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Biological evaluation of medical devices —

Part 10: Tests for irritation and delayed-type hypersensitivity

Évaluation biologique des dispositifs médicaux —
Partie 10: Essais d'irritation et d'hypersensibilité retardée

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Contents

Page

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 3.

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 part of ISO 10993 may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 10993-10 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This second edition cancels and replaces the first edition (ISO 10993-10:1995), 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*

— *Part 18: Chemical characterization of materials*

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

This part of ISO 10993 is a harmonization of numerous standards and guidelines, including BS 5736, OECD Guidelines, U.S. Pharmacopoeia and the European Pharmacopoeia. It is intended to be the basic document for the selection and conduct of tests enabling evaluation of irritation and dermal sensitization responses relevant to safety of medical materials and devices.

Annex A forms a normative part of this part of ISO 10993. Annexes B and C are for information only.

Introduction

This part of ISO 10993 assesses possible contact hazards from chemicals released from medical devices that may produce skin and mucosal irritation, eye irritation and delayed contact hypersensitivity

Some materials that are included in medical devices have been tested, and their skin or mucosal irritation or sensitization potential has been documented. Other materials and their chemical components have not been tested and may induce adverse effects when in contact with biological tissues. The manufacturer is thus obliged to evaluate each device for potential adverse effects prior to marketing.

Traditionally, small animal tests are performed prior to testing on humans to help predict human response. More recently, *in vitro* tests as well as human tests have been added as alternatives. Despite progress and considerable effort in this direction, a review of findings suggests that currently no satisfactory *in vitro* test has been devised to eliminate the requirement for *in vivo* testing. Where appropriate, the preliminary use of *in vitro* methods is encouraged for screening purposes prior to animal testing. In order to reduce the number of animals used, this part of ISO 10993 presents a step-wise approach, with review and analysis of test results at each stage. An animal test is usually required prior to human testing.

It is intended that these studies be conducted using Good Laboratory Practice and comply with regulations related to animal welfare. Statistical analysis of data is recommended and should be used whenever appropriate.

The tests included in this part of ISO 10993 are important tools for the development of safe products, provided that these are executed and interpreted by trained personnel.

Biological evaluation of medical devices —

Part 10:
Tests for irritation and delayed-type hypersensitivity

1 Scope

This part of ISO 10993 describes the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and delayed-type hypersensitivity.

This part of ISO 10993 includes

a) pretest considerations,

b) details of the test procedures, and

c) key factors for the interpretation of the results.

Instructions are given in annex A for the preparation of materials specifically in relation to the above tests.

Supplementary tests which are required specifically for devices used intradermally in the ocular, oral, rectal, penile and vaginal areas are given in annex B.

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 10993. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 10993-1:1997, *Biological evaluation of medical devices — Part 1: Evaluation and testing*

ISO 10993-2, *Biological evaluation of medical devices — Part 2: Animal welfare requirements*

ISO 10993-9, *Biological evaluation of medical devices — Part 9: Framework for identification and quantification of potential degradation products*

ISO 10993-12, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials*

ISO 10993-13, *Biological evaluation of medical devices — Part 13: Identification and quantification of degradation products from polymeric medical devices*

ISO 10993-14 *Biological evaluation of medical devices — Part 14: Identification and quantification of degradation products from ceramics*

ISO 10993-15, *Biological evaluation of medical devices — Part 15: Identification and quantification of degradation products from metals and alloys*

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

ISO 14155-1, *Clinical investigation of medical devices for human subjects — Part 1: General requirements*

ISO 14155-2, *Clinical investigation of medical devices for human subjects — Part 2: Clinical investigation plans*

3 Terms and definitions

For the purposes of this part of ISO 10993, the terms and definitions given in ISO 10993-1 and the following apply.

