FINAL
DRAFT

TECHNICAL SPECIFICATION

ISO/DTS 11796

ISO/TC **194**

Secretariat: **DIN**

Voting begins on:
2023-01-19

Voting terminates on:
2023-03-16

Biological evaluation of medical devices — Requirements for interlaboratory studies to demonstrate the applicability of validated in vitro methods to assess the skin sensitization of medical devices

Évaluation biologique des dispositifs médicaux — Guide pour les études interlaboratoires visant à démontrer l'applicabilité des méthodes in-vitro validées pour évaluer la sensibilisation cutanée des dispositifs médicaux

Reference number
ISO/DTS 11796:2023(E)

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

Contents

Page

Foreword ..... **iv**
Introduction ..... **v**
1 Scope **1**
2 Normative references **1**
3 Terms and definitions **1**
4 Consideration on the process for demonstration of the applicability domain **3**
5 Positive and negative control test samples **4**
5.1 General 4
5.2 Database of reference chemicals 5
5.3 Reference materials 8
5.3.1 General 8
5.3.2 Negative control medical device material 9
5.3.3 Positive reference material 9
6 Preparation of the test samples **9**
6.1 General 9
6.2 Preparation of the extracts 9
6.3 Spiking of the extracts 10
7 Prevalidation of candidate test methods **10**
8 Interlaboratory study **11**
8.1 General 11
8.2 Study organization 11
8.2.1 General 11
8.2.2 Management team 11
8.2.3 Lead laboratory 11
8.2.4 Sample management 11
8.2.5 Independent biostatistician 11
8.3 Sample preparation 11
8.4 Performance review 12
9 Statistical analysis **12**
10 Test report **12**
Annex A (informative) **Reference chemicals** **14**
Bibliography **28**

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, *Biological and clinical evaluation of medical devices*.

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

International Standards can be used to demonstrate the safety and compliance of medical devices. ISO 10993-10 specifies the procedure for the assessment of medical devices and their constituent materials with regard to their potential to induce skin sensitization (Type IV hypersensitivity reaction). The methods included in ISO 10993-10 are based on animal or human testing, with an annex on in vitro and in chemico tests for skin sensitization that have been validated for neat chemicals. The effort to reduce or replace the use of animals in toxicity testing has led to the development of many new non-animal methods. The test guidelines in References [56] and [57] include alternatives to animal testing methods for skin sensitization that have been previously validated to confirm their equivalence/superiority to the current in vivo methods. However, currently, none of the OECD test guideline methods are considered sufficient stand-alone replacements for in vivo tests that assess the skin sensitization potential of chemicals[1].

Current OECD test guideline methods are validated with neat chemicals and not with more complex mixtures such as medical devices or medical devices extracts. In order to use these methods in the specific context of medical devices, an evaluation is needed to verify their applicability for assessing skin sensitization of medical devices. Given the number of candidate test methods and the time that is required to assess them, it is important to ensure that the same science-based evaluation process and criteria are consistently applied to any new candidate test method. The purpose of this document is to provide a framework for the conduct of prevalidation and interlaboratory studies to assess the applicability of candidate test methods for assessing one or more key events related to OECD's adverse outcome pathway (AOP) for skin sensitization when evaluating medical devices[2].

Biological evaluation of medical devices — Requirements for interlaboratory studies to demonstrate the applicability of validated in vitro methods to assess the skin sensitization of medical devices

1 Scope

This document specifies the framework and the methodology to evaluate and demonstrate the applicability of a validated non-animal method from an OECD test guideline to assess the skin sensitizing potential of a medical device or a medical device material. This document addresses:

— database of reference chemical skin sensitizers and non sensitizers;

— reference materials;

— feasibility testing of candidate test methods, including any method optimization for use with extracts of medical devices;

— prevalidation of candidate test methods;

— interlaboratory study:

  — sample preparation and coding;

  — spiking of the extracts of negative control medical device material;

  — collection of the data;

  — statistical analysis to assess reliability and reproducibility.

The use of the approaches described in this document to assess the applicability of a candidate test method does not imply that the candidate test method can be used as stand-alone test for the evaluation of skin sensitization potential of medical devices. For certain candidate test methods, integrated approaches and/or defined approaches are needed.[1] The evaluation of skin sensitization potential of a medical device is described in ISO 10993-10.

