INTERNATIONAL STANDARD

ISO 10993-10

Third edition
2010-08-01

Biological evaluation of medical devices —

Part 10: Tests for irritation and skin sensitization

Évaluation biologique des dispositifs médicaux —

Partie 10: Essais d'irritation et de sensibilisation cutanée

Reference number
ISO 10993-10:2010(E)

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Published in Switzerland

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

This third edition cancels and replaces the second edition (ISO 10993-10:2002), which has been technically revised.

ISO 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

— *Part 1: Evaluation and testing 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 assesses possible contact hazards from chemicals released from medical devices, which may produce skin and mucosal irritation, eye irritation or skin sensitization.

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 human tissue. 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 adjuncts or 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.

This part of ISO 10993 is intended for use by professionals, appropriately qualified by training and experience, who are able to interpret its requirements and judge the outcomes of the evaluation for each medical device, taking into consideration all the factors relevant to the device, its intended use and the current knowledge of the medical device provided by review of the scientific literature and previous clinical experience.

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.

This part of ISO 10993 is based on numerous standards and guidelines, including 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.

Biological evaluation of medical devices —

Part 10:
Tests for irritation and skin sensitization

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 skin sensitization.

This part of ISO 10993 includes:

a) pretest considerations for irritation, including *in silico* and *in vitro* methods for dermal exposure;

b) details of *in vivo* (irritation and sensitization) test procedures;

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. In Annex B several special irritation tests are described for application of medical devices in areas other than skin.

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 10993-1:2009, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process*

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 document, the terms and definitions given in ISO 10993-1 and the following apply.

3.1
allergen
sensitizer
substance or material that is capable of inducing a specific hypersensitivity reaction upon repeated contact with that substance or material

3.2
blank
extraction vehicle 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 control is to evaluate possible confounding effects due to the extraction vessel, vehicle and extraction process.

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
dose
dosage
amount of test sample administered (e.g. mass, volume) expressed per unit of body weight or surface area

NOTE The terms are often used interchangeably (more commonly dosage).

3.5
erythema
reddening of the skin or mucous membrane

3.6
eschar
scab or discoloured slough of skin

3.7
extract
liquid or suspension that results from exposing a test or control material to a solvent under controlled conditions

3.8
induction
process that leads to the *de novo* generation of an enhanced state of immunological activity in an individual, to a specific material

3.9
irritant
agent that produces irritation

3.10
irritation
localized non-specific inflammatory response to single, repeated or continuous application of a substance/material

NOTE Skin irritation is a reversible reaction and is mainly characterized by local erythema (redness) of the skin.

3.11
necrosis
cell death as a direct result of irreversible changes caused by injury or disease

NOTE One should be aware that tissue repair will occur either resulting in complete functional restoration or resulting in scar formation.

3.12
negative control
any well-characterized material or substance that, 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.

3.13
oedema
swelling due to abnormal infiltration of fluid into the tissues

3.14
positive control
any well-characterized material or substance that, 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

3.15
skin corrosion
production of irreversible damage to the skin, manifested as visible necrosis through the epidermis and into the dermis, following application of a test sample

EXAMPLE The action of a compound/chemical/test sample resulting in **ulceration** of skin (see 3.19).

3.16
skin sensitization
allergic contact dermatitis
immunologically mediated cutaneous reaction to a substance

NOTE In the human, the responses can be characterized by pruritis, erythema, oedema, papules, vesicles, bullae or a combination of these. In other species the reactions can differ and only erythema and oedema can be seen.

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

3.18
test sample
material, device, device portion, component, extract or portion thereof that is subjected to biological or chemical testing or evaluation

3.19
ulceration
open sore representing loss of superficial tissue

3.20
vehicle
liquid used to moisten, dilute, suspend, extract or dissolve the test substance/material

4 General principles — Step-wise approach

The available methods for testing irritation and sensitization were developed specifically to detect skin and mucous membrane irritation and skin sensitization potential. Other types of adverse effect are generally not predicted by these tests. For medical devices that are used as implants or external communicating devices, intradermal testing is more relevant in approaching the application and so for detection of irritation activity, intracutaneous testing shall be used as described in 6.4.

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) in accordance with ISO 10993-2, *in vitro* tests in preference to *in vivo* tests shall be considered, and replacement of the latter as new *in vitro* tests are scientifically validated and become reasonably and practicably available. For the evaluation of skin irritation and corrosion, *in vitro* alternatives are available for chemicals; there are currently no internationally validated and accepted *in vitro* tests to detect sensitizers;

d) *in vivo* animal tests: in order to ensure reproducibility and sensitivity, a test of a positive-control substance for irritation and skin sensitization shall be included in each assay by the testing laboratory in order to validate the test system and demonstrate a positive response; for guinea pig sensitization assays, however, when consistency has been demonstrated over a six month or more extended period, a positive control does not need to be included in every assay, but may be run at regular intervals which shall not exceed six months.