3.1
allergen
sensitizer
substance/material which is capable of inducing specific hypersensitivity such that, on subsequent exposure to the same substance/material characteristic, allergic effects are produced

3.2
blank liquid
solvent portion treated in the same manner as the identical solvent used for the preparation of test samples but without test material, and which is intended for the determination of a background response of the solvent

3.3
challenge
elicitation
process following the induction phase in which the immunological effects of subsequent exposures in an individual to the inducing material are examined

3.4
corrosion
slow destruction of the texture or material of a tissue

EXAMPLE The action of a strong irritant.

3.5
delayed-type hypersensitization
induction of specific T-cell mediated immunological memory for an allergen to which an individual is exposed, resulting in a delayed-type hypersensitivity reaction after secondary contact with the allergen

3.6
dose
quantity to be administered to the test system at one time

3.7
erythema
reddening of the skin or mucous membrane

3.8
eschar
scab or discoloured slough of skin

3.9
induction
process that leads to the *de novo* generation of an altered state of immunological reactivity in an individual to a specific material

3.10
irritant
agent that produces irritation

3.11
irritation
localized non-specific inflammatory response to single, repeated or continuous application of a substance/material

3.12
necrosis
death of one or more cells, or portion of tissue or organ, resulting in irreversible damage

3.13
negative control
material or substance which, when tested by the procedure described, demonstrates the suitability of the procedure to yield a reproducible, appropriate negative, nonreactive or background response in the test system

3.14
oedema
swelling due to abnormal infiltration of fluid into the tissues

3.15
positive control
material or substance which, when tested by the procedure described, demonstrates the suitability of the procedure to yield a reproducible, appropriate positive or reactive response in the test system

3.16
solvent
material or substance used to moisten, dilute, suspend, extract or dissolve the test substance material

EXAMPLES Chemical, vehicle, medium, etc.

3.17
test material
material, device, device portion or component thereof that is sampled for biological or chemical testing

3.18
test sample
extract or portion of the test material that is subjected to biological or chemical testing

3.19
ulceration
open sore representing loss of superficial tissue

4 General principles — Step-wise approach

The available methods for testing irritation and sensitization were developed specifically to detect skin irritation and sensitization potential. Other types of adverse affect are generally not predicted by these tests.

This part of ISO 10993 requires a step-wise approach, which shall include one or more of the following:

a) characterization of test material, involving chemical characterization and analysis of the test sample according to the general principles described in ISO 10993-9, ISO 10993-13, ISO 10993-14, ISO 10993-15 and ISO 10993-18;

b) literature review, including an evaluation of chemical and physical properties, and information on the irritation and sensitization potential of any product constituent as well as structurally related chemicals and materials;

c) consideration of *in vitro* tests in preference to *in vivo* tests, and replacement of the latter as new *in vitro* methods become available and validated. At the present time there are no validated *in vitro* tests (other than simple screens) to detect irritants or sensitizers.

d) *in vivo* animal tests;

NOTE Acute *in vivo* animal studies are undertaken to test for materials not already classified as severe irritants or strong sensitizers by step a) or b). Materials that do not demonstrate an acute dermal irritation at single exposure may then be further evaluated following repeated exposure.

A test of a positive-control substance for skin sensitization [7] shall be run at least every six months by the testing laboratory to validate the test system and demonstrate a positive response.

e) non-invasive human tests/clinical trials.

If the material has been demonstrated not to be an irritant, a sensitizer or toxic in animals, studies on skin irritation may then be considered in humans.

5 Pretest considerations

5.1 General

It is important to emphasise that pretest considerations may result in the conclusion that testing for irritation and/or sensitization is not necessary.

The requirements given in clause 5 of 10993-1:1997 and in the subclauses below apply.

5.2 Types of material

5.2.1 Initial considerations

It shall be taken into consideration that, during manufacture and assembly of medical devices, additional chemical components may be used as processing aids, e.g. lubricants or mould-release agents. In addition to the chemical components of the starting material and manufacturing process aids, adhesive/solvent residues from assembly and also sterilant residues or reaction products resulting from the sterilization process may be present in a finished product. Whether these compounds pose a health hazard/risk depends on the leakage or degradation characteristics of the finished products.

