TECHNICAL REPORT

ISO/TR 10993-55

First edition
2023-02

Biological evaluation of medical devices —

Part 55: Interlaboratory study on cytotoxicity

Évaluation biologique des dispositifs médicaux —
Partie 55: Étude interlaboratoire sur la cytotoxicité

Reference number
ISO/TR 10993-55:2023(E)

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Published in Switzerland

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Foreword

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This document was prepared by Technical Committee ISO/TC 194, *Biological and clinical evaluation of medical devices*.

A list of all parts in the ISO 10993 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

The first edition of ISO 10993-5, published in 1992, allowed several different ways to assess cytotoxicity of medical devices and gave an imprecise description of how to perform the tests. Qualitative assays were accepted and only a small amount of guidance was given for the interpretation of the results. Not surprisingly, the first interlaboratory study in 2000 resulted in quite low reproducibility of results. Therefore, detailed protocols were included into the standard and in another study the practicability of the protocols and reference materials were evaluated. The results of this second interlaboratory study mainly influenced the revision of ISO 10993-5, which was published in 2009.

This document provides the historical report of the second interlaboratory study, conducted in 2006.

Biological evaluation of medical devices —

Part 55:
Interlaboratory study on cytotoxicity

1 Scope

This document describes the results of an international interlaboratory study conducted in 2006 to evaluate the performance of two different test protocols in terms of the cytotoxic effects in the biological evaluation of medical devices. The results of these tests were used for the revision of ISO 10993-5.[2] Furthermore, the results of these tests were used to estimate the accuracy of these test systems with living cells to define a threshold what is considered a cytotoxic effect.

NOTE The determination of cytotoxic effects has a high relevance in the biological evaluation of medical devices; according to ISO 10993-1[1], it is one of the very few tests which are proposed to be performed for every kind of device.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

No terms and definitions are listed in this document.

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

4 Participants

Twelve laboratories participated in this study[1)]. Eleven reports on a neutral red uptake (NRU) assay and ten reports on a colony formation assay (CFA) were received. Four participants were commercial test laboratories, four participants were internal test laboratories of medical device manufacturers and four laboratories were in research institutes.

The laboratories were located in six different countries: one each in Austria, France and the Netherlands, and three each in Germany, Japan and the United States.

5 Materials and sample preparation

The following materials were used for the study:

a) reference material-C [RM-C; Hatano Research Institute (HRI)]: high density polyethylene sheet;

b) RM-A (HRI): segmented polyurethane film containing 0,1 % zinc diethyldithiocarbamate (ZDEC);

1) The participating laboratories were: Deutsche Institute für Textil- und Faserforschung, Germany; Hatano Research Institute, Food and Drug Safety Center, Japan; Medical University Vienna, Austria; National Institute of Health Sciences, Japan; Envigo CRS GmbH, Germany; Terumo Corporation R&D, Japan; BD Technologies, United States; NAMSA, United States; Gambro BCT, United States and three other laboratories.

c) RM-B (HRI): segmented polyurethane film containing 0,25 % zinc dibutyldithiocarbamate (ZDBC).

RM-C (HRI), RM-A (HRI) and RM-B (HRI) have been widely used as reference materials for cytotoxicity tests of medical devices. The Food and Drug Safety Center of HRI has certified these materials. HRI agreed to provide them for the interlaboratory study. Test samples were cut (2 mm × 15 mm) and sterilized with ethylene oxide (EO) and were distributed from HRI to the participants. Extraction was then performed in the participating laboratories according to the protocols.

6 Test procedures

Two test protocols were chosen by the working group developing the tests for cytotoxicity in vitro: NRU and CFA. The NRU assay protocol is based on the protocol, which was used in a validation study of Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM).[4] The CFA protocol is based on the cytotoxicity test of the Japanese guidelines for basic biological tests of medical materials and devices.[5] These original protocols were modified to meet the requirements of this specific study (see Annexes A and B). The protocols were sent to the participants together with the test materials.

7 Results

7.1 Neutral red uptake

7.1.1 General

Eleven laboratories participated in this study. All test samples were extracted once as described in Annex A. Each concentration of the dilution series was tested in six replicates. The mean values were used to calculate the concentration producing 50 % inhibition of cell viability (IC_{50}) values.

