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**ISO 10993-12**

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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*



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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 second edition cancels and replaces the first edition (ISO 10993-12:1996), 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 8: Selection and qualification of reference materials for biological tests*

— *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*

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

This corrected version of ISO 10993-12:2002 incorporates a correction in 10.3.4, in which a note clarifies use of other media in some countries.

# Introduction

This part of ISO 10993 specifies methods of sample preparation and the selection of reference materials in the biological evaluation of medical devices. Because ISO 10993 describes many different biological assay systems, the individual parts should be consulted to ascertain if these recommendations are appropriate for specific test systems.

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 devices testing in biological systems in accordance with one or more parts of the ISO 10993 series.

Specifically, this part of ISO 10993 addresses:

— test material selection;

— selection of representative portions from a device;

— test sample preparation;

— experimental controls;

— selection of and requirements for reference materials; and

— preparation of extracts.

The applicability of this part of ISO 10993 to absorbable materials, materials that polymerize *in situ*, tissue-engineered medical products and materials of biological origin should be carefully evaluated.

## 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 10993-1:1997, *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**
**accelerated extraction**
extraction that provides a measure of the hazard potential of the device or material using conditions that shorten the time for leaching of the substances into the medium

NOTE 1 Examples of accelerated extraction conditions are elevated temperature, agitation, changing medium, etc.

NOTE 2 Accelerated extraction will not result in a chemical change in the substances being extracted.

**3.2**
**blank**
extraction medium not containing the test material, retained in a vessel identical to that which holds the test material and subjected to identical conditions to which the test material is subjected during its extraction

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

**3.3**
**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]

NOTE Standard Reference Material (SRM) is a trademark of the National Institute of Standards and Technology, Gaithersburg, MD, USA.

**3.4**
**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 simulated-use conditions

NOTE Exaggerated extraction is not intended to result in a chemical change of the material or the substances being extracted (see 10.3).

**3.5**
**experimental control**
substance with well characterized responses, which is used in a specific test system to assist in evaluating whether the test system has responded in a reproducible and appropriate manner

**3.6**
**extract**
liquid that results from extraction of test material or control

**3.7**
**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 The 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.

**3.8**
**negative control**
any well characterized material, 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 include blanks, vehicles/solvents and reference materials.

**3.9**
**positive control**
any well characterized material, 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

**3.10**
**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]

NOTE For the purposes 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.

**3.11**
**simulated-use extraction**
extraction of a test material or sample with an appropriate medium and under conditions that simulate product use, for the purpose of evaluating its potential hazard to the patient or user during its routine clinical use

**3.12**
**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

[ISO Guide 30]

**3.13**
**test material**
material, device, device portion, or component thereof subject to biological testing

**3.14**
**test sample**
test material or extract subject to biological testing

## 4 Experimental controls

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 may 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 RMs.

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 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 5 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 insure 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 test conditions specified, 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.

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

Use of an RM facilitates the comparability of the response between laboratories and assists 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, grad, and type. RMs are processed in accordance with 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 material 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).

**7.2** The same test material 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.

**8.1.1** Test samples from sterilized devices and RMs shall be handled aseptically if appropriate to the test procedure.

**8.1.2** 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.

**8.1.3** 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 10.3.2.2, 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 clean 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.

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

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

**10.2.2** To ensure that the extraction vessels do not adulterate the extract of the test materials, 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 based on common practices are as follows (see also C.5):

a) (37 ± 1) °C for (24 ± 2) h;

b) (37 ± 1) °C for (72 ± 2) h;

c) (50 ± 2) °C for (72 ± 2) h;

d) (70 ± 2) °C for (24 ± 2) h;

e) (121 ± 2) °C for (1 ± 0,1) h.

Extraction conditions described above that 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 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, extraction medium and the phase equilibrium[1)] of the material. The effects of higher temperatures or other conditions on extraction kinetics and the identity of the extractant(s) shall be considered carefully if accelerated or exaggerated extraction is used.

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

— the energy of the increased temperature can cause increased crosslinking 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 can produce degradant materials that are not typically found in the finished device under use conditions.

**10.3.2** The standard surface area can be used to determine the volume of extract 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 the configuration of the sample, a mass/volume of extracting fluid shall be used. See Table 1.



1) The phase equilibrium of a material during the 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 above 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 can affect the amount and type of extractables.