5.2.2 Ceramics, metals and alloys

These materials are normally less complex than polymers and biologically derived materials in terms of the number of chemical constituents.

5.2.3 Polymers

These materials are normally chemically more complex than those in 5.2.1 in terms of composition. A number of additives may be present and the completeness of polymerization may vary.

5.2.4 Biologically derived materials

These materials are inherently complex in their composition. They often also contain process residues, e.g.cross-linkers and anti-microbial agents. Biological materials may not be consistent from sample to sample.

The methods in this part of ISO 10993 have not been designed for testing of biologically derived materials and may therefore be less adequate. For example, the tests in this part of ISO 10993 do not consider cross-species sensitization.

5.3 Information on chemical composition

5.3.1 General

Full qualitative data on the chemical constituents of the material shall be established. Where relevant to biological safety, quantitative data shall also be obtained. If quantitative data are not obtained, the rationale shall be documented and justified.

5.3.2 Existing data sources

Qualitative and quantitative information on the composition shall be obtained where possible from the supplier of the starting material.

For polymers this often requires access to proprietary information; provision should be made for the transfer and use of such confidential information.

Qualitative information about any additional processing additives (for example, mould-release agents) shall also be obtained from appropriate members of the manufacturing chain, including converters and component manufacturers.

In the absence of any data on composition, a literature study to establish the likely nature of the starting material and any additives is recommended to assist in the selection of the most appropriate methods of analysis for the material concerned.

NOTE The composition of ceramics, metals and alloys may be in accordance with ISO or American Society of Testing Materials (ASTM) standards and/or may be specified by the user. However, in order to obtain full qualitative and quantitative details on composition, it may be necessary to request these from the supplier or manufacturer of the starting material and also from component manufacturers to ensure processing aids are also identified. Material master files held by regulatory authorities are another source of data, where they are accessible.

5.4 Material characterization

When details of composition are unavailable, or only qualitative information is available, or new or unknown substances may be expected to develop during the manufacturing process, it may be necessary to undertake analysis of a material.

Analytical methods appropriate for the material under investigation shall be used. All analytical techniques shall be justified, validated and reported and, if not already known, the pH of the material (chemical solutions) shall be measured prior to any *in vivo* or *in vitro* testing when possible. Chemical analysis (qualitative as well as quantitative) of extracts may give useful information. In this context it should also be emphasised that chemical analysis of the extract may give results that makes testing for irritation and sensitization unnecessary, as information on irritation and sensitization potential of the compounds present in the extract solution may already be available.

6 Irritation tests

6.1 *In vitro* irritation tests

Two *in vitro* methods, the rat skin Transcutaneous Electrical Resistance (TER) test and the EPISKIN test, have been internationally validated as alternative tests to assess the skin corrosivity of chemicals. However, no validated methods to assess skin irritancy yet exist.

National and international organizations continue work to develop and validate *in vitro* tests for skin irritancy in parallel with the search for alternative methods; others have been developing methods to quantify the responses of animals and humans in order to better define endpoints using non-invasive techniques. See C.1.

6.2 Factors to be considered in design and selection of *in vivo* tests

Irritation testing of medical devices can be performed with the finished product and/or extracts thereof.

Factors affecting the results of irritation studies include the following:

a) the nature of the device used in a patch test;

b) the dose of the test material;

c) the method of application of the test material;

d) the degree of occlusion;

e) the application site;

f) the duration and number of exposures;

g) the techniques used in evaluating the test.

Additional background information is provided in annex C.

While increased flexibility allows the investigator to enhance the sensitivity of the test to suit conditions of use and population exposure, consistency in procedure contributes to comparability of test results with different materials and from different laboratories.

Provisions have been included in the test procedures for evaluation of devices and materials that will have repeated and/or prolonged exposure. The study shall be designed to exaggerate the anticipated contact (time and/or concentration) in the clinical situation. This shall be borne in mind during interpretation of the result.

