

American National Standard



ANSI/AAMI/ ISO 10993-10: 2010/(R)2014

Biological evaluation of
medical devices — Part 10:
Tests for irritation and skin
sensitization

Objectives and uses of AAMI standards and recommended practices

It is most important that the objectives and potential uses of an AAMI product standard or recommended practice are clearly understood. The objectives of AAMI's technical development program derive from AAMI's overall mission: the advancement of medical instrumentation. Essential to such advancement are (1) a continued increase in the safe and effective application of current technologies to patient care, and (2) the encouragement of new technologies. It is AAMI's view that standards and recommended practices can contribute significantly to the advancement of medical instrumentation, provided that they are drafted with attention to these objectives and provided that arbitrary and restrictive uses are avoided.

A voluntary *standard* for a *medical device* recommends to the manufacturer the information that should be provided with or on the product, basic safety and performance criteria that should be considered in qualifying the device for clinical use, and the measurement techniques that can be used to determine whether the device conforms with the safety and performance criteria and/or to compare the performance characteristics of different products. Some standards emphasize the information that should be provided with the device, including performance characteristics, instructions for use, warnings and precautions, and other data considered important in ensuring the safe and effective use of the device in the clinical environment. Recommending the disclosure of performance characteristics often necessitates the development of specialized test methods to facilitate uniformity in reporting; reaching consensus on these tests can represent a considerable part of committee work. When a drafting committee determines that clinical concerns warrant the establishment of *minimum* safety and performance criteria, referee tests must be provided and the reasons for establishing the criteria must be documented in the rationale.

A *recommended practice* provides guidelines for the use, care, and/or processing of a medical device or system. A recommended practice does not address device performance *per se*, but rather procedures and practices that will help ensure that a device is used safely and effectively and that its performance will be maintained.

Although a device standard is primarily directed to the manufacturer, it may also be of value to the potential purchaser or user of the device as a frame of reference for device evaluation. Similarly, even though a recommended practice is usually oriented towards healthcare professionals, it may be useful to the manufacturer in better understanding the environment in which a medical device will be used. Also, some recommended practices, while not addressing device performance criteria, provide guidelines to industrial personnel on such subjects as sterilization processing, methods of collecting data to establish safety and efficacy, human engineering, and other processing or evaluation techniques; such guidelines may be useful to health care professionals in understanding industrial practices.

In determining whether an AAMI standard or recommended practice is relevant to the specific needs of a potential user of the document, several important concepts must be recognized:

All AAMI standards and recommended practices are *voluntary* (unless, of course, they are adopted by government regulatory or procurement authorities). The application of a standard or recommended practice is solely within the discretion and professional judgment of the user of the document.

Each AAMI standard or recommended practice reflects the collective expertise of a committee of health care professionals and industrial representatives, whose work has been reviewed nationally (and sometimes internationally). As such, the consensus recommendations embodied in a standard or recommended practice are intended to respond to clinical needs and, ultimately, to help ensure patient safety. A standard or recommended practice is limited, however, in the sense that it responds generally to perceived risks and conditions that may not always be relevant to specific situations. A standard or recommended practice is an important *reference* in responsible decision-making, but it should never *replace* responsible decision-making.

Despite periodic review and revision (at least once every five years), a standard or recommended practice is necessarily a static document applied to a dynamic technology. Therefore, a standards user must carefully review the reasons why the document was initially developed and the specific rationale for each of its provisions. This review will reveal whether the document remains relevant to the specific needs of the user.

Particular care should be taken in applying a product standard to existing devices and equipment, and in applying a recommended practice to current procedures and practices. While observed or potential risks with existing equipment typically form the basis for the safety and performance criteria defined in a standard, professional judgment must be used in applying these criteria to existing equipment. No single source of information will serve to identify a particular product as "unsafe". A voluntary standard can be used as one resource, but the ultimate decision as to product safety and efficacy must take into account the specifics of its utilization and, of course, cost-benefit considerations. Similarly, a recommended practice should be analyzed in the context of the specific needs and resources of the individual institution or firm. Again, the rationale accompanying each AAMI standard and recommended practice is an excellent guide to the reasoning and data underlying its provision.

In summary, a standard or recommended practice is truly useful only when it is used in conjunction with other sources of information and policy guidance and in the context of professional experience and judgment.

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Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization

Approved 4 September 2010 by
Association for the Advancement of Medical Instrumentation

Approved 10 September 2010 and Reaffirmed 8 October 2014 by
American National Standards Institute, Inc.

Abstract: Describes the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and skin sensitization. Includes: a) pretest considerations for irritation, including in silico and in vitro methods for dermal exposure; b) details of in vivo (irritation and sensitization) test procedures, and; c) 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 B several special irritation tests are described for application of medical devices in areas other than skin.

Keywords: biological evaluation, irritation, hypersensitivity, sensitivity, assessment

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Glossary of equivalent standards

International Standards adopted in the United States may include normative references to other International Standards. For each International Standard that has been adopted by AAMI (and ANSI), the table below gives the corresponding U.S. designation and level of equivalency to the International Standard. NOTE: Documents are sorted by international designation. The code in the US column, “(R)20xx” indicates the year the document was officially reaffirmed by AAMI. E.g., ANSI/AAMI/ISO 10993-4:2002/(R)2009 indicates that 10993-4, originally approved and published in 2002, was reaffirmed without change in 2009.

