BS EN ISO 10993-5:2009+A11:2025

BSI Standards Publication

Biological evaluation of medical devices

Part 5: Tests for in vitro cytotoxicity

Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

National foreword

This British Standard is the UK implementation of EN ISO 10993-5:2009+A11:2025. It is derived from ISO 10993-5:2009. It supersedes BS EN ISO 10993-5:2009, which is withdrawn.

The start and finish of text introduced or altered by amendment A11 is indicated in the text by tags. Tags indicating changes to CEN text carry the number of the CEN amendment. For example, text altered by CEN amendment A11 is indicated by ⟨A11⟩ ⟨A11⟩.

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 committee manager.

Contractual and legal considerations

This publication has been prepared in good faith, however no representation, warranty, assurance or undertaking (express or implied) is or will be made, and no responsibility or liability is or will be accepted by BSI in relation to the adequacy, accuracy, completeness or reasonableness of this publication. All and any such responsibility and liability is expressly disclaimed to the full extent permitted by the law.

This publication is provided as is, and is to be used at the recipient's own risk.

The recipient is advised to consider seeking professional guidance with respect to its use of this publication.

This publication is not intended to constitute a contract. Users are responsible for its correct application.

This publication has been prepared under a mandate given to the European Standards Organizations by the European Commission and the European Free Trade Association. It is intended to support requirements of the EU legislation detailed in the European Foreword. A European Annex, usually Annex ZA or ZZ, describes how this publication relates to that EU legislation.

For the Great Britain market (England, Scotland and Wales), if UK Government has designated this publication for conformity with UKCA marking (or similar) legislation, it may contain an additional National Annex. Where such a National Annex exists, it shows the correlation between this publication and the relevant UK legislation. If there is no National Annex of this kind, the relevant Annex ZA or ZZ in the body of the European text will indicate the relationship to UK regulation applicable in Great Britain. References to EU legislation may need to be read in accordance with the UK designation and the applicable UK law. Further information on designated standards can be found at www.bsigroup.com/standardsandregulation.

For the Northern Ireland market, UK law will continue to implement relevant EU law subject to periodic confirmation. Therefore Annex ZA/ZZ in the European text, and references to EU legislation, are still valid for this market.

UK Government is responsible for legislation. For information on legislation and policies relating to that legislation, consult the relevant pages of www.gov.uk.

AVA Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

© The British Standards Institution 2025
Published by BSI Standards Limited 2025

ISBN 978 0 539 36214 5

ICS 11.100.20

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

This British Standard was published under the authority of the Standards Policy and Strategy Committee on 31 July 2009.

Amendments/corrigenda issued since publication

Date	Text affected
31 March 2025	Implementation of CEN amendment A11:2025

Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN ISO 10993-5+A11

March 2025

ICS 11.100.20

Supersedes EN ISO 10993-5:1999

English Version

Biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity (ISO 10993-5:2009)

Évaluation biologique des dispositifs médicaux - Partie 5: Essais concernant la cytotoxicité in vitro (ISO 10993-5:2009)

Biologische Beurteilung von Medizinprodukten - Teil 5: Prüfungen auf In-vitro-Zytotoxizität (ISO 10993-5:2009)

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

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN Management Centre has the same status as the official versions.

CEN members are the national standards bodies of 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 United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

© 2009 CEN All rights of exploitation in any form and by any means reserved worldwide for CEN national Members.

Ref. No. EN ISO 10993-5:2009: E

Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

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.

Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

European foreword to Amendment A11

This document (EN ISO 10993-5:2009/A11:2025) has been prepared by Technical Committee CEN/TC 206 “Biological and clinical evaluation of medical devices”, the secretariat of which is held by DIN.

This Amendment to the European Standard EN ISO 10993-5:2009 shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2025, and conflicting national standards shall be withdrawn at the latest by September 2025.

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

This document amends EN ISO 10993-5:2009 with a revised European Foreword and the European Annex ZA.

