

# American National Standard

## ANSI/AAMI/ ISO 10993-5: 2009/(R)2022

Biological evaluation of  
medical devices—Part 5:  
Tests for in vitro cytotoxicity



# Biological evaluation of medical devices — Part 5: Tests for in vitro cytotoxicity

Approved 18 June 2009 by  
**Association for the Advancement of Medical Instrumentation**

Approved 23 June 2009 and reaffirmed 6 February 2014 and 9 October 2023 by  
**American National Standards Institute, Inc.**

**Abstract:** AAMI/AAMI/ISO 10993-5 describes test methods to assess the in vitro cytotoxicity of devices. These methods specify the incubation of cultured cells either directly or through diffusion with extracts of the device, and/or in contact with a device. These methods are designed to determine the biological response of mammalian cells in vitro using appropriate biological parameters.

**Keywords:** biological evaluation, medical devices, cytotoxicity

## AAMI Standard

This Association for the Advancement of Medical Instrumentation (AAMI) standard implies a consensus of those substantially concerned with its scope and provisions. The existence of an AAMI standard does not in any respect preclude anyone, whether they have approved the standard or not, from manufacturing, marketing, purchasing, or using products, processes, or procedures not conforming to the standard. AAMI standards are subject to periodic review, and users are cautioned to obtain the latest editions.

**CAUTION NOTICE:** This AAMI standard may be revised or withdrawn at any time. AAMI procedures require that action be taken to reaffirm, revise, or withdraw this standard no later than 5 years from the date of publication. Interested parties may obtain current information on all AAMI standards by calling or writing AAMI, or by visiting the AAMI website at [www.aami.org](http://www.aami.org).

All AAMI standards, recommended practices, technical information reports, and other types of technical documents developed by AAMI are *voluntary*, and their application is solely within the discretion and professional judgment of the user of the document. Occasionally, voluntary technical documents are adopted by government regulatory agencies or procurement authorities, in which case the adopting agency is responsible for enforcement of its rules and regulations.

*Published by*

Association for the Advancement of Medical Instrumentation  
901 N. Glebe Road, Suite 300  
Arlington, VA 22203  
[www.aami.org](http://www.aami.org)

© 2009 by the Association for the Advancement of Medical Instrumentation

All Rights Reserved

This publication is subject to copyright claims of ISO and AAMI. No part of this publication may be reproduced or distributed in any form, including an electronic retrieval system, without the prior written permission of AAMI. All requests pertaining to this document should be submitted to AAMI. It is illegal under federal law (17 U.S.C. § 101, *et seq.*) to make copies of all or any part of this document (whether internally or externally) without the prior written permission of the Association for the Advancement of Medical Instrumentation. Violators risk legal action, including civil and criminal penalties, and damages of \$100,000 per offense. For permission regarding the use of all or any part of this document, contact the Copyright Clearance Center.

Printed in the United States of America

**ISBN 978-1-57020-355-8**

# Contents

Page

|                                                                       |      |
|-----------------------------------------------------------------------|------|
| Glossary of equivalent standards .....                                | iv   |
| Committee representation.....                                         | vi   |
| Background of AAMI adoption of ISO 10993-5:2009 .....                 | vii  |
| Foreword.....                                                         | viii |
| Introduction .....                                                    | x    |
| 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.....                                                 | 6    |
| 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.....                                     | 8    |
| 8.5 Determination of cytotoxicity .....                               | 9    |
| 9 Test report .....                                                   | 11   |
| 10 Assessment of results.....                                         | 11   |
| Annex A (informative) Neutral red uptake (NRU) cytotoxicity test..... | 13   |
| Annex B (informative) Colony formation cytotoxicity test.....         | 21   |
| Annex C (informative) MTT cytotoxicity test.....                      | 26   |
| Annex D (informative) XTT cytotoxicity test .....                     | 32   |
| Bibliography .....                                                    | 37   |

