

Biological evaluation of medical devices

Part 5: Tests for in vitro cytotoxicity (ISO 10993-5:2009)

ICS 11.100.20

National foreword

This British Standard is the UK implementation of EN ISO 10993-5:2009. It supersedes BS EN ISO 10993-5:1999 which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee CH/194, Biological evaluation of medical devices.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Compliance with a British Standard cannot confer immunity from legal obligations.

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This European Standard was approved by CEN on 17 April 2009.

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Foreword

This document (EN ISO 10993-5:2009) has been prepared by Technical Committee ISO/TC 194 "Biological evaluation of medical devices" in collaboration with Technical Committee CEN/TC 206 "Biological evaluation of medical devices" the secretariat of which is held by NEN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by December 2009, and conflicting national standards shall be withdrawn at the latest by December 2009.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN [and/or CENELEC] shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN ISO 10993-5:1999.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EC Directives.

For relationship with EC Directives, see informative Annex ZA and ZB, which are integral parts of this document.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

Endorsement notice

The text of ISO 10993-5:2009 has been approved by CEN as EN ISO 10993-5:2009 without any modifications.

Annex ZA (informative)

Relationship between this International Standard and the Essential Requirements of EU Directive 93/42/EEC

This International Standard has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association to provide a means of conforming to Essential Requirements of the New Approach Directive 93/42/EEC on medical devices.

Once this standard is cited in the Official Journal of the European Communities under that Directive and has been implemented as a national standard in at least one Member State, compliance with the clauses of this standard given in Table ZA.1 confers, within the limits of the scope of this standard, a presumption of conformity with the corresponding Essential Requirements of that Directive and associated EFTA regulations.

Table ZA.1— Correspondence between this International Standard and Directive 93/42/EEC

Clause(s)/sub-clause(s) of this International Standard	Essential Requirements (ERs) of Directive 93/42/EEC	Qualifying remarks/Notes
4, 5, 6, 7, 8, 9, 10	Annex I: 7.1, 7.2, 7.5	

WARNING: Other requirements and other EU Directives may be applicable to the product(s) falling within the scope of this International standard.

Annex ZB (informative)

Relationship between this International Standard and the Essential Requirements of EU Directive 90/385/EEC

This International Standard has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association to provide a means of conforming to Essential Requirements of the New Approach Directive 90/385/EEC on active implantable medical devices.

Once this standard is cited in the Official Journal of the European Communities under that Directive and has been implemented as a national standard in at least one Member State, compliance with the clauses of this standard given in Table ZB.1 confers, within the limits of the scope of this standard, a presumption of conformity with the corresponding Essential Requirements of that Directive and associated EFTA regulations.

Table ZB.1 — Correspondence between this International Standard and Directive 90/385/EEC

Clause(s)/sub-clause(s) of this International Standard	Essential Requirements (ERs) of Directive 90/385/EEC	Qualifying remarks/Notes
4, 5, 6, 7, 8, 9, 10	Annex I: 9	

WARNING — Other requirements and other EU Directives may be applicable to the product(s) falling within the scope of this standard.

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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 10993-5 was prepared by Technical Committee ISO/TC 194, *Biological evaluation of medical devices*.

This third edition cancels and replaces the second edition (ISO 10993-5:1999) 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

Due to the general applicability of *in vitro* cytotoxicity tests and their widespread use in evaluating a large range of devices and materials, it is the purpose of this part of ISO 10993, rather than to specify a single test, to define a scheme for testing which requires decisions to be made in a series of steps. This should lead to the selection of the most appropriate test.

Three categories of test are listed: extract test, direct contact test, indirect contact test.

The choice of one or more of these categories depends upon the nature of the sample to be evaluated, the potential site of use and the nature of the use.

This choice then determines the details of the preparation of the samples to be tested, the preparation of the cultured cells, and the way in which the cells are exposed to the samples or their extracts.

At the end of the exposure time, the evaluation of the presence and extent of the cytotoxic effect is undertaken. It is the intention of this part of ISO 10993 to leave open the choice of type of evaluation. Such a strategy makes available a battery of tests, which reflects the approach of many groups that advocate *in vitro* biological tests.

The numerous methods used and endpoints measured in cytotoxicity determination can be grouped into the following categories of evaluation:

- assessments of cell damage by morphological means;
- measurements of cell damage;
- measurements of cell growth;
- measurements of specific aspects of cellular metabolism.

There are several means of producing results in each of these four categories. The investigator should be aware of the test categories and into which category a particular technique fits, in order that comparisons be able to be made with other results on similar devices or materials both at the intra- and interlaboratory level. Examples of quantitative test protocols are given in annexes. Guidance for the interpretation of the results is given in this part of ISO 10993.

Biological evaluation of medical devices —

Part 5: Tests for *in vitro* cytotoxicity

1 Scope

This part of ISO 10993 describes test methods to assess the *in vitro* cytotoxicity of medical devices.

These methods specify the incubation of cultured cells in contact with a device and/or extracts of a device either directly or through diffusion.

These methods are designed to determine the biological response of mammalian cells *in vitro* using appropriate biological parameters.

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, *Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management system*

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 terms and definitions given in ISO 10993-1 and the following apply.

3.1

culture vessels

vessels appropriate for cell culture including glass petri dishes, plastic culture flasks or plastic multiwells and microtitre plates

NOTE These can be used interchangeably in these methods provided that they meet the requirements of tissue culture grade and are suitable for use with mammalian cells.

3.2

positive control material

material which, when tested in accordance with this part of ISO 10993, provides a reproducible cytotoxic response