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Biological evaluation of medical devices - Part 10: Tests for skin sensitization (ISO/DIS 10993-10:2020)

Biologische Beurteilung von Medizinprodukten - Teil 10: Prüfungen auf Hautsensibilisierung (ISO/DIS 10993-10:2020)

Évaluation biologique des dispositifs médicaux -- Partie 10: Essais de sensibilisation cutanée (ISO/DIS 10993-10:2020)

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Biological evaluation of medical devices —

Part 10:
Tests for skin sensitization

Évaluation biologique des dispositifs médicaux —

Partie 10: Essais de sensibilisation cutanée

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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.

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 on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 194 *Biological and clinical evaluation*,.

This fourth edition cancels and replaces the third edition (ISO 10993-10:2010), which has been technically revised.

The main changes compared to the previous edition are as follows:

— This document now contains a description of skin sensitization testing only.

— Annex C on alternative test methods for skin sensitization has been updated.

— The testing for irritation is now described in ISO 10993-23

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.

Introduction

This document assesses possible contact hazards from chemicals released from medical devices, which may produce skin sensitization.

Some materials that are included in medical devices have been tested, and their skin sensitization potential has been documented. Especially for dental material sensitizing properties were reported see Reference [50]. 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. Since 2015, several in chemico and in vitro assays have been validated and OECD test guidelines released to assess the skin sentization potential of chemicals.[72][76][99] These test methods developed to address a specific key event may not be sufficient to conclude on the presence or absence of skin sensitisation potential of chemicals and should be considered in the context of integrated approaches such as IATA, combining them with other complementary information. It should be noted that the in vitro and in chemico tests for skin sensitization have so far been validated only for neat chemicals and not for medical device. In order to apply these assays for the testing of skin sensitization potential of medical devices, further validation for this specific area is essential. . 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 document presents a step-wise approach, with review and analysis of test results at each stage. It is intended that, for regulatory submission, sensitization study/studies be conducted using GLP/ISO 17025 as applicable to the respective country. and comply with regulations related to animal welfare. Statistical analysis of data is recommended and should 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 these are executed and interpreted by trained personnel.

This document 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 dermal sensitization responses relevant to the safety of medical materials and devices.

Biological evaluation of medical devices —

Part 10:
Tests for skin sensitization

1 Scope

This document specifies the procedure for the assessment of medical devices and their constituent materials with regard to their potential to induce skin sensitization.

This document includes:

— details of *in vivo* sensitization test procedures;

— key factors for the interpretation of the results.

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

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 10993-1:2018, *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 terms and definitions given in ISO 10993-1 and the following 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
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 1 to entry: 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
erythema
reddening of the skin or mucous membrane

3.5
extract
liquid that results from extraction of the test sample or control

[SOURCE: ISO 10993-12:2012]

3.6
induction
process that leads to the *de novo* generation of an enhanced state of immunological activity in an individual, after initial exposure to a specific material

3.7
irritant
agent that produces irritation (see 3.9)

3.8
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), swelling, itching, peeling, cracking, scaling etcetera of the skin.

3.9
negative control
well-characterized material or substance that, when evaluated by a specific test method, demonstrates the suitability of the procedure to yield a reproducible, appropriately negative, non-reactive or minimal response in the test system

[SOURCE: SOURCE; ISO 10993-12:2012]

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 tissues

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 sensitization
allergic contact dermatitis
immunologically mediated cutaneous reaction to a substance

Note 1 to entry: 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.

3.13
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
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 sensitization were developed specifically to detect skin sensitization potential. Other types of adverse effects 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.

This document 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, ISO 10993-17 and ISO 10993-18;

b) literature review, including an evaluation of chemical and physical properties, and information on the 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. There are currently a number of internationally validated and accepted *in vitro* tests to detect the skin sentization potential of chemicals; However, none of these tests has been qualified for use with medical devices.

d) *in vivo* animal tests

NOTE 1 Sensitization potential of medical device extracts 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).

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).

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

5 Pretest considerations

5.1 General

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

The requirements given in ISO 10993-1:2018, Clause 5, 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 leaching or degradation characteristics of the finished products. These components shall be taken into account for their potential 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/residual catalyst can be present and the degree or extent 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.