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

3 Terms and definitions

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

3.2
candidate test method
test method for in vitro sensitization testing of medical devices that is under evaluation

3.3
interlaboratory study
ILS
organization, performance and evaluation of measurements or tests on the same or similar items by two or more laboratories according to predetermined conditions

3.4
interlaboratory reproducibility
between-laboratory reproducibility
measure of the extent to which different qualified laboratories, using the same protocol and testing the same substances, can produce qualitatively and quantitatively similar results

Note 1 to entry: Interlaboratory reproducibility is determined during the prevalidation and validation processes, and indicates the extent to which a test can be successfully transferred between laboratories.[29]

3.5
intralaboratory reproducibility
within-laboratory reproducibility
determination of the extent that qualified people within the same laboratory can successfully replicate results using a specific protocol at different times

3.6
prevalidation
PRE
initial phase of a *validation* (3.10) small-scale study intended to obtain preliminary information on the relevance and reliability of a *candidate test method* (3.2)

3.7
test article
material (e.g. a final finished device or a reference material) that is to be used to generate a *test sample* (3.8) (e.g. using extraction)

3.8
test sample
sample (e.g. a test article extract or spiked extract vehicle) that in its present form can be evaluated by a candidate test method

3.9
test system
system (e.g. in vivo animal model, in vitro cellular model and in-silico computational models) that is used for hazard identification as part of a test method

3.10
validation
confirmation, through the provision of objective evidence, that the requirements for a specific intended use or application have been fulfilled

[SOURCE: ISO 9000:2015, 3.8.13, modified — Notes 1, 2 and 3 to entry have been deleted.]

4 Consideration on the process for demonstration of the applicability domain

The process for evaluating the applicability of a candidate test method for sensitization testing of medical devices shall include feasibility, prevalidation and interlaboratory studies (see Figure 1).

Prior to conducting a prevalidation study, a feasibility study may be needed to determine if any modification of the OECD TG protocols (e.g. dilution, solvents, incubation times, volume of test sample, stimulation index value) is necessary for the evaluation of medical devices.

Protocols for feasibility studies are not described in this document as the design of these studies should be specific to the OECD TG method.

If the candidate test method protocols planned for the prevalidation and interlaboratory studies deviate from the OECD TG protocol, the number and nature of the modifications as well as the data and documentation available (e.g. from a feasibility study) to support the modifications shall be provided. A scientific rationale for the impact of these changes on the acceptance of the method for assessing the sensitizing potential of sample tests should be provided to justify that the method used remains equivalent to the original OECD method.

The same candidate test method and protocols shall be used for both the prevalidation study and the interlaboratory study.

As the non-animal methods considered are already validated with single chemicals (but not with mixtures such as medical devices extracts) and integrated in OECD test guidelines (e.g. see Reference [91] and Reference [1] on defined approaches for sensitization) with historical data of chemicals assessment, the prevalidation step shall be conducted to:

a) prepare the standard operating procedures (SOPs), so that they can be readily used by other laboratories;

b) generate preliminary data on the reliability and relevance of the candidate test method for assessing skin sensitization of medical devices.

During the prevalidation and interlaboratory phases, evaluation of the performance of non-animal methods shall be performed with positive and negative control test samples (in accordance with Clause 5) that are representative of medical devices extracts. The accuracy, sensitivity, specificity and reproducibility shall be calculated and compared to the targeted performance values in Clause 7 and Clause 8.

If the prevalidation study does not achieve the performance criteria in accordance with Clause 7, then additional feasibility testing may be needed to optimize the assay protocol for increased accuracy, specificity and/or intralaboratory reproducibility prior to conducting a repeat prevalidation study. If the prevalidation study meets the performance criteria in accordance with Clause 7, then an interlaboratory study can be considered.

