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Biological evaluation of medical devices —

Part 23:
Tests for irritation

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 194.

A list of all parts in the ISO 10993- series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

European Foreword

The following referenced documents are indispensable for the application of this document. For undated references, the latest edition of the referenced document (including any amendments) applies. For dated references, only the edition cited applies. However, for any use of this standard 'within the meaning of Annex ZA', the user should always check that any referenced document has not been superseded and that its relevant contents can still be considered the generally acknowledged state-of-art.

When an IEC or ISO standard is referred to in the ISO standard text, this shall be understood as a normative reference to the corresponding EN standard, if available, and otherwise to the dated version of the ISO or IEC standard, as listed below.

NOTE The way in which these referenced documents are cited in normative requirements determines the extent (in whole or in part) to which they apply.

Table — Correlations between undated normative references and dated EN and ISO standards

Normative references as listed in Clause 2 of the ISO standard	Equivalent dated standard	
	EN	ISO or IEC
ISO 10993-1	EN ISO 10993-1:2019	ISO 10993-1:2018
ISO 10993-2	EN ISO 10993-2:2006	ISO 10993-2:2006
ISO 10993-9	EN ISO 10993-9:2019[a]	ISO 10993-9:2019[a]
ISO 10993-12	EN ISO 10993-12:2012	ISO 10993-12:2012
ISO 10993-13	EN ISO 10993-13:2010	ISO 10993-13:2010
ISO 10993-14	EN ISO 10993-14:2001	ISO 10993-14:2009
ISO 10993-15	EN ISO 10993-15:2019[a]	ISO 10993-15:2019[a]
ISO 10993-18	EN ISO 10993-18:2019[a]	ISO 10993-18:2019[a]
ISO 14155	EN ISO 14155:2019[a]	ISO 14155:2019[a]

[a] Under preparation. Documents are at final stage and have to be submitted to ISO/CS for FDIS vote.

NOTE This part of EN ISO 10993 refers to ISO 10993-1 which itself refers to ISO 14971. In Europe, it should be assumed that the reference to ISO 14971 is to EN ISO 14971:2012.

Introduction

This document assesses possible contact hazards from medical devices, which can produce skin, mucosal, and eye irritation.

Some materials that are included in medical devices have been tested, and their skin or mucosal irritation potential has been demonstrated. Other materials and their chemical components have not been tested and can 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, tests in small animals have been performed prior to testing on humans to help predict human responses. More recently, *in vitro* tests as well as human tests have been added as adjuncts or alternatives. For skin irritation testing of neat chemicals *in vitro* tests were developed using reconstructed human epidermis (RhE) models[32]. The method was adapted for detection of irritant chemicals in medical device extracts. The results of a large round robin study showed that some RhE models can also be used to detect the presence of irritant chemicals extracted from polymeric materials (polyvinylchloride (PVC) and silicone) commonly used in the manufacture of medical devices[5].

Irritation responses at other locations/tissues are generally not predicted by this RhE model though other *in vitro* models are available (e.g. mucosal or eye epithelial models) that might be used if qualified for use with medical devices.

It is intended that these studies be conducted using Good Laboratory Practice, or an equivalent quality system, that complies with regulations related to animal welfare. Statistical analysis of data is recommended and can be used whenever appropriate.

This document 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 document are important tools for the development of safe products, provided that they are executed and interpreted by trained personnel.

This document is based on numerous standards and guidelines, including OECD Test Guidelines (TG), 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 responses relevant to the safety of medical materials and devices.

The irritation potential of a medical device or its components can be predicted either by an *in vivo* animal irritation test or by an *in vitro* irritation test using a reconstructed human epidermis (RhE) as a model. ISO 10993-2 describes animal welfare aspects for performing animal studies for the biological evaluation of medical devices thereby also emphasizing the 3R's for replacement, reduction, and refinement of animal studies. This document describes tests to determine the irritancy of medical devices, materials or their extracts either by *in vitro* or in *vivo tests*. *In vitro* tests have preference over *in vivo* tests when appropriately validated and providing equally relevant information to that obtained from *in vivo* tests (ISO 10993-1, ISO 10993-2). A stepwise approach would be to start irritant testing with the *in vitro* RhE model. When indicated either for confirmation or further categorization of the irritant activity *in vivo* animal tests, followed by human irritant tests might be considered.

