INTERNATIONAL STANDARD

ISO 10993-5

Second edition
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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

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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 3.

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.

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

This second edition cancels and replaces the first edition (ISO 10993-5:1992), 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*

— *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 8: Guidance for reference materials*

— *Part 9: Framework for the identification and quantification of potential degradation products*

— *Part 10: Tests for irritation and sensitization*

— *Part 11: Tests for systemic toxicity*

— *Part 12: Sample preparation and reference materials*

— *Part 13: Identification and quantification of degradation products from polymers*

— *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: Methods for establishment of allowable limits for leachable substances using health-based risk assessment*

— *Part 18: Chemical characterization*

Future parts will deal with other relevant aspects of biological testing.

Introduction

Due to the general applicability of *in vitro* cytotoxicity tests and their widespread use in evaluating a large range of medical 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 which advocate *in vitro* biological tests.

The numerous methods used and end-points measured in cytotoxicity determination can be grouped into categories of evaluation type:

a) assessments of cell damage by morphological means;

b) measurements of cell damage;

c) measurements of cell growth;

d) measurements of specific aspects of cellular metabolism.

There are, therefore, several alternative means of producing results in each of these four categories. The investigator should be aware of the categories of test and into which a particular technique fits, in order that comparisons may be made with other results on similar medical devices or materials, and in order that interlaboratory tests may be conducted.

Biogical 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 either directly or through diffusion

a) with extracts of a device, and/or

b) in contact with a device.

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

2 Normative references

The following normative documents contain provisions which, through reference in this text, constitute provisions of this part of ISO 10993. For dated references, subsequent amendments to, or revisions of, any of these publications do not apply. However, parties to agreements based on this part of ISO 10993 are encouraged to investigate the possibility of applying the most recent editions of the normative documents indicated below. For undated references, the latest edition of the normative document referred to applies. Members of ISO and IEC maintain registers of currently valid International Standards.

ISO 10993-1, *Biological evaluation of medical devices — Part 1: Evaluation and testing*.

ISO 10993-12:1996, *Biological evaluation of medical devices — Part 12: Sample preparation and reference materials.*

3 Terms and definitions

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

3.1
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. For example, high-density polyethylene[1)] for synthetic polymers, and aluminium oxide ceramic rods for dental material, have been used as negative controls.

1) High-density polyethylene can be obtained from the U.S. Pharmacopeia (Rockville, Maryland, USA) and Food and Drug Safety Center, Hatano Research Institute (Ochiai 729-5, Hadanoshi, Kanagawa 257 - Japan). This information is given for the convenience of the user of this part of ISO 10993 and does not constitute an endorsement by ISO of these products.