The methods in this document have not been designed for testing of biologically derived materials and can therefore be less adequate. For example, the tests in this document 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 ASTM International 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 Skin sensitization tests

6.1 Choice of test methods

There are currently three animal assays available for the determination of the skin sensitizing potential of chemicals. These include two guinea pig assays and one murine assay. So far, the two most commonly used methods for testing for skin sensitization are the Guinea Pig Maximization Test (GPMT) and the closed-patch test (Buehler test). Of these two assays the maximization test is the most sensitive method. See Reference [9]. The closed-patch test is suitable for topical products.

The murine Local Lymph Node Assay (LLNA) was internationally accepted for testing single chemicals as a stand-alone alternative to the guinea pig assays, and is now the preferred in vivo assay for chemicals. See References [19] [32]. In some instances guinea pig assays can be necessary for the evaluation of the sensitizing potential of certain test samples. Such might be true in the case of certain metals that may give false negative findings in the LLNA or skin irritants that may give false positive findings, as well as high molecular weight substances, which do not penetrate the skin or substances that are not soluble in the recommended vehicles.

NOTE All three animal assays were developed for the detection of skin sensitizing potential of chemicals, i.e. contact dermatitis, delayed type (type IV) hypersensitivity.

In view of the provisions laid down in ISO 10993-2 on animal welfare requirements, when an *in vivo* assay is performed the LLNA shall be taken into consideration. In addition to animal welfare considerations,

the LLNA has the advantage of providing objective quantitative data that can be used to estimate the potency. Moreover, *in vitro and in chemico* alternative approaches developed for neat chemicals using a combination of different assays may identify a sensitizer. These tests include: Direct Peptide Reactivity Assay (DPRA) and the amino acid derivative reactivity assay (ADRA) according to OECD TG 442C, the KeratinoSens™ and LuSens assay according to OECD TG 442D, and the human dendritic cell activation assays (the human cell Line Activation Test (h-CLAT), the U937 Cell Line Activation Test (U-SENS) and the Interleukin-8 Reporter Gene Assay (IL-8 Luc assay) according to OECD TG 442E. Together the assays described in these test guidelines cover three key events of the now identified Adverse Outcome Pathway (AOP) for sensitization including the molecular initiating event (protein binding), induction of inflammation, and activation of dendritic cells. These test methods developed to address a specific key event may not be sufficient to conclude on the presence or absence of skin sensitisation potential of chemicals and should be considered in the context of integrated approaches such as IATA, combining them with other complementary information. In accordance with ISO 10993-2, such integrated approaches shall be taken into consideration for assessing skin sensitization potential of neat chemicals. Whether these approaches are also applicable for medical devices or medical device extracts is not (yet) known. An overview of available alternative sensitization tests for neat chemical is presented in Annex C.

6.2 Murine Local Lymph Node Assay (LLNA)

6.2.1 Principle

Following topical treatment of a test sample on the dorsum of the ears, the extent of lymphocyte proliferation is measured in the lymph nodes that drain the sites of application (ears). A response in cellular proliferation of threefold or more compared with the activity of the controls is the threshold for designating a test material as a sensitizer.

The LLNA shall be performed using a dose response approach when substances are used. For final products/medical devices it can be sufficient to test only the undiluted extract.

NOTE The Bibliography contains representative LLNA publications. Laboratories conducting this assay are encouraged to review these and other relevant publications available.

6.2.2 Test sample preparation

The test sample shall be a liquid, suspension, gel or paste such that it can be applied to the ears of the mice. Where possible, a series of doses (dilutions) shall be investigated. Otherwise, the highest concentration prepared as a chemical solution or suspension or as an extract should be used. When a strong response in the LLNA is detected with an extract dose response follow up may be necessary to evaluate the possible sensitization potency of the extract. Systemic toxicity and excessive local skin irritation can invalidate the test results; these reactions should therefore be avoided. In certain circumstances, pre-testing can be necessary.