Biological evaluation of medical devices —

Part 23:
Tests for irritation

1 Scope

This document specifies the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation. The tests are designed to predict and classify the irritation potential of medical devices, materials or their extracts according to ISO 10993-1 and ISO 10993-12.

This document includes:

— pre-test considerations for irritation, including *in silico* and *in vitro* methods for dermal exposure;

— details of in vitro and in vivo irritation test procedures;

— 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 D several special *in vivo* irritation tests are described for application of medical devices in areas other than skin. In addition, Annex E provides information for conducting human skin irritation testing.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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, *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, *Clinical investigation of medical devices for human subjects — Good clinical practice*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at http://www.electropedia.org/

— ISO Online browsing platform: available at http://www.iso.org/obp

3.1
blank test solution
blank
solution prepared in the same way as the sample measuring solution but so that it does not contain the analyte to be determined

[SOURCE: ISO 10136-1:1993, 3.8]

3.2
dose
dosage
amount of test sample administered (e.g. mass, volume) expressed per unit of body weight or surface area

Note 1 to entry: The terms are often used interchangeably (more commonly dosage).

3.3
erythema
reddening of the skin or mucous membrane

3.4
eschar
scab or discoloured slough of skin

3.5
extract
liquid or suspension that results from exposing a test or control material to an extraction vehicle under controlled conditions

3.6
irritant
agent that produces irritation

3.7
irritation
localized non-specific inflammatory response to single, repeated or continuous application of a substance/material

Note 1 to entry: Skin irritation is a reversible reaction and is mainly characterized by local erythema (redness) and swelling of the skin.

3.8
necrosis
cell death as a direct result of irreversible changes caused by injury or disease

Note 1 to entry: One should be aware that tissue repair will occur either resulting in complete functional restoration or resulting in scar formation.

3.9
negative control
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 1 to entry: In practice, negative controls include blanks, vehicles/solvents and reference materials.

3.10
oedema
swelling due to abnormal infiltration of fluid into the tissue

3.11
positive control
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

3.12
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 3.15).

3.13
test material
material, device, device portion or component thereof that is sampled for biological or chemical testing

3.14
test sample
material, device, device portion, component, extract or portion thereof that is subjected to biological or chemical testing or evaluation

3.15
ulceration
open sore representing loss of superficial tissue

3.16
vehicle
liquid used to moisten, dilute, suspend, extract or dissolve the test substance/material

3.17
vehicle control
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 1 to entry: The purpose of the vehicle control is to evaluate possible confounding effects due to the extraction vessel, vehicle and extraction process.

4 General principles — Step-wise approach

The available methods for testing irritation were developed specifically to detect skin and mucous membrane irritation potential. Other types of adverse effects, such as sensitization, are generally not predicted by these tests. Historically irritation testing was done in rabbits. 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 is indicated as described in 7.2. More recently, an *in vitro* alternative test was evaluated for skin irritancy testing of medical devices[5]. Therefore, in accordance with ISO 10993-2 the *in vitro* irritation test using

reconstructed human epidermis (RhE) shall be considered before animal testing for skin irritation is considered. When the presence of an irritant is indicated, an *in vivo* irritancy test would not be needed. When indicated either for confirmation or further categorization of the irritant activity *in vivo* animal tests, followed by human irritant tests might be considered.

This document requires a stepwise approach, which shall include one or more of the following:

— characterization of test material, involving chemical characterization and analysis of the test sample in accordance with the general principles specified in ISO 10993-9, ISO 10993-13, ISO 10993-14, ISO 10993-15 and ISO 10993-18;

— literature review, as indicated in 10993-1, including an evaluation of chemical and physical properties, and information on the irritation potential of any product constituent as well as structurally-related chemicals and materials;

NOTE Structure activity relationship can indicate irritant activity.

— in accordance with ISO 10993-2, preference for *in vitro* tests instead of *in vivo* tests shall be considered, with replacement of the latter as new *in vitro* tests are scientifically validated and become reasonably and practicably available. For the evaluation of skin irritation *in vitro* alternatives are available for use with medical devices per the methods in Clause 6.2 – 6.13.