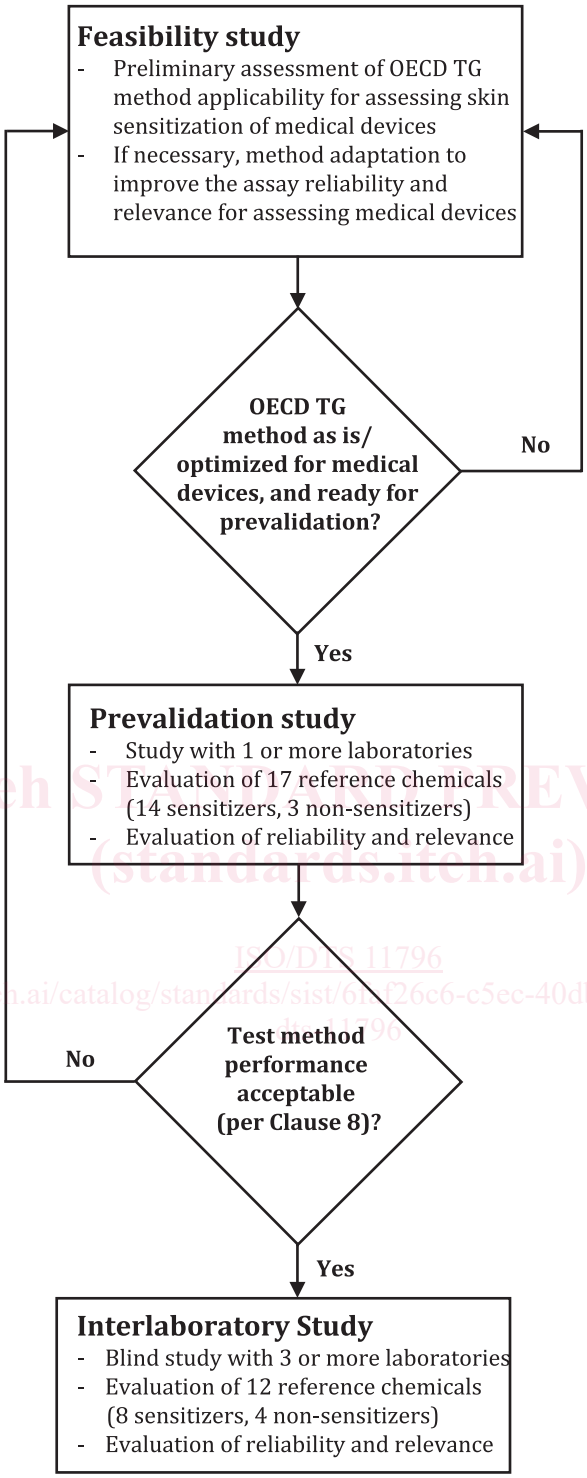

Figure 1 — General process for the evaluation of candidate test methods

5 Positive and negative control test samples

5.1 General

Due to the lack of existing positive reference test articles, samples for prevalidation and interlaboratory studies comprise negative reference material extracts spiked with a known concentration of a chemical skin sensitizer. By spiking an extract of an existing medical device material, the final composition of

test samples tested by candidate in vitro test methods can better mimic the chemical complexity of a real extract containing a low concentration of one or more chemical skin sensitizers.

For this purpose, chemical skin sensitizers and non sensitizers that can be identified in extracts of certain medical device materials have been selected. Annex A includes animal, and human data when available, to serve as a reference database for prevalidation and interlaboratory studies.

5.2 Database of reference chemicals

A list of reference chemicals was developed including chemicals:

— representative of raw materials and/or leachables found in medical devices;

— representative of a balanced range of skin sensitizer potency (weak, moderate and strong);

— supported by robust reference data on potency and no-observed-adverse-effect-levels (NOAELs) from human and animal sources, including human repeat insult patch test (HRIPT), human maximization test (HMT), local lymph node assay (LLNA), closed-patch (Buehler) test and guinea-pig maximization test (GPMT).

Additional criteria considered for selection of chemicals are:

— to be representative of the different mechanisms by which the compounds exert a sensitization effect;

— a range of physicochemical properties considered relevant to skin sensitization in Reference [1];

— commercially available compounds.

NOTE The selection of chemicals already detected in medical devices or medical device materials was based on a review of the scientific literature.[3][4][5][6][7] Particular attention was paid to the quality of the historical toxicological data for these chemicals. The estimated concentrations required to generate a threefold stimulation of proliferation in draining lymph node (EC3 values) are derived from curated databases such as SkinSenseDB,[8] the Cosmetics Europe database,[9] supplementary data from Reference [10], the integrated chemical environment (ICE) from the national toxicology program (NTP),[11] the National Toxicology Program Interagency Centre for the Evaluation of Alternative Toxicological Methods (NICEATM) LLNA Database[12] and the OECD curated database for OECD guideline 497[13][14][15].

All chemicals are presented in Table 1 with name, CAS Registry Number®[1)], medical devices application examples and the spiking concentration to be used for prevalidation and interlaboratory studies. Additional information on their physicochemical and skin sensitizing properties in humans and animals is presented in Annex A (e.g. GPMT, LLNA EC3, human potency, reference publications and complementary data).

1) CAS Registry Number® is a trademark of CAS corporation. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

