INTERNATIONAL STANDARD

ISO 10993-10

Fourth edition
2021-11

Biological evaluation of medical devices —

Part 10: Tests for skin sensitization

Évaluation biologique des dispositifs médicaux —

Partie 10: Essais de sensibilisation cutanée

Reference number
ISO 10993-10:2021(E)

© ISO 2021



COPYRIGHT PROTECTED DOCUMENT

© ISO 2021

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

© ISO 2021 – All rights reserved

Contents

Page

Foreword ..... **v**
Introduction ..... **vi**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 General principles — Step-wise approach **3**
5 Pretest considerations **4**
5.1 General 4
5.2 Types of material 4
5.2.1 Initial considerations 4
5.2.2 Ceramics, metals and alloys 4
5.2.3 Polymers 4
5.2.4 Biologically derived materials 4
5.3 Information on chemical composition 4
5.3.1 General 4
5.3.2 Existing data sources 5
6 Skin sensitization tests **5**
6.1 Choice of test methods 5
6.2 Murine local lymph node assay 6
6.2.1 Principle 6
6.2.2 Test sample preparation 6
6.2.3 Animals and husbandry 6
6.2.4 Test procedure 7
6.2.5 Treatment groups 8
6.2.6 Determination of cellular proliferation and tissue preparation 8
6.2.7 Results and interpretation 9
6.2.8 Test report 9
6.3 Guinea pig assays for the detection of skin sensitization 9
6.3.1 Principle 9
6.3.2 Choice of test sample concentrations 10
6.3.3 Induction 10
6.3.4 Challenge 10
6.4 Important factors affecting the outcome of the test 10
6.5 Guinea pig maximization test 11
6.5.1 Principle 11
6.5.2 Test sample preparation 11
6.5.3 Animals and husbandry 11
6.5.4 Test procedure 12
6.5.5 Observation of animals 14
6.5.6 Evaluation of results 14
6.5.7 Test report 15
6.6 Closed-patch test (Buehler test) 15
6.6.1 Principle 15
6.6.2 Test sample preparation 15
6.6.3 Animals and husbandry 15
6.6.4 Test procedure 16
6.6.5 Observation of animals 17
6.6.6 Evaluation of results 17
6.6.7 Test report 17
7 Key factors in interpretation of test results **18**
Annex A (normative) **Preparation of materials for skin sensitization testing** **19**

© ISO 2021 – All rights reserved

Annex B (informative) **Method for the preparation of extracts from polymeric test materials** **21**
Annex C (informative) **Non-animal methods for skin sensitization** **24**
Annex D (informative) **Background information on sensitization tests for skin sensitization** **37**
Bibliography **40**

© ISO 2021 – All rights reserved

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 of medical devices,* in collaboration with the European Committee for Standardization (CEN) Technical Committee CEN/TC 206, *Biological and clinical evaluation of medical devices,* in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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 non-animal methods for skin sensitization (formerly Annex D) 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.

© ISO 2021 – All rights reserved

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 materials, sensitizing properties were reported —see Reference [51]. 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 (background information is provided in Annex D). Since 2015, several in chemico and in vitro assays have been validated and Organization for Economic Co-operation and Development (OECD) test guidelines released to assess the skin sentization potential of chemicals.[75][79][104] An overview of available alternative skin sensitization tests for neat chemicals is given in Annex C. These test methods, each developed to address a specific key event, can possibly not be sufficient alone to conclude on the presence or absence of skin sensitization potential of chemicals and should be considered in the context of integrated approaches such as integrated approaches to testing and assessment (IATA), combining them with other complementary information. Note that the in vitro and in chemico tests for skin sensitization in Annex C have thus far been validated only for neat chemicals and not for medical devices. To confirm that they are applicable for evaluation of the skin sensitization potential of medical devices, their assays need to be assessed and validated.