— *in vivo* animal tests: to ensure reproducibility and sensitivity, a test of a positive-control substance for irritation shall be performed on a regular basis (at least once every 6 months) by the testing laboratory in order to validate the test system and demonstrate a positive response.

NOTE 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).

— Clinical studies in accordance with ISO 14155 and to ethics principles governing human clinical research, shall not be performed before the irritancy potential of a device has been established through one or more of the evaluations described by a) to d).

5 Pretest considerations

5.1 General

It is important to emphasize that pretest considerations can result in the conclusion that testing for irritation is not necessary. For example, if the pH of the test sample is ≤ 2,0 or ≥ 11,5 the material shall be considered an irritant and no further irritant testing is required [OECD TG 404].

The requirements specified in ISO 10993-1:2018, Clause 5 on the categorization of medical devices, 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 should be justified.

Also, for the *in vitro* irritation assay using reconstructed human epidermis, sterile samples should be preferably used.

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, sterilant residues or reaction products resulting from the sterilization process can be present in a finished product. Whether these components pose a health hazard/risk depends on the leaching or degradation characteristics of the finished products. These components shall be taken into account for their potential irritation activity. The following types of materials are often used in medical devices and could introduce risks for irritation

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 can be present and the completeness of polymerization can 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.

5.3 Information on chemical composition

5.3.1 General

A description of the medical device chemical constituents shall be established as per ISO 10993-18. As described by ISO 10993-1, the extent of physical and/or chemical characterization required depends on what is known about the material formulation and on the nature and duration of body contact with the medical device. At a minimum, the characterization shall address the constituent chemicals of the medical device and possible residual process aids or additives used in its manufacture. The rigour necessary in the characterization of the chemical constituents is principally determined by the nature, degree, frequency and duration of the exposure and the hazards identified for the medical device or material. 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 search is recommended to establish the likely nature of the starting material(s) and any additives, so as to assist in the selection of the most appropriate methods of analysis for the material concerned.

The chemical characterization of a medical device shall be conducted in accordance with ISO 10993-18. When quantitative chemical characterization is determined to be necessary analytical testing should be conducted 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 *In vitro* irritation tests

6.1 General

For neat chemicals the reconstructed human epidermis (RhE) may also be used to assess skin irritation [OECD TG 439]. In addition, the RhE test was found to be suitable to detect irritant activity in extracts from polymeric medical materials (PVC and silicone) that contained known irritants.[5] It is likely that also for leachables from other types of materials the assay will detect the presence of irritant activity.

6.2 *In vitro* reconstructed human epidermis (RhE) model.

The *in vitro* RhE model for testing irritation was developed specifically to detect skin irritation potential for neat chemicals[2][11][OECD TG 439]. The method was adapted for detection of irritant chemicals in medical device extracts.[4][5][10][12][16][17][18] The RhE model was found equally sensitive to detect irritant activity when compared to the human patch testing and intracutaneous rabbit test[13]. Hence, the RhE test as described in this standard can replace the *in vivo* rabbit test for skin irritation. Irritation responses at other locations/tissues are generally not predicted by this RhE model though other *in vitro* models are available (e.g. mucosal or eye epithelial models) that might be used if qualified for use with medical devices.

6.2.1 Test system — Reconstructed human epidermis (RhE) model

The RhE model consists of normal human-derived epidermal keratinocytes, which have been cultured to form a multilayered highly differentiated model of the human epidermis. It consists of organized basal, spinous and granular layers, and a multilayered stratum corneum containing intercellular lamellar lipid layers arranged in patterns analogous to those found *in vivo*. In general normal human keratinocytes obtained from healthy volunteer donors are cultured for a number of days on a membrane or filter at an air-liquid interface to form the three- dimensional epidermal model comprising the main basal, supra basal, spinous and granular layers and a functional stratum corneum. Both polar (e.g. saline) and non polar (e.g. sesame oil) extracts can be directly added to the apical surface of RhE constructs. The model was found to show predictive outcomes when compared to human patch testing and rabbit intracutaneous tests[13].

Materials not suitable for extraction (e.g. liquids, gels, pastes, and particulates) can also be added to the apical surface of the RhE constructs directly. The suitability of these uses should be justified.