Other normatively referenced International Standards may be under consideration for U.S. adoption by AAMI; therefore, this list should not be considered exhaustive.

International designation	U.S. designation	Equivalency
IEC 60601-1:2005 Technical Corrigendum 1 and 2	ANSI/AAMI ES60601-1:2005 and ANSI/AAMI ES60601-1:2005/A2:2010 ANSI/AAMI ES60601-1:2005/C1:2009 (amdt)	Major technical variations C1 Identical to Corrigendum 1 & 2
IEC 60601-1-2:2007	ANSI/AAMI/IEC 60601-1-2:2007	Identical
IEC 60601-2-2:2009	ANSI/AAMI/IEC 60601-2-2:2009	Identical
IEC 60601-2-4:2002	ANSI/AAMI DF80:2003/(R)2010	Major technical variations
IEC 60601-2-19:2009	ANSI/AAMI/IEC 60601-2-19:2009	Identical
IEC 60601-2-20:2009	ANSI/AAMI/IEC 60601-2-20:2009	Identical
IEC 60601-2-21:2009	ANSI/AAMI/IEC 60601-2-21:2009	Identical
IEC 60601-2-24:1998	ANSI/AAMI ID26:2004/(R)2009	Major technical variations
IEC 60601-2-47:2001	ANSI/AAMI EC38:2007	Major technical variations
IEC 60601-2-50:2009	ANSI/AAMI/IEC 60601-2-50:2009	Identical
IEC 80001-1:20xx ¹	ANSI/AAMI/IEC 80001-1:2010	Identical
IEC 80601-2-30:2009 and Technical Corrigendum 1	ANSI/AAMI/IEC 80601-2-30:2009 and ANSI/AAMI/IEC 80601-2-30:2009/ C1:2009 (amdt) – consolidated text	Identical (with inclusion) C1 Identical to Corrigendum 1
IEC 80601-2-58:2008	ANSI/AAMI/IEC 80601-2-58:2008	Identical
IEC/TR 60878:2009	ANSI/AAMI/IEC TIR60878:2003	Identical
IEC/TR 62296:2009	ANSI/AAMI/IEC TIR62296:2009	Identical
IEC 62304:2006	ANSI/AAMI/IEC 62304:2006	Identical
IEC/TR 62348:2006	ANSI/AAMI/IEC TIR62348:2006	Identical
IEC/TR 62354:2009	ANSI/AAMI/IEC TIR62354:2009	Identical
IEC/TR 80002-1:2009	ANSI/IEC/TR 80002-1:2009	Identical
ISO 5840:2005	ANSI/AAMI/ISO 5840:2005/(R)2010	Identical
ISO 7198:1998	ANSI/AAMI/ISO 7198:1998/2001/(R)2010	Identical
ISO 7199:2009	ANSI/AAMI/ISO 7199:2009	Identical
ISO 8637:2010	ANSI/AAMI/ISO 8637:2010	Identical
ISO 8638:2010	ANSI/AAMI/ISO 8638:2010	Identical
ISO 10993-1:2009	ANSI/AAMI/ISO 10993-1:2009	Identical
ISO 10993-2:2006	ANSI/AAMI/ISO 10993-2:2006	Identical
ISO 10993-3:2003	ANSI/AAMI/ISO 10993-3:2003/(R)2009	Identical
ISO 10993-4:2002 and Amendment 1:2006	ANSI/AAMI/ISO 10993-4:2002/(R)2009 and Amendment 1:2006/(R)2009	Identical
ISO 10993-5:2009	ANSI/AAMI/ISO 10993-5:2009	Identical
ISO 10993-6:2007	ANSI/AAMI/ISO 10993-6:2007	Identical
ISO 10993-7:2008	ANSI/AAMI/ISO 10993-7:2008	Identical
ISO 10993-9:2009	ANSI/AAMI/ISO 10993-9:2009	Identical
ISO 10993-10:2010	ANSI/AAMI/ISO 10993-10:2010	Identical
ISO 10993-11:2006	ANSI/AAMI/ISO 10993-11:2006	Identical
ISO 10993-12:2007	ANSI/AAMI/ISO 10993-12:2007	Identical
ISO 10993-13:2010	ANSI/AAMI/ISO 10993-13:20xx ²	Identical
ISO 10993-14:2001	ANSI/AAMI/ISO 10993-14:2001/(R)2006	Identical
ISO 10993-15:2000	ANSI/AAMI/ISO 10993-15:2000/(R)2006	Identical
ISO 10993-16:2010	ANSI/AAMI/ISO 10993-16:2010	Identical
ISO 10993-17:2002	ANSI/AAMI/ISO 10993-17:2002/(R)2008	Identical
ISO 10993-18:2005	ANSI/AAMI BE83:2006	Major technical variations