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

For the relationship with EU Legislation, see informative Annex ZA, which is an integral part of this document.

Any feedback and questions on this document should be directed to the users’ national standards body/national committee. A complete listing of these bodies can be found on the CEN website.

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, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

A11 Annex ZA
(informative)

Relationship between this European Standard the General Safety and Performance Requirements of Regulation (EU) 2017/745 aimed to be covered

This European standard has been prepared under M/575 to provide one voluntary means of conforming to the General Safety and Performance Requirements of Regulation (EU) 2017/745 of 5 April 2017 concerning medical devices [OJ L 117] and to system or process requirements including those relating to quality management systems, risk management, post-market surveillance systems, clinical investigations, clinical evaluation or post-market clinical follow-up.

Once this standard is cited in the Official Journal of the European Union under that Regulation, compliance with the normative clauses of this standard given in Table ZA.1 and application of the edition of the normatively referenced standards as given in Table ZA.2 confers, within the limits of the scope of this standard, a presumption of conformity with the corresponding General Safety and Performance Requirements of that Regulation, and associated EFTA Regulations.

Where a definition in this harmonised standard differs from a definition of the same term set out in Regulation (EU) 2017/745, the differences shall be indicated in the Annex Z. For the purpose of using this standard in support of the requirements set out in Regulation (EU) 2017/745, the definitions set out in this Regulation prevail.

Where the European standard is an adoption of an International Standard, the scope of this document can differ from the scope of the European Regulation that it supports. As the scope of the applicable regulatory requirements differ from nation to nation and region to region the standard can only support European regulatory requirements to the extent of the scope of the European Regulation for medical devices ((EU) 2017/745).

NOTE 1 Where a reference from a clause of this standard to the risk management process is made, the risk management process needs to be in compliance with Regulation (EU) 2017/745. This means that risks have to be ‘reduced as far as possible’, ‘reduced to the lowest possible level’, ‘reduced as far as possible and appropriate’, ‘removed or reduced as far as possible’, ‘eliminated or reduced as far as possible’, ’removed or minimized as far as possible’, or ‘minimized’, according to the wording of the corresponding General Safety and Performance Requirement.

NOTE 2 The manufacturer’s policy for determining **acceptable risk** must be in compliance with General Safety and Performance Requirements 1, 2, 3, 4, 5, 8, 9, 10, 11, 14, 16, 17, 18, 19, 20, 21 and 22 of the Regulation.

NOTE 3 When a General Safety and Performance Requirement does not appear in Table ZA.1, it means that it is not addressed by this European Standard.

AVA Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

Table ZA.1 — Correspondence between this European Standard and Annex I of Regulation (EU) 2017/745 [OJ L 117] and to system or process requirements including those relating to quality management systems, risk management, post-market surveillance systems, clinical investigations, clinical evaluation or post-market clinical follow-up

General Safety and Performance Requirements of Regulation (EU) 2017/745	Clause(s) / sub-clause(s) of this EN	Remarks / Notes
10.1 a), b) and d)	4.1, 4.2.2, 4.2.3, 4.3.2, 4.3.3, 4.3.4, 5, 6, 7, 8.1, 8.2, 8.3, 8.4, 8.5, 9, 10	This document only provides a means to determine the biological response of mammalian cells in vitro (cytotoxicity) associated with materials and substances used for design and manufacture (flammability, absorption, distribution, metabolism and excretion are not covered), the compatibility between the materials and substances used and biological tissues, cells and body fluids as well as the impact of processes on material properties. 10.1 is only partly covered by this document, as it does not provide requirements for design and manufacture.
10.2	4.1, 4.2.2, 4.2.3, 4.3.2, 4.3.3, 4.3.4, 5, 6, 7, 8.1, 8.2, 8.3, 8.4, 8.5, 9, 10	This document only provides a means to determine the biological response of mammalian cells in vitro (cytotoxicity) associated with devices taking account of the intended purpose and evaluate risks posed by contaminants and residues to patients or users. 10.2 is only partly covered by this document, as it does not provide requirements for design, manufacture and packaging, nor does it address risks to persons involved in the transport or storage of devices.
10.4.1 (first paragraph)	4.1, 4.2.2, 4.2.3, 4.3.2, 4.3.3, 4.3.4, 5, 6, 7, 8.1, 8.2, 8.3, 8.4, 8.5, 9, 10	This document only provides a means to determine the biological response of mammalian cells in vitro (cytotoxicity) associated with medical devices and aids in evaluation of risks posed by substances or particles, including wear debris, degradation products and processing residues, that may be released from the device. 10.4.1 (first paragraph) is only partly covered by this document, as it does not provide requirements for design and manufacture.
10.6	4.1, 4.2.2, 4.2.3, 4.3.2, 4.3.3, 4.3.4, 5, 6, 7, 8.1, 8.2, 8.3, 8.4, 8.5, 9, 10	This document only provides a means to determine the biological response of mammalian cells in vitro (cytotoxicity) associated with medical devices and aids in evaluation of risks posed by particles that may be released from the device. 10.6 is only partly covered by this document, as it does not provide special attention to nanomaterials, or for design and manufacture.