## 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.

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                       | ANSI/AAMI ES60601-1:2005                                           | Major technical variations              |
| 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                                                | 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                                                | 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 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                               |
| ISO 5840:2005                          | ANSI/AAMI/ISO 5840:2005                                            | Identical                               |
| ISO 7198:1998                          | ANSI/AAMI/ISO 7198:1998/2001/(R)2004                               | Identical                               |
| ISO 7199:2009                          | ANSI/AAMI/ISO 7199:2009                                            | Identical                               |
| ISO 8637:2004                          | ANSI/AAMI RD16:2007                                                | Major technical variations              |
| ISO 8638:2004                          | ANSI/AAMI RD17:2007                                                | Major technical variations              |
| ISO 10993-1:2003                       | ANSI/AAMI/ISO 10993-1:2003                                         | Identical                               |
| ISO 10993-2:2006                       | ANSI/AAMI/ISO 10993-2:2006                                         | Identical                               |
| ISO 10993-3:2003                       | ANSI/AAMI/ISO 10993-3:2003                                         | 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 10993-9:1999                       | ANSI/AAMI/ISO 10993-9:1999/(R)2005                                 | Identical                               |
| ISO 10993-10:2002 and Amendment 1:2006 | ANSI/AAMI BE78:2002/(R)2008<br>ANSI/AAMI BE78:2002/A1:2006/(R)2008 | Minor technical variations<br>Identical |
| ISO 10993-11:2006                      | ANSI/AAMI/ISO 10993-11:2006                                        | Identical                               |
| ISO 10993-12:2007                      | ANSI/AAMI/ISO 10993-12:2007                                        | Identical                               |
| ISO 10993-13:1998                      | ANSI/AAMI/ISO 10993-13:1999/(R)2004                                | 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 10993-16:1997                      | ANSI/AAMI/ISO 10993-16:1997/(R)2009                                | 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              |
| 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                               |

| International designation                       | U.S. designation                                     | Equivalency                 |
|-------------------------------------------------|------------------------------------------------------|-----------------------------|
| ISO/TS 11135-2:2008                             | ANSI/AAMI/ISO TIR11135-2:2008                        | Identical                   |
| ISO 11137-1:2006                                | ANSI/AAMI/ISO 11137-1:2006                           | 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                           | Identical                   |
| ISO 11138-1: 2006                               | ANSI/AAMI/ISO 11138-1:2006                           | Identical                   |
| ISO 11138-2: 2006                               | ANSI/AAMI/ISO 11138-2:2006                           | Identical                   |
| ISO 11138-3: 2006                               | ANSI/AAMI/ISO 11138-3:2006                           | Identical                   |
| ISO 11138-4: 2006                               | ANSI/AAMI/ISO 11138-4:2006                           | Identical                   |
| ISO 11138-5: 2006                               | ANSI/AAMI/ISO 11138-5:2006                           | Identical                   |
| ISO/TS 11139:2006                               | ANSI/AAMI/ISO 11139:2006                             | Identical                   |
| ISO 11140-1:2005                                | ANSI/AAMI/ISO 11140-1:2005                           | 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 11737-2:1998                                | ANSI/AAMI/ISO 11737-2:1998                           | 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                             | 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 14161:2000                                  | ANSI/AAMI/ISO 14161:2000                             | Identical                   |
| ISO 14937:2000                                  | ANSI/AAMI/ISO 14937:2000                             | Identical                   |
| ISO/TR 14969:2004                               | ANSI/AAMI/ISO TIR14969:2004                          | Identical                   |
| ISO 14971:2007                                  | ANSI/AAMI/ISO 14971:2007                             | Identical                   |
| ISO 15223-1:2007 and A1:2008                    | ANSI/AAMI/ISO 15223-1:2007 and A1:2008               | Identical                   |
| ISO 15225:2000 and A1:2004                      | ANSI/AAMI/ISO 15225:2000/(R)2006 and A1:2004/(R)2006 | 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/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 and A1:2005               | Identical                   |
| ISO 25539-2:2008                                | ANSI/AAMI/ISO 25539-2:2008                           | Identical                   |
| ISO 81060-1:2007                                | ANSI/AAMI/ISO 81060-1:2007                           | Identical                   |

## Committee representation

### Association for the Advancement of Medical Instrumentation Cytotoxicity Working Group

The adoption of ISO 10993-5 as a revision of ANSI/AAMI/ISO 10993-5:1999 was initiated by the AAMI Cytotoxicity Working Group under the auspices of the AAMI Biological Evaluation Committee. U.S. representatives played an active part in developing the ISO standard. Committee approval of the standard does not necessarily imply that all committee and working group members voted for its approval.