¹ In production as of 13 October 2010

² Final approval pending as of 13 October 2010

International designation	U.S. designation	Equivalency
ISO/TS 10993-19:2006	ANSI/AAMI/ISO TIR10993-19:2006	Identical
ISO/TS 10993-20:2006	ANSI/AAMI/ISO TIR10993-20:2006	Identical
ISO 11135-1:2007	ANSI/AAMI/ISO 11135-1:2007	Identical
ISO/TS 11135-2:2008	ANSI/AAMI/ISO TIR11135-2:2008	Identical
ISO 11137-1:2006	ANSI/AAMI/ISO 11137-1:2006/(R)2010	Identical
ISO 11137-2:2006 (2006-08-01 corrected version)	ANSI/AAMI/ISO 11137-2:2006	Identical
ISO 11137-3:2006	ANSI/AAMI/ISO 11137-3:2006/(R)2010	Identical
ISO 11138-1: 2006	ANSI/AAMI/ISO 11138-1:2006/(R)2010	Identical
ISO 11138-2: 2006	ANSI/AAMI/ISO 11138-2:2006/(R)2010	Identical
ISO 11138-3: 2006	ANSI/AAMI/ISO 11138-3:2006/(R)2010	Identical
ISO 11138-4: 2006	ANSI/AAMI/ISO 11138-4:2006/(R)2010	Identical
ISO 11138-5: 2006	ANSI/AAMI/ISO 11138-5:2006/(R)2010	Identical
ISO/TS 11139:2006	ANSI/AAMI/ISO 11139:2006	Identical
ISO 11140-1:2005	ANSI/AAMI/ISO 11140-1:2005/(R)2010	Identical
ISO 11140-3:2007	ANSI/AAMI/ISO 11140-3:2007	Identical
ISO 11140-4:2007	ANSI/AAMI/ISO 11140-4:2007	Identical
ISO 11140-5:2007	ANSI/AAMI/ISO 11140-5:2007	Identical
ISO 11607-1:2006	ANSI/AAMI/ISO 11607-1:2006	Identical
ISO 11607-2:2006	ANSI/AAMI/ISO 11607-2:2006	Identical
ISO 11737-1: 2006	ANSI/AAMI/ISO 11737-1:2006	Identical
ISO 11737-2:2009	ANSI/AAMI/ISO 11737-2:2009	Identical
ISO 13408-1:2008	ANSI/AAMI/ISO 13408-1:2008	Identical
ISO 13408-2:2003	ANSI/AAMI/ISO 13408-2:2003	Identical
ISO 13408-3:2006	ANSI/AAMI/ISO 13408-3:2006	Identical
ISO 13408-4:2005	ANSI/AAMI/ISO 13408-4:2005	Identical
ISO 13408-5:2006	ANSI/AAMI/ISO 13408-5:2006	Identical
ISO 13408-6:2006	ANSI/AAMI/ISO 13408-6:2006	Identical
ISO 13485:2003	ANSI/AAMI/ISO 13485:2003/(R)2009	Identical
ISO 14155-1:2003	ANSI/AAMI/ISO 14155-1:2003/(R)2008	Identical
ISO 14155-2:2003	ANSI/AAMI/ISO 14155-2:2003/(R)2008	Identical
ISO 14160:1998	ANSI/AAMI/ISO 14160:1998/(R)2008	Identical
ISO 14161:2009	ANSI/AAMI/ISO 14161:2009	Identical
ISO 14708-3:2008	ANSI/AAMI/ISO 14708-3:2008	Identical
ISO 14708-4:2008	ANSI/AAMI/ISO 14708-4:2008	Identical
ISO 14708-5:2010	ANSI/AAMI /ISO 14708-5:2010	Identical
ISO 14937:2009	ANSI/AAMI/ISO 14937:2009	Identical
ISO/TR 14969:2004	ANSI/AAMI/ISO TIR14969:2004	Identical
ISO 14971:2007	ANSI/AAMI/ISO 14971:2007/(R)2010	Identical
ISO 15223-1:2007 and A1:2008	ANSI/AAMI/ISO 15223-1:2007 and A1:2008	Identical
ISO 15223-2:2010	ANSI/AAMI/ISO 15223-2:2010	Identical
ISO 15225:2010	ANSI/AAMI/ISO 15225:2010	Identical
ISO 15674:2009	ANSI/AAMI/ISO 15674:2009	Identical
ISO 15675:2009	ANSI/AAMI/ISO 15675:2009	Identical
ISO 15882:2008	ANSI/AAMI/ISO 15882:2008	Identical
ISO 15883-1:2006	ANSI/AAMI ST15883-1:2009	Major technical variations
ISO/TR 16142:2006	ANSI/AAMI/ISO TIR16142:2005	Identical
ISO 17664:2004	ANSI/AAMI ST81:2004	Major technical variations
ISO 17665-1:2006	ANSI/AAMI/ISO 17665-1:2006	Identical (with inclusions)
ISO/TS 17665-2:2009	ANSI/AAMI/ISO TIR17665-2:2009	Identical
ISO 18472:2006	ANSI/AAMI/ISO 18472:2006	Identical
ISO/TS 19218:2005	ANSI/AAMI/ISO 19218:2005	Identical
ISO 22442-1:2007	ANSI/AAMI/ISO 22442-1:2007	Identical
ISO 22442-2:2007	ANSI/AAMI/ISO 22442-2:2007	Identical
ISO 22442-3:2007	ANSI/AAMI/ISO 22442-3:2007	Identical
ISO 25539-1:2003 and A1:2005	ANSI/AAMI/ISO 25539-1:2003/(R)2009 and A1:2005/(R)2009	Identical
ISO 25539-2:2008	ANSI/AAMI/ISO 25539-2:2008	Identical
ISO 27186:2010	ANSI/AAMI/ISO 27186:2010	Identical
ISO 81060-1:2007	ANSI/AAMI/ISO 81060-1:2007	Identical
ISO 81060-2:2009	ANSI/AAMI/ISO 81060-2:2009	Identical