If the pH of the test sample is less than or equal to 2 or equal to or greater than 11,5, the material shall be declared an irritant and no further testing is required. However, experimental evidence suggests that acidity and alkalinity of the test material are not the only factors to be considered in relation to the capacity of a material to produce severe injury. The concentration of the test material, its period of contact, and many other physical and chemical properties are also important.

NOTE For products intended to be used extensively on normal and compromised skin, no substantial risk is normally accepted; however, many products, in spite of a potential to irritate, are fully acceptable because of their inherent benefit or intended biological activity.

6.3 Animal skin irritation test

6.3.1 Principle

An assessment is made of the potential of the material under test to produce dermal irritation in a relevant animal model.

The rabbit is the preferred test animal.

6.3.2 Test material

If the test material is a solid or a liquid, it shall be prepared as specified in annex A.

In order to demonstrate the sensitivity of the assay it is advisable to include, in addition to the negative control, a positive control on each animal. As there are two test sites and two control sites on each animal, a maximum of two test materials may be applied together with the control materials, provided that the same vehicle is used.

6.3.3 Animals and husbandry

Healthy young adult albino rabbits of either sex from a single strain, weighing not less than 2 kg, shall be used.

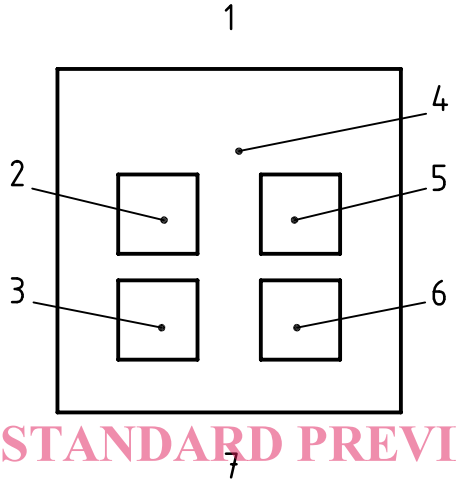
The animals shall be acclimatized and cared for as specified in ISO 10993-2.

If irritation is anticipated, consideration shall be given to testing in one animal first. Unless a well-defined positive response [score greater than 2 for either erythema or oedema (see Table 1)] is observed, a minimum of two further animals shall be used. If no response is expected, initial testing may be conducted using three animals. If the response in the test using the minimum of three animals is equivocal, further testing shall be considered.

6.3.4 Test procedure

6.3.4.1 Preparation of animals

The condition of the skin is a critical factor. Use only animals with healthy intact skin. Fur is generally clipped within 24 h to 4 h of testing on the backs of the animals a sufficient distance on both sides of the spine for application and observation of all test sites (approximately 10 cm × 15 cm). Fur may be re-clipped to facilitate observation and/or to accommodate repeated exposures. Depilatories may be used by trained technicians, if the process has been validated at the testing facility. If repeated exposure is required, follow the procedures in 6.3.4.2, 6.3.4.3 or 6.3.4.4, repeated for a maximum of 21 days.



Key

1	Cranial end	5	Control site
2	Test site	6	Test site
3	Control site	7	Caudal end
4	Clipped dorsal region		

Figure 1 — Location of skin application sites

6.3.4.2 Application of powder or liquid sample

Apply 0,5 g or 0,5 ml of the test material directly to each test skin site as shown in Figure 1. For solid and hydrophobic materials, there is no need for moistening. If the material is a powder, it should be slightly moistened with water or other suitable solvent before application (see annex A).

Cover the application sites with a 2,5 cm × 2,5 cm non-occlusive dressing (such as an absorbent gauze patch) and then wrap the application site with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites with permanent ink. Remove residual test material by appropriate means, such as washing with lukewarm water or other suitable non-irritating solvent, and careful drying.

6.3.4.3 Application of extracts and extract vehicle

Apply the appropriate extract(s) to the 2,5 cm × 2,5 cm absorbent gauze patches. Use a volume of extract sufficient to saturate the gauze; generally 0,5 ml per patch. Apply one patch on each side of the animal as shown in Figure 1. Apply a control patch of gauze moistened with the extract vehicle as indicated in Figure 1.

