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**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**

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*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* <sup>23</sup>

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## Foreword

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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 document 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](http://www.iso.org/directives)).

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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](http://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<sup>[1]</sup>.

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<sup>[2]</sup>.

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# 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:

- the database of reference chemical skin sensitizers and non-skin 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;
- the interlaboratory study:
  - sample preparation and coding;
  - spiking of the extracts from the negative control medical device material;
  - data collection;
  - 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 a stand-alone test for evaluating the skin sensitization potential of medical devices. For certain candidate test methods, integrated approaches and/or defined approaches are needed.<sup>[1]</sup> 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 skin 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.<sup>[29]</sup>

**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**

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 skin sensitization testing of medical devices shall include feasibility, prevalidation and interlaboratory studies (see [Figure 1](#)).

The required sample size was determined according to Reference [80]. The basis for the determination was that the candidate test methods for the application to medical device extracts or medical device material extracts, predict the spiked extracts (non-skin sensitizer versus skin sensitizer) as good as the original OECD test method with the neat chemicals used for spiking the extracts. To demonstrate concordance of results, it was assumed that at least 90 % of the test samples were predicted concordantly in the candidate test method as compared to the original OECD test method. It was also assumed that the concordance was not below 70 %. Considering a one-sided proportion of 0,05 and a power of 0,8, a sample size of 28 was calculated. The sample size was increased to 29 to account for potential drop-out of chemicals, e.g. due to commercial unavailability.

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 skin 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 skin 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 concordance of results (compared to the original OECD test method) 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 can be needed to optimize the assay protocol for increased concordance 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.

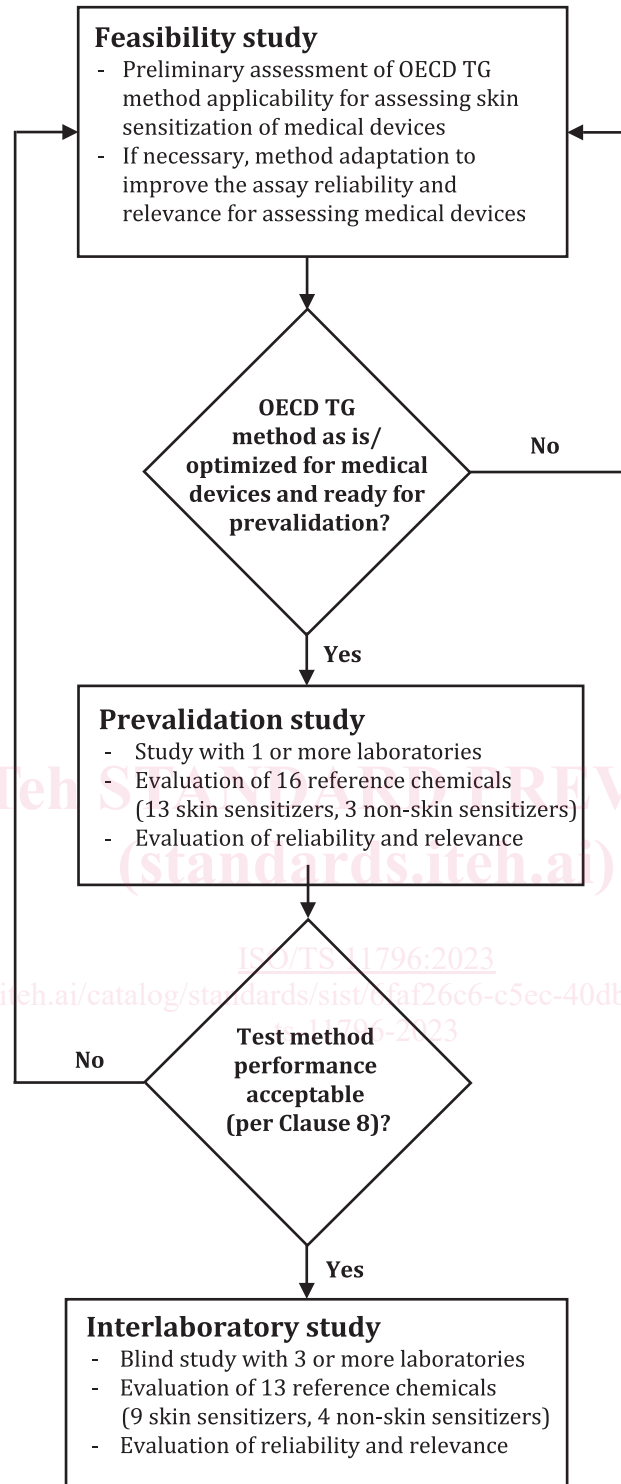


Figure 1 — General process for the evaluation of candidate test methods

## 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 are comprised of 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 evaluated by 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-skin sensitizers that can be identified in extracts of certain medical device materials have been selected. [Annex A](#) contains a database of reference chemicals with animal, and human data when available, that will be used in the 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, strong and extreme);
- 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 skin sensitization effect;
- a range of physicochemical properties considered relevant to skin sensitization in Reference [\[1\]](#);
- commercially available compounds.