AVA Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

Table ZA.2 — Normative references from clause 2 of this document and their corresponding European publications

Column 1 Reference in Clause 2	Column 2 International Standard Edition	Column 3 Title	Column 4 Corresponding European Standard Edition
ISO 10993-1	ISO 10993-1:2018	Biological evaluation of medical devices — Part 1: Evaluation and testing within a risk management process	EN ISO 10993-1:2020
ISO 10993-12	ISO 10993-12:2021	Biological evaluation of medical devices - Part 12: Sample preparation and reference materials	EN ISO 10993-12:2021

The documents listed in the Column 1 of Table ZA.2, in whole or in part, are normatively referenced in this document, i.e. are indispensable for its application. The achievement of the presumption of conformity is subject to the application of the edition of Standards as listed in Column 4 or, if no European Standard Edition exists, the International Standard Edition given in Column 2 of Table ZA.2.

WARNING 1 — Presumption of conformity stays valid only as long as a reference to this European standard is maintained in the list published in the Official Journal of the European Union. Users of this standard should consult frequently the latest list published in the Official Journal of the European Union.

WARNING 2 — Other Union legislation may be applicable to the product(s) falling within the scope of this standard. 〈A11〉

Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

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.

Documents
Document Sharing Hub

Full document details: BS EN ISO 10993-5:2009+A11:2025

Contents

Page

Foreword ... iv
Introduction ... vi
1 Scope ... 1
2 Normative references ... 1
3 Terms and definitions ... 1
4 Sample and control preparation ... 2
4.1 General ... 2
4.2 Preparation of liquid extracts of material ... 3
4.3 Preparation of material for direct-contact tests ... 4
4.4 Preparation of controls ... 5
5 Cell lines ... 5
6 Culture medium ... 5
7 Preparation of cell stock culture ... 6
8 Test procedures ... 6
8.1 Number of replicates ... 6
8.2 Test on extracts ... 6
8.3 Test by direct contact ... 7
8.4 Test by indirect contact ... 7
8.5 Determination of cytotoxicity ... 9
9 Test report ... 10
10 Assessment of results ... 11
Annex A (informative) Neutral red uptake (NRU) cytotoxicity test ... 12
Annex B (informative) Colony formation cytotoxicity test ... 19
Annex C (informative) MTT cytotoxicity test ... 24
Annex D (informative) XTT cytotoxicity test ... 29
Bibliography ... 34

© ISO 2009 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 10993-5:2009+A11:2025

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*

© ISO 2009 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 10993-5:2009+A11:2025

— *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]

© ISO 2009 – All rights reserved

Documents
Document Sharing Hub
Full document details: BS EN ISO 10993-5:2009+A11:2025

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.

© ISO 2009 – All rights reserved
Documents
Document Sharing Hub
Full document details: BS EN ISO 10993-5:2009+A11:2025