7.1.2 Sodium lauryl sulfate as positive control

The laboratories used different internal reference materials as positive controls. It was therefore decided that all participants use the same common chemical substance as positive control and sodium lauryl sulfate (SLS, CAS Registry Number® 151-21-3[2]) was selected for this purpose. Table 1 summarizes the results.

Table 1 — IC_{50}-values of SLS in the NRU assay

Laboratory	IC_{50} µg/ml
1	34,0
2	83,0
3	62,4
4	77,0
5	85,9
6	75,6
7	67,8
8	47,8
9	49,8
10	62,1
11	22,0

2) CAS Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

The variation of the IC_{50} was from 22,0 µg/ml to 85,9 µg/ml, the mean IC_{50} was (60,7 ± 20,4) µg/ml.

In Annex A, an IC_{50}-value between 70 µg/ml and 116 µg/ml was requested as acceptance criterion. This was an error in ISO 10993-5:2009 and will be removed in the next edition. Historical IC_{50}-values are typical for a specific laboratory but cannot be compared between laboratories.

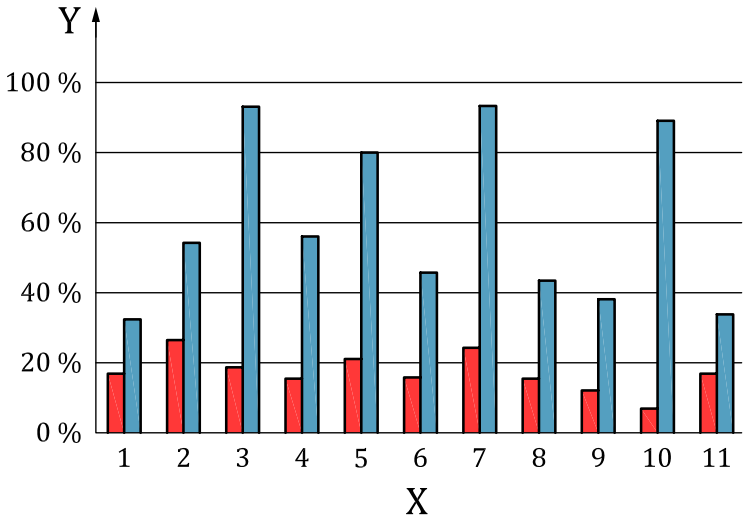
7.1.3 Test samples

The three different test samples RM-A, RM-B and RM-C were extracted as described in Annex A and the extracts were diluted as defined. The cell viabilities at different extract concentrations were determined as described in Annex A. The IC_{50} was determined from the concentration-response. This was done by using validated software, which is available in public, see Reference [6]. The results of the eleven participants are summarized in Table 2 and illustrated in Figure 1. Initially, the testing was conducted using the following concentrations of the RM-A extract: 0,25 %, 0,5 %, 1,0 %, 2,0 %, 3,0 % and 4,0 %. Unexpectedly, IC_{50}-values were higher than expected from the colony formation assay, because the neutral red assay is less sensitive probably due to the shorter exposure time. Therefore, the laboratories repeated the test with the following concentrations of the RM-A extract: 5 %, 10 %, 20 %, 30 %, 40 % and 50 %.

Table 2 — IC_{50}-values of sample extracts in the NRU assay

Laboratory	IC_{50} %		
	RM-A	RM-B	RM-C
1	16,5	32,0	—
2	26,4	54,0	—
3	18,5	93,2	—
4	15,3	56,6	—
5	20,6	79,6	—
6	15,6	45,6	—
7	24,3	93,3	—
8	15,1	43,3	—
9	11,7	38,2	—
10	6,7	89,4	—
11	16,7	33,6	—

Results for RM-A varied from 6,7 % to 26,4 %, mean IC_{50} was (17,0 ± 5,5) %. Results for RM-B varied from 32,0 % to 93,3 %, the mean IC_{50} was (59,9 ± 24,4) %.