Committee representation

Association for the Advancement of Medical Instrumentation

Biological Evaluation of Medical Devices Committee

The adoption of ISO 10993-10:2010 as an American National Standard was initiated by the AAMI Biological Evaluation of Medical Devices Committee, which also functions as a U.S. Technical Advisory Group to the relevant work in the International Organization for Standardization (ISO). U.S. representatives from the AAMI Irritation and Sensitization Working Group (U.S. Sub-TAG for ISO/TC 194/WG 08), played an active part in developing the ISO standard.

At the time this document was published, the **AAMI Biological Evaluation of Medical Devices Committee** had the following members:

- Cochairs* Ronald P. Brown, FDA/CDRH
Jon Cammack, PhD, DABT, ISIS Services
- Members* James M. Anderson, MD, PhD, Case Western Reserve University (Independent Expert)
Ronald P. Brown, FDA/CDRH
Jon Cammack, PhD, DABT, ISIS Services
Joseph Carraway, DVM, NAMSA
Richard W. Hutchinson, DVM, PhD, DABT, Johnson & Johnson
Anita Y. Sawyer, Becton Dickinson & Company
Michael F. Wolf, Medtronic Inc.
- Alternates* Jesus W. Casas, MD, MSc, Medtronic Inc.

At the time this document was published, the **AAMI Irritation and Sensitization Working Group** had the following members:

- Cochairs* Gloria H. Frost, PhD, Cardinal Health (MP&S)
Niranjan Goud, PhD, Boston Scientific Corporation
- Members* Carolyn Braithwaite, CaridianBCT Sterilization Services Inc.
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Gloria H. Frost, PhD, Cardinal Health (MP&S)
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NOTE—Participation by federal agency representatives in the development of this standard does not constitute endorsement by the federal government or any of its agencies.



Background of ANSI/AAMI adoption of ISO 10993-10:2010

As indicated in the foreword to the main body of this document (page ix), the International Organization for Standardization (ISO) is a worldwide federation of national standards bodies. The United States is one of the ISO members that took an active role in the development of this standard.

ISO 10993-10:2010 was developed by Working Group (WG) 08, Irritation and Sensitization Working Group, of ISO Technical Committee (TC) 194, *Biological evaluation of medical devices*, to assess possible contact hazards from chemicals released from medical devices, which may produce skin and mucosal irritation, eye irritation or skin sensitization.

U.S. participation in this ISO/TC 194/WG 08 is organized through the U.S. Technical Advisory Group for ISO/TC 194, administered by the Association for the Advancement of Medical Instrumentation (AAMI) on behalf of the American National Standards Institute (ANSI). U.S. experts made a considerable contribution to this standard.

AAMI encourages its committees to harmonize their work with international standards as much as possible. Upon review of ISO 10993-10, the AAMI Biological Evaluation of Medical Devices Committee and the AAMI Irritation and Sensitization Working Group decided to adopt 10993-10, verbatim, as a revision of ANSI/AAMI BE78:2002/(R)2008, *Biological evaluation of medical devices - Part 10: Tests for irritation and delayed-type hypersensitivity*, and its 2006 amendment.

This edition of ISO 10993-10 has been revised from the previous edition to include additional methods for testing irritation or sensitization that have been developed in recent years, such as a section on Murine Local Lymph Node Assay as another method for testing chemicals for skin sensitization. The annexes (both normative and informative) have also been improved and additional special test methods and extraction protocols are now included.

AAMI and ANSI procedures require that standards be reviewed and, if necessary, revised every five years to reflect technological advances that may have occurred since publication.

AAMI (and ANSI) have adopted other ISO documents. See the Glossary of Equivalent Standards for a list of ISO standards adopted by AAMI, which gives the corresponding U.S. designation and the level of equivalency with the ISO standard.

As used within the context of this document, “shall” indicates requirements strictly to be followed to conform to the recommended practice. “Should” indicates that among several possibilities, one is recommended as particularly suitable, without mentioning or excluding others, or that a certain course of action is preferred but not necessarily required, or that (in the negative form) a certain possibility or course of action should be avoided but is not prohibited. “May” is used to indicate that a course of action is permissible within the limits of the recommended practice. “Can” is used as a statement of possibility and capability. Finally, “must” is used only to describe “unavoidable” situations, including those mandated by government regulation.

