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Biological evaluation of medical devices —

Part 10:
Tests for irritation and sensitization

Évaluation biologique des dispositifs médicaux —

Partie 10: Essais d'irritation et de sensibilisation

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Contents

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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.

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.

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

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

— *Part 1: Guidance on selection of tests*

— *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 cytotoxicity: in vitro methods*

— *Part 6: Tests for local effects after implantation*

— *Part 7: Ethylene oxide sterilization residuals*

— *Part 9: Degradation of materials related to biological testing* [Technical Report]

— *Part 10: Tests for irritation and sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymers*

— *Part 14: Identification and quantification of degradation products from ceramics*

— *Part 15: Identification and quantification of degradation products from coated and uncoated metals and alloys*

— *Part 16: General guidance on toxicokinetic study design for degradation products and leachables*

— *Part 17: Glutaraldehyde and formaldehyde residues in industrially sterilized medical devices*

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. Pharmacopeia and the European Pharmacopoeia. It is intended to be the overall guidance document for the selection and conduct of tests enabling evaluation of irritation and senitization responses relevant to material and device safety.

Annexes A, B and C form an integral part of this part of ISO 10993. Annexes D, E and F are for information only.

Introduction

This part of ISO 10993 assesses possible contact hazards from device-released chemicals that may produce skin and mucosal irritation, eye irritation, and delayed contact sensitization.

Some materials that are included in these 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 act differently when exposed to biological tissues. It is incumbent upon the manufacturer to evaluate each device for its human toxic potential prior to marketing.

Traditionally, small animal tests are performed prior to human testing to help predict human response. More recently, *in vitro* tests have been added as an alternative. 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 as screening tests prior to animal testing. In order to reduce the number of animals used, these standards use a step-wise approach with review and analysis of test results at each stage.

It is incumbent upon the investigator to conduct these studies using good scientific laboratory practices, complying with regulations related to animal welfare. Since the number of animals is restricted, the data obtained may be insufficient to warrant the application of statistics.

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Biological evaluation of medical devices —

Part 10:
Tests for irritation and sensitization

1 Scope

This part of ISO 10993 describes test methods:

a) to evaluate the potential of devices and their constituent materials to produce irritation; and

b) to evaluate the potential of devices and their constituent materials to produce sensitization.

These test methods are recommended for most categories of device and mode of body contact given in ISO 10993-1. Of the tests listed, those appropriate to the end use of the device are to be selected. Guidance is also given for the preparation of materials specifically in relation to the above tests.

NOTE 1 Guidance on the conduct of supplementary tests which may be required specifically for use in the oral, rectal, penile and vaginal areas is given in annex D.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this part of ISO 10993. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below. Members of IEC and ISO maintain registers of currently valid International Standards.

ISO 10993-1:1992, *Biological evaluation of medical devices — Part 1: Guidance on selection of tests.*

ISO 10993-12:—[1], *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials.*

3 Definitions

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

3.1 (allergic contact) sensitization; delayed contact hypersensitivity: Allergic response involving immunological systems that have been activated by prior exposure.

3.2 irritation: Localized inflammatory response to single, repeated or continuous application of the test substance, without involvement of an immunological mechanism.

3.3 oedema: Swelling due to abnormal infiltration of fluid into the tissues.

3.4 erythema: Reddening of the skin or mucous membrane.

3.5 eschar: Scab or discoloured slough of skin.

3.6 corrosion: Production of irreversible tissue damage at the site of contact with the skin following the application of a test substance.

1) To be published.

3.7 ulceration: Open sore representing loss of superficial tissue.

3.8 necrosis: Death of cells and/or tissues.

3.9 negative control: Substance that closely resembles the test substance in form and, when tested in accordance with this part of ISO 10993, is neither an irritant nor a sensitizer.

3.10 positive control: Substance that, when tested in accordance with this part of ISO 10993, gives a reproducible irritation or sensitization response.

3.11 solvent: Substance (chemical, vehicle, medium, etc.) used to moisten, dilute, suspend, extract or dissolve the test substance.

3.12 reagent control: Solvent used to moisten, dilute, suspend, extract or dissolve the test substance, which is evaluated concurrently with the moistened, diluted, suspended, extracted or dissolved test substance.