Cover the application sites with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites with permanent ink. Remove residual test material by appropriate means, such as washing with lukewarm water or other suitable non-irritating solvent and careful drying.

6.3.4.4 Application of solid sample

Apply the samples of the test material directly to the skin on each side of each rabbit as shown in Figure 1. Similarly, apply the control samples to each rabbit. When testing solids (which may be pulverised if considered necessary), the test material shall be moistened sufficiently with water or, where necessary, an alternative solvent, to ensure good contact with the skin (see annex A). When solvents are used, the influence of the solvent on irritation of skin by the test material shall be taken into account

Cover the application sites with 2,5 cm × 2,5 cm non-occlusive dressings (such as a gauze patch) and then wrap the application sites with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites with permanent ink. Remove residual test material by appropriate means, such as washing with lukewarm water or other suitable non-irritating solvent and careful drying.

6.3.5 Observation of animals

6.3.5.1 General

Use of natural or full-spectrum lighting is highly recommended to visualize the skin reactions. Describe and score the skin reactions for erythema and oedema according to the scoring system given in Table 1 for each application site at each time interval, and record the results for the test report.

NOTE Histological or non-invasive techniques of evaluating the skin reaction(s) may assist in certain cases.

6.3.5.2 Single-exposure tests

For single-exposure tests, record the appearance of each application site at 1 h, 24 h, 48 h and 72 h following removal of the patches. Extended observation may be necessary if there are persistent lesions, in order to evaluate the reversibility or irreversibility of the lesions. This need not exceed 14 days.

Table 1 — Scoring system for skin reaction

Reaction	Primary Irritation Score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Total possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

6.3.5.3 Repeated-exposure tests

Repeated exposure shall only be carried out after completion of an acute single-exposure test (after at least 72 h of observation).

For repeated-exposure tests, record the appearances of the application site at 1 h after removal of the patches and immediately prior to the next application. The number of exposures may vary.

After the last exposure, note the appearance of each application site at 1 h, 24 h, 48 h and 72 h following removal of the patches. Extended observation may be necessary if there are persistent lesions, in order to evaluate the reversibility or irreversibility of the lesions. This need not exceed 14 days.

6.3.6 Evaluation of results

For single exposure tests, determine the Primary Irritation Index (PII) as follows.

Use only 24 h, 48 h and 72 h observations for calculations. Observations made prior to dosing or after 72 h to monitor recovery are not used in the determination.

For each animal, add together the Primary Irritation Scores for the test material for both erythema and oedema at each time point and divide the sum by the total number of observations. (One observation in this context includes both erythema and oedema at each test site.) When blank liquid or negative control is used, calculate the Primary Irritation Score for the controls and subtract that score from the score using the test material to obtain the Primary Irritation Score. Add the scores for each animal and divide the total by the number of animals. This value is the Primary Irritation Index.

For repeated exposure, determine the Cumulative Irritation Index as follows.

For each animal, add together the Primary Irritation Scores for both erythema and oedema at each time specified. Divide this total by the total figure of observations to obtain the Irritation Score per animal.

Add together the Irritation Scores of all animals and divide by the total number of animals. This value is the Cumulative Irritation Index.

The Cumulative Irritation Index is compared to the categories of Irritation Response defined in Table 2 and the appropriate Response category is recorded for the report.

NOTE The categories of Cumulative Irritation Index are based on the data relating the Primary Irritation Index (PII) for chemicals in rabbits to the primary irritation response in humans for a number of chemicals that have been tested in both species.

For any response, record the maximum Primary Irritation Score from Table1 for each animal, the time of onset of the response and the time to maximum response.

The Primary or Cumulative Irritation Index is characterized by number (score) and description (Response category) in Table 2. In case different extracts have been tested, the one giving the highest PII determines the Response category.

Table 2 — Irritation Response categories in rabbit

Mean score	Response category
0 to 0,4	Negligible
0,5 to 1,9	Slight
2 to 4,9	Moderate
5 to 8	Severe