concept is derived from the agreement of the OECD polymer group regarding health concerns and the exemption of polymers from testing (see Reference [17]). The report pointed out the following four parameters, which are important for judging the health risk of polymers:

a) number-average molecular weight of the polymer;

b) content of low-molecular-weight chemical species ;

c) presence of reactive functional groups (see Reference [18]);

d) presence of bioavailable metals.

NOTE LMWCs are defined as low-molecular-weight chemical substances with a molecular weight not exceeding 1 000 Da.

When performing a biological evaluation of polymeric devices, extraction practices are needed for preparing test samples (except in the case of implantation, direct-contact haemocompatibility and direct contact cytotoxicity tests). Annex C points out that exaggerated extraction is appropriate for hazard identification. Exhaustive extraction using organic solvents is another practice that might prove useful in hazard identification of polymeric devices, especially those for long-term use.

The rationale for this practice is based on the following considerations:

— for hazard identification, it is recommended that, as far as possible, the total amount of extractable substance be obtained from the polymeric device and applied appropriately to each test system;

— several papers showed that body fluids such as serum have a potency comparable with those of organic solvents, such as ethanol and methanol, when extracting chemicals (phthalate, 4,4’-methylenedianiline, bisphenol-A) from polymeric materials[20][23][25][26];

— organic solvent extraction is routinely used in the field of polymer analysis for identifying/quantifying LMWCs of polymers.

D.2 Selection of appropriate solvents for extracting LMWCs from polymeric devices

There are a wide variety of polymers and their additives. Therefore, no single solvent is universally applicable to exhaustive extraction for all polymers. Standard techniques for separating LMWCs from high polymeric fractions that are routinely used in the field of polymer analysis are helpful for selecting appropriate solvents for the exhaustive extraction.

The typical technique should be to first perform a stepwise extraction to select the appropriate organic solvent in order to then extract cross-linked polymers. The selected solvent should dissolve maximum amounts of non-polar LMWCs, but not the polymer itself. Care should be taken that the solvent does not result in a chemical change of the material, creating artefacts. For more information on the stepwise extraction using various solvents, see References [27] and [33]. Recommendations for solvents applicable to the purpose described above are given in References [27], [28] and [33]. Typically those are methanol, ethanol, acetone, methylethylketone, chloroform (2-propanol/hexane), diethylether, and hexane (from polar to non-polar).

In general, the LMWCs of toxicological concern are lipid-soluble ones with a partition coefficient or log octanol/water coefficient of 2,0 or more as these chemicals might not be readily excreted compared to the polar chemicals. The rate of migration of these LMWCs is very likely to be controlled by diffusion processes in the polymer unless there is a partitioning in the external phase. The situation is equivalent to migration into infinite volume and corresponds to exhaustive extraction conditions (see References [21] and [22]). The following set of polar and non-polar solvents can be used for preliminary experiments to solubilize the majority of LMWCs of toxicological concern: methanol, acetone, 2-propanol/hexane (50:50) and hexane. For highly crosslinked polymers, solvents that swell the polymers are desirable as they would enable a more timely completion of the experiment.

Volatility of the solvent is another factor for consideration when choosing the extraction solvent. Solvents with high boiling points are not appropriate because decomposition of LMWCs can be expected during evaporation of the solvent from the extract to obtain the evaporation residue, a process which will be applied to biological test systems.

D.3 Other points to consider when designing the extraction condition

D.3.1 For determination of residual levels of LMWCs of polymeric materials, exhaustive extractions should be carried out as described in D.3.3. This will also provide the maximum amount of extractable per sample (worst-case scenario) which should be used for further chemical characterization and for toxicological tests.

D.3.2 Extractions can be done at 37 °C or at elevated temperatures in order to accelerate the experiment. However, analysts are advised that elevated temperatures can cause chemical reactions (or decomposition), producing additional extract compounds. Also, if elevated temperatures are used, they should be chosen so that no additional curing or cross-linking of the polymers takes place during the extraction experiment.

D.3.3 For exhaustive extractions, the duration of the extraction cannot be prescribed in advance but can be dealt with in the following manner. A series of successive extractions is carried out by exposing the sample to the solvent for a period of time, replacing the solvent with fresh solvent and again exposing the sample for a period of time, and repeating the process. When the level of the residue for *n*th successive extraction is one tenth (0,1) of the level in the first extraction, the extraction may be deemed complete. It is possible that this condition may not occur because of extremely slow migration of the higher molecular weight material. The individual residue levels are accumulated to give the cumulative value and, via the sample/solvent ratio, referred back to sample levels and finally back to device levels. Total residue level from the device should be recorded in terms of the “percentage of extractables”.

D.4 Usages of residue obtained by exhaustive extraction in biological evaluation

The residue thus obtained is dissolved in an appropriate solvent and may be used as a test sample for the following purposes in biological evaluation.

a) As a test sample in biological tests, the residue may often be a mixture of chemical substances. By applying the residue to various biological test methods, we can evaluate the potential hazard of each biological endpoint. Application of the residue to test systems is described in corresponding parts of ISO 10993 and their annexes, e.g. ISO 10993-3 and ISO 10993-10.

b) For chemical characterization, chemical analysis data of the residue provide useful information for characterizing material and/or its component chemicals (see ISO 10993-18). Such information may sometimes give a conclusion that there is no need of further testing, based on toxicological equivalence with clinically established material.

