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Biologisk värdering av medicintekniska produkter – Del 5: Prövning för cytotoxicitet in vitro (ISO 10993-5:2009)

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

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Europastandarden EN ISO 10993-5:2009 gäller som svensk standard. Detta dokument innehåller den officiella engelska versionen av EN ISO 10993-5:2009.

Denna standard ersätter SS-EN ISO 10993-5, utgåva 1.

The European Standard EN ISO 10993-5:2009 has the status of a Swedish Standard. This document contains the official English version of EN ISO 10993-5:2009.

This standard supersedes the Swedish Standard SS-EN ISO 10993-5, edition 1.

"WARNING: The use of this standard may involve hazardous materials, operations and equipment. This standard does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use."

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EUROPEAN STANDARD

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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.

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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of an identical text or by endorsement, at the latest by December 2009, and conflicting national standards shall be withdrawn at the latest by December 2009.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.

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Endorsement notice

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

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[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.

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 recognise 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.

4.2.3.2 With the exception of circumstances given below, the extraction shall be conducted under one of the following conditions and shall be applied according to the device characteristics and specific conditions for use:

a) (24 ± 2) h at (37 ± 1) °C;

b) (72 ± 2) h at (50 ± 2) °C;

c) (24 ± 2) h at (70 ± 2) °C;

d) (1 ± 0,2) h at (121 ± 2) °C.