NOTE 1 Sensitization can at the moment only be determined by an *in vivo* assay. This can be accomplished by using the local lymph node assay (LLNA) in mice, the occluded patch test in guinea pigs or the guinea pig maximization test (GPMT). For single chemicals the LLNA is now the preferred assay for determining the sensitizing potential. See References [69] [88] [90].

NOTE 2 *In vivo* animal tests are appropriate when test materials cannot be characterized and risk assessments cannot be undertaken using information obtained by the means set out in a), b) and c).

NOTE 3 For sensitization assays in guinea pigs, ten animals are normally used for positive control once every six months. Fewer guinea pigs can be used when an assay with a positive control substance is performed more frequently than once every six months. At least five test animals with a positive substance and five control animals should be used.

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.

Clinical studies in accordance with ISO 14155-1, ISO 14155-2 and to ethics principles shall not be performed before the results of the other evaluations in a) to d) are known.

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 ISO 10993-1:2009 and the following apply.

Non-sterile samples shall be investigated by topical investigation only, as the possibility of microbial contamination of the test sample could confound the final assay interpretation. In cases where the sterility of a test sample cannot be guaranteed, but the sample is still considered to be non-contaminated, intradermal administration may be justified.

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 components pose a health hazard/risk depends on the leakage or degradation characteristics of the finished products. These components shall be taken into account for their potential irritation/sensitization activity.

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 5.2.2 in terms of composition. A number of reaction products/impurities/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 can be inconsistent from sample to sample.

The methods in this part of ISO 10993 have not been designed for testing of biologically derived materials and can 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, so as to assist in the selection of the most appropriate methods of analysis for the material concerned.

The chemical composition of finalized products shall be determined in accordance with ISO 10993-18.

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

6 Irritation tests

6.1 *In vitro* irritation tests

In vitro methods, the rat skin Transcutaneous Electrical Resistance (TER) test and the Human skin model test, have been internationally validated and accepted as alternative tests to assess the skin corrosivity of chemicals (OECD Guidelines 430[9] and 431[10]). National and international organizations continue working 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 F.1).

NOTE In 2007 the ECVAM Scientific Advisory Committee (ESAC) evaluated the validation process of an *in vitro* human skin model for the determination of skin irritation of chemicals. See Reference [101]. The use of *in vitro* human skin models for assessing the potential of chemicals to induce skin irritation is described in Annex D.

The *in vitro* test for skin irritation has so far been validated only for neat chemicals and not for medical device extracts. In order to apply these assays for the testing of irritation potential of medical devices, further validation for this specific area is essential.

6.2 *In vivo* irritation tests — 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 F.

Whilst flexibility with respect to the precise protocol followed 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 $\leqslant$ 2,0 or $\geqslant$ 11,5, the material shall be considered 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.

In exceptional cases where further risk characterization/assessment is needed, it might be necessary to test materials which are either an irritant or have a pH outside the range mentioned above. These cases shall be justified and documented.

6.3 Animal 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.

The sensitivity of the assay shall be demonstrated. This can be done by including a positive control in the assay. However, the use of a positive control to confirm sensitivity is only warranted when the testing laboratory has not within the previous six months produced positive results using the test method.

NOTE A suitable positive control is sodium lauryl sulphate (SLS).

6.3.3 Animals and husbandry

Three healthy young adult albino rabbits of either sex from a single strain, weighing not less than 2 kg, shall be used. 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 additional animals shall be used. If the response in the test using the minimum of three animals is equivocal, further testing shall be considered.

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

Table 1 — Scoring system for skin reaction

Reaction	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
Maximal possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

6.3.4 Test procedure

6.3.4.1 Preparation of animals

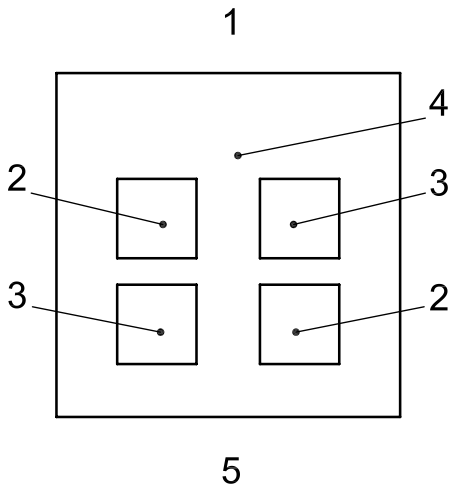
The condition of the skin is a critical factor. Use only animals with healthy intact skin. Fur is generally clipped within 24 h to 4 h of testing on the backs of the animals, a sufficient distance on both sides of the spine for application and observation of all test sites (approximately 10 cm × 15 cm). 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 6.3.4.2.1, 6.3.4.2.2 or 6.3.4.2.3, repeated for a maximum of 21 d.

6.3.4.2 Application of test sample

6.3.4.2.1 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 vehicle 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.



Key

1 cranial end
2 test site
3 control site
4 clipped dorsal region
5 caudal end

Figure 1 — Location of skin application sites

6.3.4.2.2 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 shown 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.

6.3.4.2.3 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 pulverized 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 caused 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.