The concepts incorporated into this standard should not be considered inflexible or static. This standard, like any other, must be reviewed and updated periodically to assimilate progressive technological developments. To remain relevant, it must be modified as technological advances are made and as new data comes to light.

Suggestions for improving this standard are invited. Comments and suggested revisions should be sent to Standards Department, AAMI, 4301 N. Fairfax Dr. Suite 301, Arlington, VA 22203-1633.

NOTE—Beginning with the ISO foreword on page ix, this American National Standard is identical to ISO 10993-10:2010.

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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 10993-10 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This third edition cancels and replaces the second edition (ISO 10993-10:2002), which has been technically revised.

ISO 10993 consists of the following parts, under the general title *Biological evaluation of medical devices*:

- *Part 1: Evaluation and testing within a risk management process*
- *Part 2: Animal welfare requirements*
- *Part 3: Tests for genotoxicity, carcinogenicity and reproductive toxicity*
- *Part 4: Selection of tests for interactions with blood*
- *Part 5: Tests for in vitro cytotoxicity*
- *Part 6: Tests for local effects after implantation*
- *Part 7: Ethylene oxide sterilization residuals*
- *Part 9: Framework for identification and quantification of potential degradation products*
- *Part 10: Tests for irritation and skin sensitization*
- *Part 11: Tests for systemic toxicity*
- *Part 12: Sample preparation and reference materials*

- *Part 13: Identification and quantification of degradation products from polymeric medical devices*
- *Part 14: Identification and quantification of degradation products from ceramics*
- *Part 15: Identification and quantification of degradation products from metals and alloys*
- *Part 16: Toxicokinetic study design for degradation products and leachables*
- *Part 17: Establishment of allowable limits for leachable substances*
- *Part 18: Chemical characterization of materials*
- *Part 19: Physico-chemical, morphological and topographical characterization of materials* [Technical Specification]
- *Part 20: Principles and methods for immunotoxicology testing of medical devices* [Technical Specification]

Introduction

This part of ISO 10993 assesses possible contact hazards from chemicals released from medical devices, which may produce skin and mucosal irritation, eye irritation or skin sensitization.

Some materials that are included in medical devices have been tested, and their skin or mucosal irritation or sensitization potential has been documented. 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. More recently, *in vitro* tests as well as human tests have been added as adjuncts or alternatives. Despite progress and considerable effort in this direction, a review of findings suggests that currently no satisfactory *in vitro* test has been devised to eliminate the requirement for *in vivo* testing. 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 part of ISO 10993 presents a step-wise approach, with review and analysis of test results at each stage. An animal test is usually required prior to human testing.

It is intended that these studies be conducted using Good Laboratory Practice and comply with regulations related to animal welfare. Statistical analysis of data is recommended and should be used whenever appropriate.

This part of ISO 10993 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 part of ISO 10993 are important tools for the development of safe products, provided that these are executed and interpreted by trained personnel.

This part of ISO 10993 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 irritation and dermal sensitization responses relevant to safety of medical materials and devices.

Biological evaluation of medical devices — Part 10: Tests for irritation and skin sensitization

1 Scope

This part of ISO 10993 describes the procedure for the assessment of medical devices and their constituent materials with regard to their potential to produce irritation and skin sensitization.

This part of ISO 10993 includes:

- a) pretest considerations for irritation, including *in silico* and *in vitro* methods for dermal exposure;
- b) details of *in vivo* (irritation and sensitization) test procedures;
- c) 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 B several special irritation tests are described for application of medical devices in areas other than skin.

2 Normative references

The following referenced documents are indispensable for the application 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 10993-1:2009, *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 14155-1, *Clinical investigation of medical devices for human subjects — Part 1: General requirements*

ISO 14155-2, *Clinical investigation of medical devices for human subjects — Part 2: Clinical investigation plans*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 10993-1 and the following apply.

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 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

dose

dosage

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

NOTE The terms are often used interchangeably (more commonly dosage).

3.5

erythema

reddening of the skin or mucous membrane

3.6

eschar

scab or discolored slough of skin

3.7

extract

liquid or suspension that results from exposing a test or control material to a solvent under controlled conditions

3.8

induction

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

3.9

irritant

agent that produces irritation

3.10

irritation

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

NOTE Skin irritation is a reversible reaction and is mainly characterized by local erythema (redness) of the skin.

3.11

necrosis

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

NOTE One should be aware that tissue repair will occur either resulting in complete functional restoration or resulting in scar formation.

3.12

negative control

any 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 In practice, negative controls include blanks, vehicles/solvents and reference materials.

3.13

oedema

swelling due to abnormal infiltration of fluid into the tissues

3.14

positive control

any 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

3.15

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 3.19).

3.16

skin sensitization

allergic contact dermatitis

immunologically mediated cutaneous reaction to a substance

NOTE 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.

3.17

test material

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

3.18

test sample

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

3.19

ulceration

open sore representing loss of superficial tissue

3.20

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 irritation and sensitization were developed specifically to detect skin and mucous membrane irritation and skin sensitization potential. Other types of adverse effect 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 and so for detection of irritation activity, intracutaneous testing shall be used as described in 6.4.