Bibliography

[1] ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

[2] ISO 10993-3:2003, *Biological evaluation of medical devices — Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*

[3] ISO 10993-5, *Biological evaluation of medical devices — Part 5: Tests for* in vitro *cytotoxicity*

[4] ISO 10993-10:2010, *Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization*

[5] ISO 10993-18, *Biological evaluation of medical devices — Part 18: Chemical characterization of materials*

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

[7] ISO Guide 31, *Reference materials — Contents of certificates and labels*

[8] ISO Guide 33, *Uses of certified reference materials*

[9] ISO Guide 34, *General requirements for the competence of reference material producers*

[10] ISO Guide 35, *Reference materials — General and statistical principles for certification*

[11] NF S 90-701, *Medico-Surgical Equipment, Biocompatibility of Materials and Medical Devices, Methods for Extraction*, 1988

[12] BRAYBROOK, J.H., MACKAY, G.A. *Supercritical fluid extraction of polymer additives for use in biocompatibility testing*, Polymer International, **27**, 1992, pp. 157-164

[13] UPHILL, P.F., CHRISTOPHER, D.H. *Developing a Positive Control for Cytotoxicity Testing of Medical Device Materials*, Medical Device Technology, Nov./Dec. 1990, pp. 24-27

[14] United States Pharmacopeia/National Formulary, <88> Biological Reactivity Tests, In Vivo

[15] MHLW Notification (Tsuuchi), *Principles for Biological Safety Evaluation of Medical Devices*. Iyakushin No.0213001, 2003.02.13

[16] Memorandum (Jimu-renraku), *Guidelines for Specific Biological Tests relevant to the Principles*, issued by the MHLW Notification No.0213001, 2003.02.13, Iryokiki-Shinsa No.36, 2003.03.19

[17] OECD Environment Directorate, Chemical group and management committee, Third Meeting of OECD Experts on Polymers (Tokyo, 14-16 April 1993), Chairman's Report

[18] EPA Proposed Rule 40, CFR Part 723 (58FR 7679, February 8, 1993)

[19] ASH, M. and I. *Handbook of Plastic and Rubber Additives, An International Guide to More than 13000 Products by Trade Name, Chemical, Function, and Manufacturer*, Gover, USA, 1995 (ISBN 0-566-07594-6)

[20] MATSUOKA, A., HAISHIMA, Y., HASEGAWA, C., MATSUDA, Y., TSUCHIYA, T. Organic-solvent extraction of model biomaterials for use in the *in vitro* chromosome aberration test. *J. Biomed. Mater. Res.*, Part A, **86**, 2008, pp. 13-22

[21] REID, R.C., SIDMAN, K.R., SCHWOPE, A.D., TILL, D.E. *Ind. Eng. Chem. Prod. Res. Dev.*, **19(4)**, 1980, pp. 580-587

[22] REID, R.C., SCHWOPE, A.D., SIDMAN, K.R. Modeling the migration of additives from polymer films to foods and food simulating liquids, MIT Industrial Liaison Program Report 1-14-84, Directory of Current Research: 3.04.077

[23] TSUJI, K., MIZUMACHI, S., IIDA, K., OBA, T. *Kobunshi Ronbunshu*, **34(4)**, 1977, pp. 287-290

[24] OBA, T., TSUJI, K., NAKAMURA, A., SHINTANI, H., MIZUMACHI, S., KIKUCHI, H., KANIWA, M.A., KOJIMA, S., KANOHTA, K., KAWASAKI, Y., FURUYA, T., MATSUMOTO, K., TOBE, M. *Artificial Organs*, **8(4)**, 1984, pp. 429-435

[25] SHINTANI, H., NAKAMURA, A. *J. Biomed. Mater. Res.*, **25**, 1991, pp. 1275-1286

[26] HAISHIMA, Y., HAYASHI, Y., YAGAMI, T., NAKAMURA, A. *J. Biomed. Mater. Res. (Appl. Biomater.)*, **58**, 2001, pp. 209-215

[27] The Japan Society for Analytical Chemistry, Research Committee of Polymer Analysis, *Polymer Analysis Handbook*, pp. 549-558, Kinokuniya-Shoten, Tokyo, 1995 (ISBN 4-314-10110-5 C3043)

[28] FUCHS O. Solvents and Non-solvents for Polymers in *Polymer Handbook* (third edition), edited by BRANDRUP, J. and IMMERGUT, E.H., VII/379-VII/407, Wiley Interscience, 1989

[29] VONDRACEK, P. and DOLEZEL. B. Biostability of Medical Elastomers: A Review, *Biomater.*, **5**, 1984, p. 209

[30] ADAMS, W.P., ROBINSON, J.B., ROHRICH, R.J. Lipid Infiltration as a Possible Biologic Cause of Silicone Gel Breast Implant Aging, *Plast. Reconstr. Surg.*, **101**, 1998, p. 64

[31] European Pharmacopoeia 6.0, 3.1 Materials for Containers and Containers, pp. 337-370, 2008

[32] MHLW Notification by Director, OMDE, Yakushokuki-hatsu 0301 No.20, March 1, 2012. Basic Principles of Biological Safety Evaluation Required for Application for Approval to Market Medical Devices

[33] VOGEL, A. *Vogel's Textbook of Practical Organic Chemistry: Experimental Techniques* (fifth edition), Chapter 2, Revised by FURNISS, B.A. et al., John Wiley & Sons, Inc., New York, 1989

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