4 General principles, step-wise approach

This part of ISO 10993 advocates a step-wise approach which may include any or all of the following:

a) literature review;

b) *in vitro* tests (if available and when validated);

c) *in vivo* tests;

d) non-invasive human tests/clinical trials.

The first stage is a literature review and shall include an evaluation of chemical and physical properties, and information on structually related chemicals and materials. If not already known, the pH and pKa of the material (liquid, solution or extracts of materials) shall be measured prior to any *in vivo* or *in vitro* testing.

The second stage provides for *in vitro* assessments. These should always be considered in preference to *in vivo* tests and should replace these as new *in vitro* methods become available and validated.

At the third stage acute *in vivo* studies are undertaken to test for materials not already classified as severe irritants or strong sensitizers by stages a) or b). Materials that do not demonstrate an acute potential may be further evaluated following repeated exposure.

At the present time there are no validated *in vitro* tests (other than simple screens) to detect irritants or sensitizers; guidance is therefore provided only in the conduct of *in vivo* tests in species other than humans.

It is not necessary to use positive controls in every *in vivo* test. A positive control should be run periodically to validate the test system and demonstrate a positive response.

If assessment is not possible using the above stages, consideration should be given to non-invasive testing in humans.

5 Irritation tests

5.1 Factors to be considered in design and selection of tests

Factors affecting the results of irritation studies include

a) the patch test unit;

b) the degree of occlusion;

c) application of the test substance;

d) the application site;

e) the duration of exposure; and

f) the techniques used in evaluating the test.

Additional background information is provided in annex E.

While increased flexibility will allow 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 investigator, in consultation with the device manufacturer, should design the study to exaggerate the anticipated contact (time and/or concentration) in the clinical situation. While use of an exaggerated concentration or extract of the material is acceptable, this should be borne in mind during interpretation of the results.

For products intended to be used extensively on normal and abnormal 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.

If the pH of the test material is less than or equal to 2 or equal to or greater than 11,5, the material may 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 substance to produce severe injury. The concentration of the test material, its period of contact, and many other physical and chemical properties are also important.

As dose levels in test procedures can be exaggerated, a positive test does not necessarily exclude the material from use.

5.2 Skin irritation test

5.2.1 Principle

Assessment of the potential of the material under test to produce dermal irritation.

5.2.2 Test material

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

If the test material is to be tested as an extract, it shall be prepared as specified in annex B.

5.2.3 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 annex C.

One animal shall initially be used to evaluate the test material.

A well-defined response in the one animal obviates the need for additional testing.

Unless a well-defined response is observed for solid or liquid materials, a minimum of two further animals shall be used. For extracts, a minimum of two further animals per extract shall be used.

If the response in the test using the minimum of three animals is equivocal or not clear, additional testing shall be considered.

5.2.4 Test procedure

5.2.4.1 Preparation of animals

On the day before the test, closely clip the fur 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). Use only animals with healthy intact skin.

NOTE 2 Abrasion of the test site is not necessary, as evidence indicates similar responses between abraded and non-abraded sites.

If repeated exposure is required, follow the procedures in 5.2.4.2, 5.2.4.3 or 5.2.4.4, repeated for a maximum of 21 days.

5.2.4.2 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. If the substance is a powder, it should be slightly moistened with water or other suitable solvent before application.

Cover the application sites with a 25 mm × 25 mm non-occlusive dressing (such as a gauze patch) and then wrap the application site with a semi-occlusive bandage for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites. Remove residual test substance by appropriate means, such as washing with lukewarm water or other suitable, non-irritating solvent, and careful drying.