This part of ISO 10993 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 and ISO 10993-18;
- b) literature review, including an evaluation of chemical and physical properties, and information on the irritation and 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. For the evaluation of skin irritation and corrosion, *in vitro* alternatives are available for chemicals; there are currently no internationally validated and accepted *in vitro* tests to detect sensitizers;
- d) *in vivo* animal tests: in order to ensure reproducibility and sensitivity, a test of a positive-control substance for irritation and skin sensitization shall be included in each assay by the testing laboratory in order to validate the test system and demonstrate a positive response; for guinea pig sensitization assays, however, when consistency has been demonstrated over a six month or more extended period, a positive control does not need to be included in every assay, but may be run at regular intervals which shall not exceed six months.

NOTE 1 Sensitization 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). For single chemicals the LLNA is now the preferred assay for determining the sensitizing potential. See References [69] [88] [90].

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).

NOTE 3 For sensitization assays in guinea pigs, ten animals are normally used for positive control once every six months. Fewer guinea pigs can be used when an assay with a positive control substance is performed more frequently than once every six months. At least five test animals with a positive substance and five control animals should be used.

e) Non-invasive human tests/clinical trials; if the material has been demonstrated not to be an irritant, a sensitizer or toxic in animals, studies on skin irritation may then be considered in humans.

Clinical studies in accordance with ISO 14155-1, ISO 14155-2 and to ethics principles shall not be performed before the results of the other evaluations in a) to d) are known.

5 Pretest considerations

5.1 General

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

The requirements given in Clause 5 of ISO 10993-1:2009 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 leakage or degradation characteristics of the finished products. These components shall be taken into account for their potential irritation/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 may be present and the completeness of polymerization may 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 part of ISO 10993 have not been designed for testing of biologically derived materials and can therefore be less adequate. For example, the tests in this part of ISO 10993 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 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 Irritation tests

6.1 *In vitro* irritation tests

In vitro methods, the rat skin Transcutaneous Electrical Resistance (TER) test and the Human skin model test, have been internationally validated and accepted as alternative tests to assess the skin corrosivity of chemicals (OECD Guidelines 430^[9] and 431^[10]). National and international organizations continue working to develop and validate *in vitro* tests for skin irritancy in parallel with the search for alternative methods; others have been developing methods to quantify the responses of animals and humans in order to better define endpoints using non-invasive techniques (see F.1).

NOTE In 2007 the ECVAM Scientific Advisory Committee (ESAC) evaluated the validation process of an *in vitro* human skin model for the determination of skin irritation of chemicals. See Reference [101]. The use of *in vitro* human skin models for assessing the potential of chemicals to induce skin irritation is described in Annex D.

The *in vitro* test for skin irritation has so far been validated only for neat chemicals and not for medical device extracts. In order to apply these assays for the testing of irritation potential of medical devices, further validation for this specific area is essential.

6.2 *In vivo* irritation tests — Factors to be considered in design and selection of *in vivo* tests

Irritation testing of medical devices can be performed with the finished product and/or extracts thereof.

Factors affecting the results of irritation studies include the following:

- a) the nature of the device used in a patch test;
- b) the dose of the test material;
- c) the method of application of the test material;
- d) the degree of occlusion;
- e) the application site;
- f) the duration and number of exposures;
- g) the techniques used in evaluating the test.

Additional background information is provided in Annex F.

Whilst flexibility with respect to the precise protocol followed allows the investigator to enhance the sensitivity of the test to suit conditions of use and population exposure, consistency in procedure contributes to comparability of test results with different materials and from different laboratories.

Provisions have been included in the test procedures for evaluation of devices and materials that will have repeated and/or prolonged exposure. The study shall be designed to exaggerate the anticipated contact (time and/or concentration) in the clinical situation. This shall be borne in mind during interpretation of the result.

If the pH of the test sample is ≤ 2.0 or ≥ 11.5 , the material shall be considered an irritant and no further testing is required. However, experimental evidence suggests that acidity and alkalinity of the test

material are not the only factors to be considered in relation to the capacity of a material to produce severe injury. The concentration of the test material, its period of contact, and many other physical and chemical properties are also important.

In exceptional cases where further risk characterization/assessment is needed, it might be necessary to test materials which are either an irritant or have a pH outside the range mentioned above. These cases shall be justified and documented.

6.3 Animal irritation test

6.3.1 Principle

An assessment is made of the potential of the material under test to produce dermal irritation in a relevant animal model.

The rabbit is the preferred test animal.

6.3.2 Test material

If the test material is a solid or a liquid, it shall be prepared as specified in Annex A.

The sensitivity of the assay shall be demonstrated. This can be done by including a positive control in the assay. However, the use of a positive control to confirm sensitivity is only warranted when the testing laboratory has not within the previous six months produced positive results using the test method.

NOTE A suitable positive control is sodium lauryl sulphate (SLS).