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

|            |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
|------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cochairs   | Lisa Olson, WuXi AppTec<br>Vana Poovala, Philips Healthcare                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Members    | Stephanie Board, St Jude Medical Inc.<br>Carolyn Braithwaite, CaridianBCT Sterilization Services Inc.<br>Kimbrell Darnell, CR Bard<br>Todd A. Festerling, NAMSA<br>Gloria H. Frost, PhD, Cardinal Health (MP&S)<br>Niranjan Goud, PhD, Boston Scientific Corporation<br>Victoria M. Hitchins, PhD, FDA/CDRH<br>Robert Dennis Houlsby, Abbott Laboratories<br>Carl Johnson, Baxter Healthcare Corporation<br>Daniel Edward McLain, PhD CNS DABFE, Walker Downey & Associates Inc.<br>Lisa Olson, WuXi AppTec<br>Vana Poovala, Philips Healthcare<br>Robert T. Przygoda, PhD, Johnson & Johnson<br>Bobbi Rushton-Castro, BS, Nelson Laboratories Inc.<br>Sharmilee Sawant, Kimberly-Clark Corporation<br>Anita Y. Sawyer, Becton Dickinson & Company<br>Nancy J. Stark, PhD, Clinical Device Group Inc |
| Alternates | Jon Cammack, PhD DABT, Baxter Healthcare Corporation<br>Anthony J. DeMarinis, BS MS CQA CQM, CR Bard<br>William H. Houser, Abbott Laboratories<br>Danny Hutson, Cardinal Health (MP&S)<br>Michelle A. Lee, Nelson Laboratories Inc.<br>Byron Lewis, Boston Scientific Corporation<br>Christina Oval, NAMSA<br>Jeffrey Toy, PhD, FDA/CDRH                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

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

|            |                                                                                                                                                                                                                                                                                                 |
|------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cochairs   | Jon Cammack, PhD DABT, Baxter Healthcare Corporation<br>Melvin E. Stratmeyer, PhD, FDA/CDRH                                                                                                                                                                                                     |
| Members    | James M. Anderson, MD PhD, Case Western Reserve University (Independent Expert)<br>Jon Cammack, PhD DABT, Baxter Healthcare Corporation<br>Joseph Carraway, DVM, NAMSA<br>Anita Y. Sawyer, Becton Dickinson & Company<br>Melvin E. Stratmeyer, PhD, FDA/CDRH<br>Michael F. Wolf, Medtronic Inc. |
| Alternates | Jesus W. Casas, MD MSc, Medtronic Inc.<br>Donald E. Marlowe, FDA/CDRH                                                                                                                                                                                                                           |

---

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 AAMI adoption of ISO 10993-5:2009

As indicated in the foreword to the main body of this document (page viii), 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.

International Standard ISO 10993-5:2009 was developed as a revision to the 1999 edition by Working Group (WG) 5, *Cytotoxicity*, of Technical Committee ISO/TC 194, *Biological evaluation of medical devices*. In this new edition, key changes include introducing a threshold for cytotoxicity to clarify when a test sample may be considered cytotoxic and adding test methods to informative annexes to provide more choice for evaluating cytotoxicity of test samples.

U.S. participation in this ISO TC is organized through the U.S. Technical Advisory Group for ISO/TC 194, administered by the Association for the Advancement of Medical Instrumentation on behalf of the American National Standards Institute. The U.S. made a considerable contribution to this International Standard.

AAMI encourages its committees to harmonize their work with international standards as much as possible. Upon review of ISO 10993-5, the AAMI Cytotoxicity Working Group decided to adopt 10993-5, verbatim, as a revision of ANSI/AAMI/ISO 10993-5:1999.

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

AAMI (and ANSI) have adopted other ISO standards. 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 in 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, 901 N. Glebe Road, Suite 300, Arlington, VA 22203.

---

NOTE—Beginning with the foreword on page viii, this American National Standard is identical to ISO 10993-5:2009.