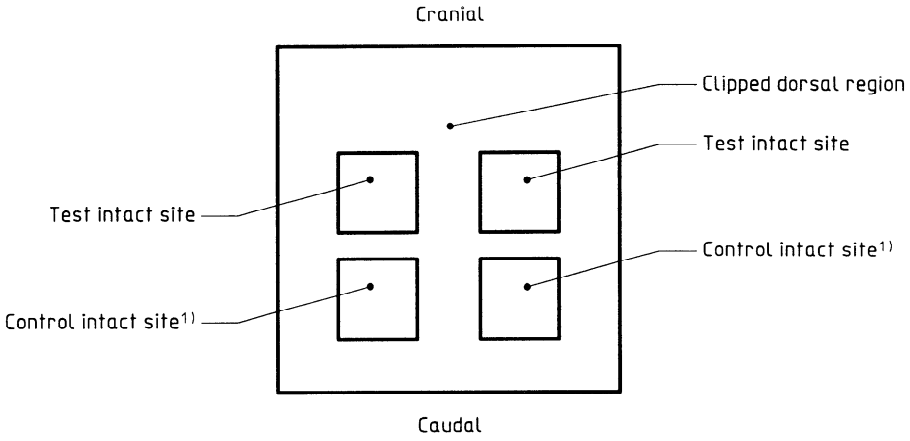
5.2.4.3 Extracts and extractants

Apply the appropriate extract(s) to the 25 mm × 25 mm four-ply gauze patches (0,5 ml per patch), one patch on each side of the animal as shown in figure 1. Apply a control patch of gauze moistened with the extracting medium to the other side.

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

5.2.4.4 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.



1) If sample preparation requires this type of control.

Figure 1 — Location of skin application sites

When testing solids (which may be pulverized if considered necessary), the test substance shall be moistened sufficiently with water or, where necessary, an alternative solvent, to ensure good contact with the skin. When solvents are used, the influence of the solvent on irritation of skin by the test substance shall be taken into account.

Cover the application sites with 25 mm × 25 mm non-occlusive dressings (such as a gauze patch) and then wrap the application sites with a semi-occlusive bandage for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites. Remove residual test substance by appropriate means, such as washing with lukewarm water or other suitable, non-irritating solvent, and careful drying.

5.2.5 Observation of animals

For acute (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.

For repeated exposure, record the appearances of the application site at 1 h after removal of the patches and immediately prior to the next application. 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.

Describe and grade the skin reactions for erythema and oedema according to the classification system given in table 1 for each application site at each time interval and record the results for the test report.

NOTE 3 Histological and non-invasive techniques may assist in certain cases.

5.2.6 Evaluation of results

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

For each animal, add together the Primary Irritation Scores for the test material for both erythema and oedema at each time specified and divide by the total number of observations (six: two at each time specified). When vehicle controls are used, calculate the Primary Irritation Score for the vehicle controls and subtract that score from the score for the test material to obtain the Primary Irritation Score.

Only use 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.

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.

Table 1 — Classification system for skin reaction

Reaction	Numerical grading
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

NOTE — Other adverse changes at the skin sites shall be recorded and reported.

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

Add the Irritation Scores of each animal 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 Cumulative Irritation Index defined in table 2 and the appropriate Category is recorded for the report.

NOTE 4 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, determine the Maximum Irritation Response, the time of onset of the response and the time to maximum response.

The Primary or Cumulative Irritation Index is characterized by number and description in table 2.

Table 2 — Irritation response categories in rabbit

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

5.2.7 Presentation of results

The test report shall include

a) a description of the test material(s) or device;

b) the intended use/application of the test material(s) or device;

c) a detailed description of the method employed in preparing the test material or device;

d) the test animals;

e) method of application to the test sites;

f) how the site readings were performed and a record of the observations;

g) assessment of the results.

5.3 Ocular irritation test

5.3.1 Principle

Assessment of the potential of the material under test to produce ocular irritation.

5.3.2 Exclusion from test

Materials and/or final products which have demonstrated definite corrosion or severe irritation in a dermal study shall not be further tested for eye irritation. Strongly acidic or alkaline substances (pH $\leqslant$ 2 or $\geqslant$ 11,5) shall not be tested owing to their predictive corrosive properties. These products shall be considered eye irritants.

5.3.3 Test material

If the test material is a liquid, instil 0,1 ml undiluted into the lower conjunctival sac of one eye.

If the test material is a solid or granular product, grind to a fine dust. When gently compacted, instil that amount which occupies a volume of 0,1 ml and does

not weigh more than 100 mg into the lower conjunctival sac of one eye.

NOTE 5 Some products may not be amenable to testing directly in the eye. Mechanical damage can result in making the test useless.

If the test material is contained in a pump spray, expel and instil 0,1 ml as for liquids.

If the test material is contained in an aerosol container, examine by either

a) spraying a single burst of 1 s duration at a distance of 10 cm directed at the open eye; or

b) expelling the aerosol into a cool container and treating as for a liquid.

If the test material is such that it can only be applied as an extract, prepare extracts as described in annex B. Instil a 0,1 ml aliquot of the extract into the lower conjunctival sac of one eye.