6.3.3 Animals and husbandry

Three healthy young adult albino rabbits of either sex from a single strain, weighing not less than 2 kg, shall be used. If irritation is anticipated, consideration shall be given to testing in one animal first. Unless a well-defined positive response [score greater than 2 for either erythema or oedema (see Table 1)] is observed, a minimum of two additional animals shall be used. If the response in the test using the minimum of three animals is equivocal, further testing shall be considered.

The animals shall be acclimatized and cared for as specified in ISO 10993-2.

Table 1 — Scoring system for skin reaction

Reaction	Irritation score
Erythema and eschar formation	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate erythema	3
Severe erythema (beet-redness) to eschar formation preventing grading of erythema	4
Oedema formation	
No oedema	0
Very slight oedema (barely perceptible)	1
Well-defined oedema (edges of area well-defined by definite raising)	2
Moderate oedema (raised approximately 1 mm)	3
Severe oedema (raised more than 1 mm and extending beyond exposure area)	4
Maximal possible score for irritation	8
Other adverse changes at the skin sites shall be recorded and reported.	

6.3.4 Test procedure

6.3.4.1 Preparation of animals

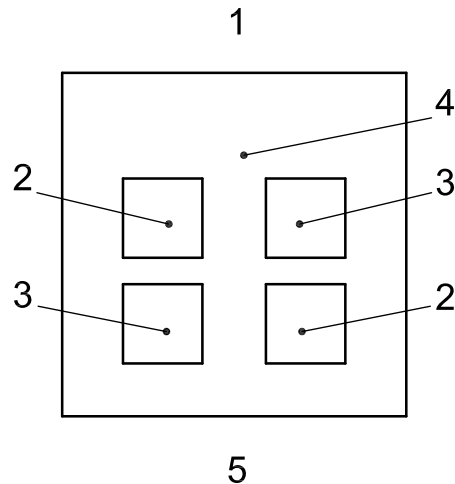
The condition of the skin is a critical factor. Use only animals with healthy intact skin. Fur is generally clipped within 24 h to 4 h of testing on the backs of the animals, a sufficient distance on both sides of the spine for application and observation of all test sites (approximately 10 cm × 15 cm). Fur may be re-clipped to facilitate observation and/or to accommodate repeated exposures. Depilatories may be used by trained technicians, if the process has been validated at the testing facility. If repeated exposure is required, follow the procedures in 6.3.4.2.1, 6.3.4.2.2 or 6.3.4.2.3, repeated for a maximum of 21 d.

6.3.4.2 Application of test sample

6.3.4.2.1 Application of powder or liquid sample

Apply 0.5 g or 0.5 ml of the test material directly to each test skin site as shown in Figure 1. For solid and hydrophobic materials, there is no need for moistening. If the material is a powder, it should be slightly moistened with water or other suitable vehicle before application (see Annex A).

Cover the application sites with a 2.5 cm × 2.5 cm non-occlusive dressing (such as an absorbent gauze patch) and then wrap the application site with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites with permanent ink. Remove residual test material by appropriate means, such as washing with lukewarm water or other suitable non-irritating solvent, and careful drying.



Key

- 1 cranial end
- 2 test site
- 3 control site
- 4 clipped dorsal region
- 5 caudal end

Figure 1 — Location of skin application sites

6.3.4.2.2 Application of extracts and extract vehicle

Apply the appropriate extract(s) to the 2.5 cm × 2.5 cm absorbent gauze patches. Use a volume of extract sufficient to saturate the gauze, generally 0.5 ml per patch. Apply one patch on each side of the animal as shown in Figure 1. Apply a control patch of gauze moistened with the extract vehicle as shown in Figure 1.

Cover the application sites with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites with permanent ink. Remove residual test material by appropriate means, such as washing with lukewarm water or other suitable non-irritating solvent and careful drying.

6.3.4.2.3 Application of solid sample

Apply the samples of the test material directly to the skin on each side of each rabbit as shown in Figure 1. Similarly, apply the control samples to each rabbit. When testing solids (which may be pulverized if considered necessary), the test material shall be moistened sufficiently with water or, where necessary, an alternative solvent, to ensure good contact with the skin (see Annex A). When solvents are used, the influence of the solvent on irritation of skin caused by the test material shall be taken into account.

Cover the application sites with 2.5 cm × 2.5 cm non-occlusive dressings (such as a gauze patch) and then wrap the application sites with a bandage (semi-occlusive or occlusive) for a minimum of 4 h. At the end of the contact time, remove the dressings and mark the positions of the sites with permanent ink. Remove residual test material by appropriate means, such as washing with lukewarm water or other suitable non-irritating solvent and careful drying.

6.3.5 Observation of animals

6.3.5.1 General

Use of natural or full-spectrum lighting is highly recommended to visualize the skin reactions. Describe and score the skin reactions for erythema and oedema according to the scoring system given in Table 1, for each application site at each time interval, and record the results for the test report.

NOTE Histological or non-invasive techniques of evaluating the skin reaction(s) can assist in certain cases.

6.3.5.2 Single-exposure test

For single-exposure tests, record the appearance of each application site at (1 ± 0.1) h, (24 ± 2) h, (48 ± 2) h and (72 ± 2) h following removal of the patches. Extended observation can be necessary if there are persistent lesions, in order to evaluate the reversibility or irreversibility of the lesions over a period of time not exceeding 14 d.