**Table 1 — Standard surface areas and extract liquid volumes**

| Thickness<br>mm | Extraction ratio<br>(surface area or mass/volume)<br>± 10 % | Forms of material |
|---|---|---|
| < 0,5 | 6 $cm^2$/ml | film, sheet, tubing wall |
| 0,5 to 1,0 | 3 $cm^2$/ml | tubing wall, slab, small molded items |
| > 1,0 | 1,25 $cm^2$/ml | larger molded item(s) |
| 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 |
| NOTE While there are no standardized methods available at present for testing absorbents and hydrocolloids, the following is a suggested protocol:<br>Determine the "absorption capacity" of the material, i.e. the amount of extract liquid absorbed per gram of the material. The test sample shall be 0,1 g/ml beyond the absorptive capacity of the material. | | |

**10.3.2.1** 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.

**10.3.2.2** Materials shall be cut into small pieces before extraction to enhance submersion in the extract media, except when otherwise inappropriate (see, for example, 10.3.3). For polymers, pieces approximately 10 mm × 50 mm or 5 mm × 25 mm are appropriate.

**10.3.3** 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 can have surface properties that differ from those of the bulk material.

**10.3.4** Extraction using both polar and non-polar solvents shall be performed. Examples of extraction media are:

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

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

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

NOTE In some countries other media, having known effects on the material and the biological system, and appropriate to the nature and use of the device or the methods for hazard identification, may be considered as acceptable alternatives.

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

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

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

**10.3.8** 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.

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

**10.3.10** 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).

### 10.4 Extraction conditions for hazard identification and risk estimation in 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** The test samples for materials that cure *in situ* (e.g. cements, adhesives and pre-polymer mixtures) shall represent the curing point at which the material is placed *in situ* and the maximum curing time during use *in situ* (i.e. simulate the minimum and maximum cures during clinical use).

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 may be appropriate, so that the designated dimensions or mass 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; and

d) extraction techniques, as appropriate, including documentation of extraction medium, 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 also Table A.1).

**Table A.1 — Summary of available RMs and controls for those tests in ISO 10993 which do not require specific RMs or controls**

| Test | Positive control [a] | 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<br>PUR 2541 |

[a] Abbreviations in this Table refer to specific materials available from sources designated in Clauses 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)], 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[12)], segmented polyurethane films containing zinc diethyl-[13) 14)] or dibutyl-dithio-carbamate[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.

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2) High-density polyethylene (Negative Control Plastic RS) can be obtained from the US Pharmacopeia (Rockville, MD USA). This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

3) HDPE film: RM-C Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 Japan; TEL 81-463-82-4751, FAX: 81-463-82-9627, E-mail: RM.Office@fdsc.or.jp. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

4) HDPE sheet: RM-D Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 Japan; TEL 81-463-82-4751, FAX: 81-463-82-9627, E-mail: RM.Office@fdsc.or.jp. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

5) HDPE rod: RM-E Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 Japan; TEL 81-463-82-4751, FAX: 81-463-82-9627, E-mail: RM.Office@fdsc.or.jp. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

6) PE 140 tubing: AG, D-8673 Rehau, Germany. PE film is available from Hoechst AG, D-6230 Frankfurt 80, Germany. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

7) Biomaterials Program, Devices and Technology Branch, National Heart, Lung and Blood Institute, NIH, 312 Federal Building, 7550 Wisconsin Ave., Bethesda, MD 20892, USA. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

8) SIK 8363 tubing: Rehau AG, D-8673 Rehau, Germany. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

9) PVC 7506 and PVC 7536 tubing: Rehau AG, D-8673 Rehau, Germany. PVC-DEHP and PVC-TEHTM film is available from Hoechst AG, D-6230 Frankfurt 80, Germany. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

10) PUR 2541 tubing: Rehau AG, D-8673 Rehau, Germany. PU film is available from Frontline Filmblasning, S-60003 Norrkoping, Sweden. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

11) PP 146 tubing is available from Rehau AG, D-8673 Rehau, Germany. PP film is available from Hoechst AG, D-6230 Frankfurt 80, Germany. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

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]. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

13) Polyurethane film — ZDEC: RM-A; Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 Japan; TEL 81-463-82-4751, FAX: 81-463-82-9627, E-mail: RM.Office@fdsc.or.jp. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

14) Polyurethane rod — ZDEC: RM-F; Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 Japan; TEL 81-463-82-4751, FAX: 81-463-82-9627, E-mail: RM.Office@fdsc.or.jp. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

15) Polyurethane film — ZDEC: RM-B; Hatano Research Institute/Food and Drug Safety Center, 729-5 Ochiai Hadano, Kanagawa 257-8523 Japan; TEL 81-463-82-4751, FAX: 81-463-82-9627, E-mail: RM.Office@fdsc.or.jp. This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of the product.

# Annex B
(informative)

# General principles and practices of test material 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 10993-1:1997, 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 thermal processing, and whether it is virgin or regrind and, if regrind, the specification for the maximum allowable regrind.

Materials that may be resterilized 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 resterilized 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.

Bioprosthetic, i.e. animal-tissue-derived, materials 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 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 material. The effect of extraction procedures shall be considered in the design of tests of a material if particulates are generated by the extraction conditions.