---

## 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

**NOTE** The purpose of the positive control is to demonstrate an appropriate test system response. For example, an organotin-stabilized polyurethane<sup>1)</sup> has been used as positive control for solid materials and extracts. Dilutions of phenol, for example, have been used as a positive control for extracts. In addition to a material, pure chemicals can also be used to demonstrate the performance of the test system.

### 3.3

#### **blank**

extraction vehicle not containing the test sample, retained in a vessel identical to that which holds the test sample and subjected to conditions identical to those to which the test sample is subjected during its extraction

**NOTE** The purpose of the blank is to evaluate the possible confounding effects due to the extraction vessel, vehicle and extraction process.

### 3.4

#### **negative control material**

material which, when tested in accordance with this part of ISO 10993, does not produce a cytotoxic response

**NOTE** The purpose of the negative control is to demonstrate background response of the cells. For example, high-density polyethylene<sup>2)</sup> for synthetic polymers, and aluminum oxide ceramic rods for dental material have been used as negative controls.

### 3.5

#### **test sample**

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

### 3.6

#### **subconfluency**

approximately 80 % confluency, i.e. the end of the logarithmic phase of growth

## 4 Sample and control preparation

### 4.1 General

The test shall be performed on

a) an extract of the test sample

and/or

b) the test sample itself.

---

1) The ZDEC and ZDBC polyurethanes are available from the Food and Drug Safety Center, Hatano Research Institute, Ochiai 729-5, Hadanoshi, Kanagawa 257, Japan.

2) High-density polyethylene can be obtained from the U.S. Pharmacopeia (Rockville, MD, USA) and from the Food and Drug Safety Center, Hatano Research Institute (Ochiai 729-5, Hadanoshi, Kanagawa 257, Japan).

The information given in 1) and 2) is for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of these products. Equivalent products may be used if they can be shown to lead to the same results.

Sample preparation shall be in accordance with ISO 10993-12.

Negative and positive controls shall be included in each assay.

## **4.2 Preparation of liquid extracts of material**

### **4.2.1 Principles of extraction**

Extracting conditions should attempt to simulate or exaggerate the clinical use conditions so as to determine the potential toxicological hazard without causing significant changes in the test sample, such as fusion, melting or any alteration of the chemical structure, unless this is expected during clinical application. Due to the nature of certain materials (e.g. biodegradable materials), alteration of the chemical structure can occur during the extraction procedure.

**NOTE** The concentration of any endogenous or extraneous substances in the extract, and hence the amount exposed to the test cells, depends on the interfacial area, the extraction volume, pH, chemical solubility, diffusion rate, osmolarity, agitation, temperature, time and other factors.

For devices that involve mixing two or more components in the patient to arrive at the final device (for example bone cement), the final device should not be washed prior to extraction. Washing the test sample can reduce or remove residuals present on the device. If the test sample is to be used in a sterile environment, a sterilized test sample should be used to extract chemical constituents.

### **4.2.2 Extraction vehicle**

The choice of the extraction vehicle(s) taking into account the chemical characteristics of the test sample shall be justified and documented. For mammalian cell assays one or more of the following vehicles shall be used:

- a) culture medium with serum;
- b) physiological saline solution;
- c) other suitable vehicle.

The choice of vehicle should reflect the aim of the extraction. Consideration shall be given to the use of both a polar and a non-polar vehicle. Culture medium with serum is the preferred extraction vehicle. The use of culture medium with serum is preferred for extraction because of its ability to support cellular growth as well as extract both polar and non-polar substances. In addition to culture medium with serum, use of medium without serum should be considered in order to specifically extract polar substances (e.g. ionic compounds). Other suitable vehicles include purified water and dimethyl sulfoxide (DMSO). DMSO is cytotoxic in selected assay systems at greater than 0.5 % (volume fraction). The cellular exposure concentration of extractables in DMSO will be lower due to the greater dilution as compared to extraction in culture medium with serum.

**NOTE 1** Different types of serum (e.g. foetal, bovine/calf serum, newborn calf serum) might be used and the choice of the serum is dependent on the cell type.

**NOTE 2** It is important to recognize that serum/proteins are known to bind, to some extent, extractables.

### **4.2.3 Extraction conditions**

**4.2.3.1** The extraction shall be performed in sterile, chemically inert, closed containers by using aseptic techniques, in accordance with ISO 10993-12.