According to the sample size considerations, a total of 29 chemicals are required. For each candidate test method evaluated, the results obtained for the original OECD test methods with neat chemicals will be collected or, when not available, generated experimentally.

**NOTE** The selection of chemicals already detected in medical devices or medical device materials was based on a review of References [\[3\]](#)[\[4\]](#)[\[5\]](#)[\[6\]](#) and [\[7\]](#). 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<sup>®1</sup>, 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).

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1) CAS Registry Number<sup>®</sup> is a trademark of the American Chemical Society (ACS). 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 industry	Spiking concentration 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	ILS	Hydratropic aldehyde	93-53-8	—	6,3
S	PRE	Alpha-hexylcinnamaldehyde	101-86-0	Ink, lubricants, etc.	10,8
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 phthalate	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-Dinitrochlorobenzene (DNCB)	97-00-7	—	0,06
S	ILS	Formaldehyde (act. 37 %)	50-00-0	Sterilization-low temperature steam and formaldehyde (LTSF)	0,3
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 (NiSO <sub>4</sub> )	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

**Key**

S skin sensitizer

NS non-skin sensitizer

w weight

v volume

The database of reference chemicals contains 29 chemicals, 7 non-skin sensitizers and 22 skin sensitizers. The selected chemicals are distributed in two sets. The first set of 16 chemicals (13 skin sensitizers and 3 non-skin sensitizers) which shall be used for the prevalidation phase, are labelled "PRE" in Table 1 and are included in Clause A.1. The second set of 13 chemicals (9 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, 13 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 industry	Spiking concentration w/v %
S	ILS	$\alpha$ -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	Antibacterial agent	1
NS	ILS	Glycerol	56-81-5	Lubricant	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 skin sensitizer

NS non-skin sensitizer

w weight

v volume

The database of reference chemicals contains 29 chemicals, 7 non-skin sensitizers and 22 skin sensitizers. The selected chemicals are distributed in two sets. The first set of 16 chemicals (13 skin sensitizers and 3 non-skin sensitizers) which shall be used for the prevalidation phase, are labelled "PRE" in Table 1 and are included in Clause A.1. The second set of 13 chemicals (9 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, 13 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<sup>[16]</sup> as represented in Figure 2: 9 weak, 8 moderate, 3 strong and 2 extreme skin sensitizers.

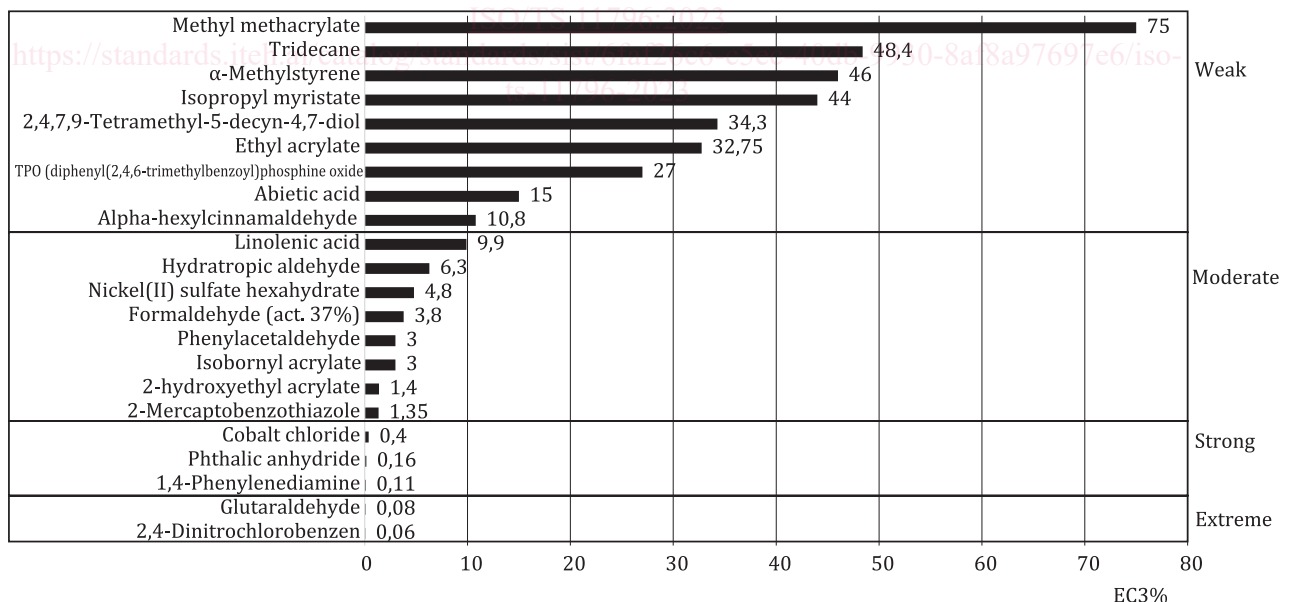


Figure 2 — Distribution of EC3 values of the reference chemical skin sensitizers

The physicochemical properties considered relevant to skin sensitization in the supporting document to Reference [1] are presented in Table 2 for the 29 chemicals selected compared to the 167 chemicals of the OECD database<sup>[13]</sup>.