A commonly used vehicle for substances/chemicals is an acetone olive oil (AOO) 4:1 mixture. Liquid samples that are hydrophilic and/or do not adequately adhere to the skin of the ear should be modified to adhere to the test site. This can be obtained by adding a thickening agent like carboxy methyl cellulose or hydroxyethyl cellulose (0,5 % w/v) or by a surfactant such as Pluronic® L92 with a volume fraction of 1%. For water soluble chemicals, dimethyl sulfoxide (DMSO) or dimethyl formamide (DMF) are preferred above the surfactant Pluronic® L92. See Reference [34]. Alternatively, other extract vehicles can be used, as mentioned. See Reference [33]. The effect of additions to the extract media and/or changes in vehicle composition shall be validated and documented. This might be done by experiments using weak to moderate sensitizers as commonly used as positive control. In addition, spiking of the test sample with a positive control might be performed in order to demonstrate that the LLNA is still able to detect the presence of potential sensitizers in the prepared extract. The methods of extraction are described in ISO 10993-12.

For each administration, a separate extract shall be prepared.

NOTE For polymers, information on a specific method for preparation of extracts is given in Annex B.

6.2.3 Animals and husbandry

Healthy female non-pregnant mice of the CBA/Ca or CBA/J or BALB/c strain shall be used, unless another strain is validated. See References [33] [41] [42]. Several mouse strains have been reported as acceptable (DBA/2, B6C3F1). See Reference [35]. The mice shall be seven to twelve weeks of age; the mice in each study shall be matched in age (within a one-week age range).

Husbandry and selection of animals shall be in accordance with ISO 10993-2. The mice, routinely acclimatized to the laboratory, shall be individually identified. For certain test samples, individual housing can be necessary. This shall be justified and documented.

Animals shall be uniquely identified by methods not to include ear punches or ear tags.

When group housing is performed, cross contamination and unwanted oral intake should be taken into consideration.

6.2.4 Test procedure

For chemicals, the LLNA is generally performed in a dose-response manner. For solid medical devices, samples to be tested shall be extracts. In these cases, only a single dose is available for testing. In general, the extract can be investigated undiluted. However, when the extract contains highly toxic components, this can result in a negative response in the LLNA due to toxicity. It is therefore recommended, when investigating highly toxic extracts (see ISO 10993-5) to perform the LLNA in a dose-response manner and to dilute the extract. In addition, when a strong response is detected in the LLNA a dose response follow up can be conducted to evaluate the possible sensitization potency of the extract. Consideration should be given to confirming positive responses in a guinea pig model of sensitization.

In order to ensure reproducibility and sensitivity, a test of a positive-control substance for skin sensitization shall be included by the testing laboratory in order to validate the test system and demonstrate a positive response. Well-known weak to moderate contact allergens, e.g. mercaptobenzothiazole, hexyl cinnamic aldehyde, or benzocaine, shall be used as positive control. The examples mentioned might not be suitable for each vehicle used for sample preparation (e.g. water based vehicle); in such cases, another positive control might be selected. ASTM F2148 indicates that in such circumstances formalin and 2,4-dinitrochlorobenzene (DNCB) should be used as positive controls. This shall be justified and documented.

While inclusion of a concurrent positive control group is recommended, there may be situations in which only periodic testing (i.e. at intervals ≤ 6 months) of the positive control test substance may be adequate. This the case for laboratories that conduct the LLNA regularly (i.e. conduct the LLNA at a frequency of no less than once per month) and have an established historical PC database that demonstrates the laboratory's ability to obtain reproducible and accurate results with PCs. Adequate proficiency with the LLNA can be successfully demonstrated by generating consistent positive results with the positive control in at least 10 independent tests conducted within a reasonable period of time (i.e. less than one year).

The individual body weights shall be recorded at initiation and at the end of the study. In order to detect potential toxicity of the test sample, clinical observation shall be performed and recorded during the study.

Using a positive control only once every six months can have consequences for the results obtained in the previous six months period when this positive control shows a negative outcome. Reference [33] states that periodic testing (i.e. at intervals ≤ 6 months) of the positive control substance can be considered in laboratories that conduct the LLNA regularly (i.e. conduct the LLNA at a frequency of no less than once per month) and that have a history and a documented proficiency for obtaining consistent results with positive controls. It is important to realize that the decision to only include a positive control periodically instead of concurrently could have ramifications on the adequacy and acceptability of negative study results generated without a concurrent positive control during the interval between each periodic positive control study. For example, if a false negative result is obtained in the periodic positive control test, all negative test substance results obtained in the interval between the last acceptable periodic positive control test and the unacceptable periodic positive control test could be