Table 1 — Database of reference chemicals for PRE and ILS

S/NS	Set	Name	CAS	Application in the medical device (MD) industry	**Spiking concentration** weight per volume (*w/v*) %
S	PRE	Glutaraldehyde	111–30–8	Disinfectant and crosslinker for animal derived tissue product	0,08
S	PRE	1,4-Phenylenediamine	106–50–3		0,11
S	PRE	Phthalic anhydride	85–44–9		0,16
S	PRE	Cobalt chloride	7646–79–9	Orthopaedic implants, stents, pacemakers	0,4
S	PRE	Phenylacetaldehyde	122–78–1		3
S	PRE	Hydratropic aldehyde	93–53–8		6,3
S	PRE	Methyl oleate	112–62–9	Ink, lubricants, etc.	8,5
S	PRE	Linolenic acid	463–40–1	Lubricants	9,9
S	PRE	Ethyl acrylate	140–88–5	Acrylates, methacrylates and monomers Found in adhesives, wearable devices, wound dressings	10
S	PRE	TPO (Diphenyl(2,4,6-trimethylbenzoyl)phosphine oxide)	75980–60–8	Photo-initiator for additive manufacturing in many light-cured acrylic polymers) such as composite materials, dental fillers, etc.	27
S	PRE	2,4,7,9-Tetramethyl-5-decyn-4,7-diol	126–86–3	Plastic and rubber products	34,3
S	PRE	Isopropyl Myristate	110–27–0	Plasticizer, lubricant	44
S	PRE	Tridecane	629–50–5	solvent, rubber industry	48,4
S	PRE	Methyl methacrylate	80–62–6	Bone cement, dental materials, hearing aids	75
NS	PRE	Diethyl phtalate	84–66–2	Solvent, plasticizer, extractable associated with polyethylene and PET	1
NS	PRE	1,3-diphenylguanidine	102–06–7	Rubber accelerators Found in gloves	1
NS	PRE	Zinc oxide	1314–13–2	Anti-bacterial and anti-biofilm activity	1
S	ILS	2,4-Dinitrochlorobenzen DNCB	97–00–7		0,06
S	ILS	Formaldehyde (act. 37 %)	50–00–0	Sterilization-low temperature steam and formaldehyde (LTSF)	0,3

Key

S sensitizer

NS non sensitizer

The database of reference chemicals contains 29 chemicals, 7 non sensitizers and 22 skin sensitizers. The selected chemicals are distributed in two sets. The first set of 17 chemicals (14 sensitizers and 3 non sensitizers) which shall be used for the prevalidation phase, are labelled "PRE" in Table 1 and included in Clause A.1. The second set of 12 chemicals (8 sensitizers and 4 non sensitizers) which shall be used for the interlaboratory study phase, are labelled "ILS" in Table 1 and included in Clause A.2. The size of this set, 12 chemicals, represents half the number of chemicals originally used for the validation of OECD test methods. It was considered appropriate from a statistical point of view because minor deviation of the protocols during the feasibility study are unlikely to affect the transferability and interlaboratory reproducibility.

Table 1 *(continued)*

S/NS	Set	Name	CAS	Application in the medical device (MD) industry	**Spiking concentration** weight per volume (*w/v*) %
S	ILS	Isobornyl acrylate (IBOA)	5888–33–5	Plastic materials, valves, tubes lining, stoppers, sealants, coatings, inks, glues Found in adhesives, wearable devices such as glucose monitoring sensors, insulin patch pumps and some wound dressings	1
S	ILS	2-Mercaptobenzothiazole (MBT)	149–30–4	Rubber accelerators Gloves	1,35
S	ILS	2-hydroxyethyl acrylate	818–61–1	Acrylates, methacrylates and monomers Wound dressings, EKG electrodes, Contact lenses	1,4
S	ILS	Nickel(II) sulfate hexahydrate (NiSO4)	10101–97–0 7786–81–4	Nickel alloys and stainless steels in implantable medical devices	4,8
S	ILS	Abietic acid	514–10–3	Adhesives Wound dressing	15
S	ILS	α-Methylstyrene	98–83–9	Intermediate used in the manufacture of plasticizers, resins and polymers	46
NS	ILS	Chlorobenzene	108–90–7	Intermediate in rubber, solvent in adhesives	1
NS	ILS	Octanoic acid	124–07–2	Lubricant	1
NS	ILS	Glycerol	56–81–5		1
NS	ILS	Lactic Acid	50–21–5	Monomer of polymer polylactic acid (PLA). PLA is commonly used in biodegradable polymers for drug delivery systems, tissue engineering, temporary and long-term implantable devices, etc.	1

Key

S sensitizer

NS non sensitizer

The database of reference chemicals contains 29 chemicals, 7 non sensitizers and 22 skin sensitizers. The selected chemicals are distributed in two sets. The first set of 17 chemicals (14 sensitizers and 3 non sensitizers) which shall be used for the prevalidation phase, are labelled "PRE" in Table 1 and included in Clause A.1. The second set of 12 chemicals (8 sensitizers and 4 non sensitizers) which shall be used for the interlaboratory study phase, are labelled "ILS" in Table 1 and included in Clause A.2. The size of this set, 12 chemicals, represents half the number of chemicals originally used for the validation of OECD test methods. It was considered appropriate from a statistical point of view because minor deviation of the protocols during the feasibility study are unlikely to affect the transferability and interlaboratory reproducibility.

The selected chemicals are representative of chemicals previously found in leaching studies of medical devices extracts (see Annex A). The skin sensitizers cover the different potency categories according to the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) classification[16] as represented in Figure 2: 8 weak, 9 moderate, 3 strong and 2 extreme skin sensitizers.