6.3.5.3 Repeated-exposure test

Repeated exposure shall only be carried out after completion of an acute single-exposure test [after at least (72 ± 2) h of observation].

For repeated-exposure tests, record the appearances of the application site at (1 ± 0.1) h after removal of the patches and immediately prior to the next application. The number of exposures can vary.

After the last exposure, note the appearance of each application site at (1 ± 0.1) h, (24 ± 2) h, (48 ± 2) h and (72 ± 2) h following removal of the patches. Extended observation can be necessary if there are persistent lesions, in order to evaluate the reversibility or irreversibility of the lesions. This need not exceed a period of 14 d.

6.3.6 Evaluation of results

For single exposure tests, determine the primary irritation index (PII) as follows.

Use only (24 ± 2) h, (48 ± 2) h and (72 ± 2) h observations for calculations. Observations made prior to dosing or after 72 h to monitor recovery are not used in the determination.

After the 72 h grading, all erythema grades plus oedema grades (24 ± 2) h, (48 ± 2) h and (72 ± 2) h are totaled separately for each test sample and blank for each animal. The primary irritation score for an animal is calculated by dividing the sum of all the scores by 6 (two test/observation sites, three time points).

To obtain the primary irritation index for the test sample add all the primary irritation scores of the individual animals and divide by the number of animals (generally three).

When blank or negative control is used, calculate the primary irritation score for the controls and subtract that score from the score using the test material to obtain the primary irritation score.

For repeated exposure assays the primary irritation score for each animal shall be calculated according to the principle mentioned above, taking into consideration all evaluation points. For repeated exposure, determine the cumulative irritation index as follows.

Add together the irritation scores of all animals and divide by the total number of animals. This value is the cumulative irritation index.

The cumulative irritation index is compared with the categories of irritation response given in Table 2 and the appropriate response category is recorded for the report.

NOTE The categories of cumulative irritation index are based on the data relating the primary irritation index for chemicals in rabbits to the primary irritation response in humans for a number of chemicals that have been tested on both species.

For any response, record the maximum primary irritation score given in Table 1 for each animal, the time of onset of the response and the time to maximum response.

The primary or cumulative irritation index is characterized by number (score) and description (response category) given in Table 2. In case different extracts have been tested, the one giving the highest PII determines the response category.

Table 2 — Primary or cumulative irritation index categories in a rabbit

Mean score	Response category
0 to 0.4	Negligible
0.5 to 1.9	Slight
2 to 4.9	Moderate
5 to 8	Severe

6.3.7 Test report

The test report shall include:

- a description of the test material(s) or device;
- the intended use/application of the test material(s) or device;
- a detailed description of the method employed in preparing the test sample or test material;
- a description of the test animals;
- the method of application to the test sites and type (semi-occlusive or occlusive) of bandage material;
- how the sites were marked, and the readings performed;
- records of the observations;
- number of exposures and intervals between them, when repeated exposures were carried out;
- evaluation of the results.

6.4 Animal intracutaneous (intradermal) reactivity test

6.4.1 Introduction

For medical devices that are used as an implant, the use of an intracutaneous (intradermal) reactivity test is indicated. An assessment is made of the potential of the material under test to produce irritation following intradermal injection of extracts of the material.

6.4.2 Exclusion from test

Any material shown to be a skin, eye or mucosal tissue irritant or material with a pH ≤ 2.0 or ≥ 11.5 shall not be tested intradermally. In exceptional cases where further risk characterization/assessment is needed, it might be necessary to test materials which are either an irritant or have a pH outside the range mentioned above. These cases shall be justified and documented.

6.4.3 Test sample

The test sample shall be an extract prepared in accordance with Annex A. As there are multiple test sites on each animal, several test samples might be applied together with the appropriate negative controls or blank.

6.4.4 Animals and husbandry

Healthy young adult albino rabbits of either sex from a single strain, weighing not less than 2 kg, shall be used. The animals shall be acclimatized and cared for as specified in ISO 10993-2. A minimum of three animals shall initially be used to evaluate the test material. If irritation is anticipated, consideration shall be given to testing in one animal first. Unless a well-defined positive response [score greater than 2 for either erythema or oedema (see Table 1)] is observed, a minimum of two additional animals shall be used. If the response in the test using the minimum of three animals is equivocal, further testing shall be considered.

6.4.5 Test procedure

Within a 4 h to 18 h period before testing, closely clip the fur on the backs of the animals, allowing a sufficient distance on both sides of the spine for injection of the extracts.

Inject intracutaneously 0.2 ml of the extract obtained with polar or non-polar solvent at five sites on one side of each rabbit. Use the smallest needle appropriate to the viscosity of the test material for the intradermal injections.

An example of the arrangements of the injection sites is presented in Figure 2.

Similarly, inject 0.2 ml of the polar or non-polar solvent control on five sites of the contralateral side of each rabbit (for example, see Figure 2).

If other solvents are used, repeat the above steps for the extract obtained with the other solvents and the solvent controls.