Under conditions identical with those used above, prepare reagent controls, using both the polar and the non-polar solvent, in the absence of the test material.

5.3.4 Animals and husbandry

Healthy young adult albino rabbits of either sex from a single strain weighing 2 kg to 3 kg shall be used.

The animals shall be acclimatized and cared for as specified in annex C.

One animal shall initially be used to evaluate the test material.

A well-defined response in the one animal obviates the need for additional testing.

Unless a well-defined response is observed for solid or liquid materials, a minimum of two further animals shall be used. For extracts, a minimum of two further animals per extract shall be used.

If the response in the test using the minimum of three animals is equivocal or not clear, additional testing shall be considered.

5.3.5 Test procedure

No longer than 24 h before commencement of the test, visually examine both eyes of each rabbit for evidence of ocular abnormality. If either eye shows any abnormality, the rabbit shall be replaced.

When the eyes are examined, sodium fluorescein 2 % BP may be used to visualize any corneal damage. The use of an ophthalmoscope, hand slit-lamp, or other suitable device, is recommended.

Instil the test material as specified in 5.3.3.

Following instillation hold the eyelids together for approximately 1 s.

NOTE 6 The contralateral eye of each animal serves as an untreated control.

If repeated exposure of the material is anticipated and the test material has not demonstrated a significant response in the acute test, a repeat exposure study may be conducted. The duration of the exposure should bear resemblance to the length of use of the test material/device in the clinical situation.

5.3.6 Observation of animals

For animals receiving a single instillation of test material, examine both eyes of each animal approximately 1 h, 24 h, 48 h and 72 h after instillation.

Extended observation may be necessary if there are persistent lesions in order to determine the progress of the lesions or their reversal; this need not exceed 21 days. Extended observation cannot be justified for animals with severe lesions.

Grade and record any reactions observed in accordance with the scale for grading ocular lesions given in table 3.

For animals receiving multiple instillations of test material, examine both eyes of each animal immediately before and approximately 1 h after each instillation.

If there is evidence of irritation after the last treatment, the observations may be extended. Extended observation may be necessary if there is persistent corneal involvement or other ocular irritation in order to determine the progress of the lesions and their reversibility.

Grade and record any reactions observed in accordance with the scale for grading ocular lesions given in table 3.

Withdraw an animal immediately from the study and humanely kill it, if at any time it shows

a) very severe ocular damage (e.g. sloughing and ulceration of conjunctival membrane, corneal perforation, blood or pus in the anterior chamber); or

b) blood-stained or purulent discharge; or

c) significant corneal ulceration.

Withdraw from the study any animal showing maximum effects on the grading system in table 3 — absence of a light reflex (iridial response 2) or corneal opacity (grade 4) without evidence of recovery within 24 h or maximum conjunctival inflammation (chemosis grade 4 together with redness grade 3) — without evidence of recovery within 48 h, and kill it humanely.

Table 3 — Classification system for grading ocular lesions

Reaction	Numerical grading
1. Cornea	
Degree of opacity (most dense area used)	
No opacity	0
Scattered or diffuse areas, details of iris clearly visible	1 *
Easily discernible translucent areas, details of iris slightly obscured	2 *
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 *
Opaque, iris invisible	4 *
Area of cornea involved	
One-quarter (or less), not zero	0
Greater than one-quarter, but less than half	1
Greater than half, but less than three-quarters	2
Greater than three-quarters, up to whole area	3
2. Iris	
Normal	0
Folds above normal, congestion swelling, circumcorneal injection (any or all or combination of these), iris still reacting to light (sluggish reaction is positive)	1 *
No reaction to light, haemorrhage gross destruction (any or all of these)	2 *
3. Conjunctivae	
Redness (refers to palpebral and bulbar conjunctiva excluding cornea and iris)	
Vessels normal	0
Vessels definitely injected above normal	1
More diffuse, deeper crimson red, individual vessels not easily discernible	2 *
Diffuse beefy red	3 *
Chemosis	
No swelling	0
Any swelling above normal (include nictitating membrane)	1
Obvious swelling with partial eversion of lids	2 *
Swelling with lids about half-closed	3 *
Swelling with lids about half-closed to completely closed	4 *
Discharge	
No discharge	0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)	1
Discharge with moistening of the lids and hairs just adjacent to lids	2
Discharge with moistening of lids and hairs, and considerable area round the eye	3
* positive result	