Where appropriate, the preliminary use of in vitro methods is encouraged for screening purposes prior to animal testing. 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, skin sensitization studies be conducted using GLP or ISO/IEC 17025 as applicable to the respective country and comply with regulations related to animal welfare. Statistical analyses of data are recommended and used whenever appropriate. This document includes important tools for the development of safe products and is intended for use by professionals, appropriately qualified by training and experience, who can 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.

This document is based on numerous standards and guidelines, including OECD Guidelines, US Pharmacopoeia and the European Pharmacopoeia. It is intended to be the basic document for the selection and conduct of tests enabling the evaluation of dermal sensitization responses relevant to the safety of medical materials and devices.

© ISO 2021 – All rights reserved

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

ISO 10993-18, *Biological evaluation of medical devices — Part 18: Chemical characterization of medical device materials within a risk management process*

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 terminology databases for use in standardization at the following addresses:

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

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

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

© ISO 2021 – All rights reserved

3.2
allergic contact dermatitis
clinical diagnosis based on an observed immunologically-mediated cutaneous reaction to a substance

3.3
blank
extraction *vehicle* (3.17) not containing the *test material* (3.15), 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.

3.4
challenge
process following the *induction* (3.8) phase, in which the immunological effects of subsequent exposures in an individual to the inducing material are examined

3.5
elicitation
immunological reaction to exposure to a sensitizer in a previously sensitized individual

3.6
erythema
reddening of the skin or mucous membrane

3.7
extract
liquid that results from extraction of the *test sample* (3.16) or control

[SOURCE: ISO 10993-12:2021, 3.6]

3.8
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

3.9
irritant
agent that produces *irritation* (3.10)

3.10
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 symptoms like local *erythema* (3.6) (redness), swelling, itching, peeling, cracking and scaling of the skin.

3.11
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

Note 1 to entry: In practice, negative controls include *blanks* (3.3), *vehicles* (3.17)/solvents and reference materials.

[SOURCE: ISO 10993-12:2021, 3.10, modified — Note 1 to entry has been replaced.]

© ISO 2021 – All rights reserved

3.12
oedema
swelling due to abnormal infiltration of fluid into the tissues

3.13
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

3.14
skin sensitization
T-cell mediated delayed-type hypersensitivity reaction induced by low molecular weight reactive chemicals (allergens) comprising two phases, induction and elicitation

Note 1 to entry: In humans, the responses can be characterized by pruritis, *erythema* (3.6), *oedema* (3.12), papules, vesicles, bullae or a combination of these. In other species, the reactions can differ and only erythema and oedema can be seen.

3.15
test material
material, device, device portion or component thereof that is sampled for biological or chemical testing

3.16
test sample
material, device, device portion, component, *extract* (3.7) or portion thereof that is subjected to biological or chemical testing or evaluation

3.17
vehicle
liquid used to moisten, dilute, suspend, *extract* (3.7) 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.

This document requires a step-wise approach, considering that any stage can result in the conclusion that further testing for skin sensitization is not necessary:

a) literature and supplier information review, including chemical and physical properties, and information on the skin sensitization potential of any medical device constituent as well as structurally-related chemicals and materials; refer to ISO 10993-1 for details; conduct risk assessment based on existing information to determine whether skin sensitization risk is acceptable or whether further testing is necessary;

b) additional characterization and risk assessment, if needed, of the device material, involving chemical characterization and analysis of the test sample according to the general principles described in ISO 10993-18;

c) in vitro tests shall be considered in preference to in vivo tests in accordance with ISO 10993-2, and the replacement of the latter as new in vitro tests are scientifically validated and become reasonably and practicably available;

NOTE There are currently a number of internationally validated and accepted in vitro tests to detect the skin sensitization potential of chemicals; however, these in vitro tests are not yet validated for medical devices. Work is ongoing for some of these tests to qualify them for use with medical devices.

d) in vivo animal tests are only 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).

© ISO 2021 – All rights reserved