6.2.2 Principle of the method

Endpoints: Cell viability determination is based on cellular reduction of MTT ((3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) and subsequent conversion to a purple formazan salt that is quantitatively measured after extraction from the tissues.[8][15] The cell viability in treated tissues is expressed as a percentage of the negative vehicle control. The percent reduction in viability is used to predict the irritation potential.

NOTE 1 Reduced tissue survival was accompanied by IL-1α release.[12][16][18] Tissue culture media from the exposure can be collected and kept frozen at a minimum -20 °C for possible analysis of cytokines.

Brief Procedure: Studies performed with polymeric biomaterials specifically manufactured to contain irritant chemicals at low concentrations indicated that a prolonged exposure is needed compared to the OECD TG 439 protocol for neat chemicals. An incubation period of no less than 18 h up to 24 h exposure at 37 ºC for exposure to potentially low concentrations of irritants in extracts from biomaterials is sufficient for predicting irritation *in vitro* by reduction of tissue viability below 50 %.[3][5][12][16][18] Both 18 and 24 h exposure showed similar results in both RhE models evaluated in the round robin study using medical device extracts[12][16][18].

Tissues are incubated at 37 °C, 5 % CO_2 and 95 % relative humidity (RH) following the addition of the test and control extracts.

Exposure to the test sample extract is terminated by rinsing with Dulbecco's phosphate buffered saline (DPBS), or PBS without Ca++ and Mg++. After washing the tissues are manually dried. The viability is assessed by incubating the tissues for 3 h with MTT solution in a 24-well plate (1 mg/ml; 0,3 ml per well). The formazan crystals are extracted using an appropriate amount (depending on the RhE model used) of isopropanol for 2 h at room temperature (RT). Two or three aliquots (depending on the instructions of the supplier) per tissue of extracted formazan will be added to 96-well plates (200 µl/ well) and quantified spectrophotometrically at 570 nm.

For direct inoculation assays, a 1 % (v/v) solution of Sodium Dodecyl Sulfate (SDS, see 6.4.3) in sesame oil and in saline solution of NaCl 0,9 % can be used as positive controls and DPBS or PBS treated epidermis are used as the negative control, respectively. For extracted assays, a verified irritant infused control extracted in sesame oil and in saline solutions of NaCl 0,9 % can be used as positive controls.

NOTE 2 Aliquots of culture media collected after 18 h or 24 h exposure can be stored frozen (at a minimum of -20 °C) for potential cytokine (IL-1α) measurements as a complementary endpoint to cell viability. IL-1α measurement determines the inflammation component to the assessment of skin irritation in addition to the cell damage component determined indirectly by the MTT test for cell viability.

Vehicle controls shall include saline (NaCl 0,9 %) solution and sesame oil that have undergone the ISO 10993-12 medical device extraction procedure. For each treated tissue the viability is expressed as a percent relative to negative DPBS or PBS treated control tissues (mean).

Known limitations of the method: The method is not applicable to gases and aerosols. It is also not considered applicable to evaluate irritation by direct contact of solid materials as close contact over the whole test surface cannot be guaranteed.

Known cases of test-compounds requiring specific controls: Some chemicals can directly reduce the MTT reagent (e.g., electrophiles, test articles with high pH), while other chemicals can directly color the tissue or the cells. Such test sample properties can only interfere if sufficient amounts of the chemical are still present on the tissue at the end of the exposure period. In these cases, a special procedure allowing the quantification of the "true" MTT reduction should be applied. A protocol for determination of possible interactions with MTT is provided in reference [18A]. The use of specific and adapted controls will enable the calculation of true tissue viability after subtracting the unspecific Optical Densities due to direct chemical MTT reduction and/or chemical residual color extracted from the tissues.

6.2.3 Prediction Model (PM)

This prediction model is based on the prediction model of the OECD TG 439 and data further generated during the optimization of the medical device (MD) protocol[3][5][13][16][18].

If cell viability after the exposure is ≤ 50 %: the test sample is classified as Irritant (I).

If cell viability after the exposure is > 50 %: the test sample is classified as Non-Irritant (NI).

The cell viability test shall be conducted with both polar (e.g. saline) and non-polar (e.g. sesame oil) test extracts. If at least one of the extracts shows a positive effect (viability ≤ 50 %) the test sample of the medical device is considered to have irritant potential. The device or the device component tested shall