Key

X laboratory number

Y IC_{50}

reference material A

reference material B

Figure 1 — Comparison of IC_{50}-values of sample extracts in the NRU assay

7.2 Colony formation assay

7.2.1 General

The test samples RM-A, RM-B and RM-C were those materials, which were already recommended to be used as reference materials in the Japanese Guidelines for the colony formation assay. For the study, only these materials were used by the laboratories, to assess the differences between individual laboratories. The test samples were extracted as described in Annex B and the extracts were diluted as proposed. The cell viabilities at different extract concentrations were determined by counting the colonies formed [plating efficiency (PE)]. IC_{50} was calculated from the dose-response curve as the dose with 50 % PE which was calculated from the line which passed through a dose with higher PE and a dose with lower PE than 50 %.

Ten of the twelve participants in the NRU study also participated in the CFA and communicated their results. All test samples were extracted once as described in Annex B. Each concentration of the dilution series was tested in triplicate. The mean values were used to calculate IC_{50}-values.

The plating efficiency of the controls in the different laboratories is listed in Table 3.

Table 3 — Control PE of the CFA in the participating laboratories

Laboratory	Plating efficiency %
1	68,0
2	62,8
3	75,7
4	101,5
5	92,2
7	71,2
8	108,7
9	85,0
10	75,0

Table 3 *(continued)*

Laboratory	Plating efficiency %
12	106,3

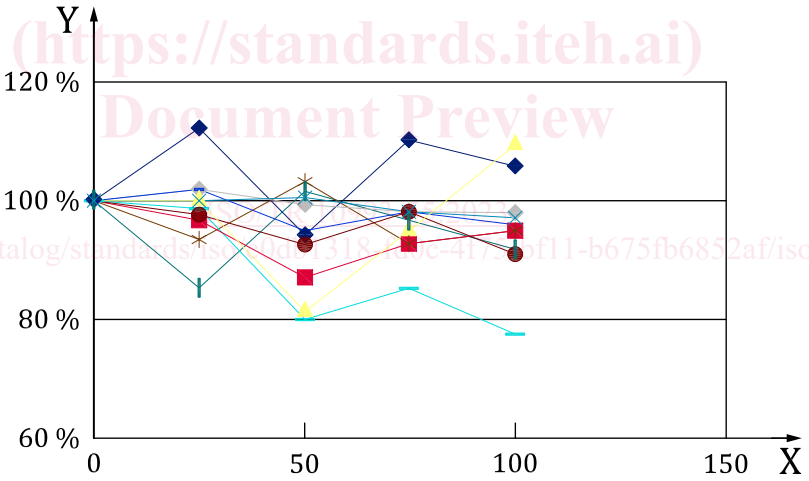
The PE in the controls varied from 62,8 % to 108,7 %, mean value was (84,6 ± 15,8) %.

7.2.2 Negative reference material

The test sample RM-C is certified not to give any positive response in the test. Nevertheless, the extract of RM-C was used in this study to detect the variation of results in this biological system. The results of the ten participants are summarized in Table 4 and illustrated in Figure 2.

Table 4 — Plating efficiencies of RM-C in the CFA

Concentration of RM-C %	Plating efficiency %									
	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 7	Lab 8	Lab 9	Lab 10	Lab 12
0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0	100,0
25	112,0	96,8	100,0	100,2	93,3	97,8	85,3	102,4	98,7	101,9
50	94,0	87,1	81,5	100,5	103,1	92,6	101,8	94,9	79,6	99,4
75	110,0	92,9	96,0	97,5	92,9	98,2	96,6	98,5	85,3	98,4
100	106,0	94,8	110,1	97,2	95,1	91,2	92,0	96,1	77,8	98,1



Key

X extract concentration, %
Y platting efficiency of the control, %
laboratory 1
laboratory 2
laboratory 3
laboratory 4
laboratory 5
laboratory 7
laboratory 8
laboratory 9
laboratory 10
laboratory 12

Figure 2 — Plating efficiencies of RM-C in the CFA

7.2.3 Positive reference materials

Extracts of the test samples RM-A and RM-B were applied in the CFA as indicated in Annex B. The results of the ten participants are summarized in Table 5 and Table 6 and illustrated in Figure 3 and Figure 4.