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Biological evaluation of medical devices —

Part 5: Tests for *in vitro* cytotoxicity

Évaluation biologique des dispositifs médicaux —

Partie 5: Essais concernant la cytotoxicité in vitro

Reference number
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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

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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*

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— *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]

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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.

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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

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NOTE The purpose of the positive control is to demonstrate an appropriate test system response. For example, an organotin-stabilized polyurethane[1)] 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[2)] for synthetic polymers, and aluminium 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.

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

Negative and positive controls shall be included in each assay.

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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.

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