SLOVENSKI STANDARD
oSIST prEN ISO 19337:2024

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Nanotehnologije - Značilnosti delovnih suspenzij nanoobjektov za in vitro teste za oceno inherentne toksičnosti nanoobjektov (ISO 19337:2023)

Nanotechnologies - Characteristics of working suspensions of nano-objects for in vitro assays to evaluate inherent nano-object toxicity (ISO 19337:2023)

Nanotechnologien - Eigenschaften von Arbeitssuspensionen von Nanoobjekten für In-vitro-Assays zur Bewertung der inhärenten Nanoobjekt-Toxizität (ISO 19337:2023)

Nanotechnologies - Caractéristiques des suspensions de nano-objets utilisées pour les tests in vitro évaluant la toxicité inhérente aux nano-objets (ISO 19337:2023)

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Nanotechnologies — Characteristics of working suspensions of nano-objects for in vitro assays to evaluate inherent nano-object toxicity

Nanotechnologies — Caractéristiques des suspensions de nano-objets utilisées pour les tests in vitro évaluant la toxicité inhérente aux nano-objets

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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.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO document should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 229, *Nanotechnologies.*

This second edition cancels and replaces the first edition (ISO/TS 19337:2016) which has been technically revised.

The main changes are as follows:

— “the flow of measurements” has been improved as shown in Figure A.1;

— the status of Annex A has been changed from informative to normative;

— “5.2 Endotoxin” has been replaced by “5.5 Contamination”.

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

Before nano-objects enter onto the market, their possible impact on human health and the environment should be carefully evaluated.

In vitro toxicity assays using cultured cells are frequently used as a tool in screening materials for possible hazardous properties. The testing provides essential information for understanding the mechanisms of biological effects induced by the materials. However, nano-objects require specific considerations with respect to the in vitro toxicity assays, because of their unique behaviour in cell culture medium. For example, immediately after the introduction of nano-object samples into the culture medium, the nano-objects can undergo changes in

a) ionic dissolution,

b) corona formation, or

c) aggregation/agglomeration

leading to alteration in particles size and sedimentation. Therefore, it is critical to consider the above-mentioned phenomena in clarifying if the observed effects are related to the tested nano-object itself or from uncontrolled sources and to avoid false interpretation of assay results. For example, the corona formation, metal ion release from the nano-objects and impurities (residual from the nano-object synthesis process) can interfere with some in vitro assays[1], producing inaccurate results. Additionally, the formation of agglomerates and aggregates can alter the toxicity of a suspension. It is important to carefully assess and describe the characteristics of the suspension of nano-objects being tested.

Therefore, the rigorous characterization of the working suspension prior and during in vitro toxicity assays on these characteristics is essential to exclude the in vitro experimental artefacts. In this document, the essential characteristics related to these three phenomena and applicable measurement methods were summarized. On the other hands, this document does not include a strategy to select the appropriate techniques from the applicable measurement methods because the working suspensions that contain nano-object samples for in vitro toxicity assays has the different materials components, concentration and sizes; thus, the appropriate selection is depending on the investigators. While the related informative annexes and the list of references in the Bibliography are included in this document to assist with appropriate method selection by investigators to make allowance for the characterization method selection, optional methods are also given in this document. In Clause 6, the details of the characterization methods/procedures are described; therefore, the appropriateness of the characterization can be judged.

The intention of this document is for reliable test results on nano-object toxicity to be shared and communicated among stakeholders of nano-objects, such as regulators, general public, manufacturers and end users.

Nanotechnologies — Characteristics of working suspensions of nano-objects for in vitro assays to evaluate inherent nano-object toxicity

1 Scope

This document describes the characteristics of working suspensions of nano-objects to be considered when conducting in vitro assays to evaluate inherent nano-object toxicity. In addition, the document identifies applicable measurement methods for these characteristics.

This document is applicable to nano-objects, and their aggregates and agglomerates greater than 100 nm.

This document intends to help clarify whether observed toxic effects come from tested nano-objects themselves or from uncontrolled sources.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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/TS 80004-2, *Nanotechnologies — Vocabulary — Part 2: Nano-objects*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO/TS 80004-2 and the following apply.

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/

3.1
culture medium
aqueous solution of nutrients required for cell growth

3.2
secondary particle
agglomerate/aggregate of primary particle(s), proteins and other medium components

3.3
stability
properties to remain unchanged over a given time under stated or reasonably expected conditions of storage and use for an in vitro toxicity assay

3.4
working suspension
suspension prepared for an in vitro assay that includes *culture medium* (3.1) and nano-object sample

3.5
contamination
trace amounts of extrinsic substances present in the nano-object samples that affect cellular growth

4 Abbreviated terms

AAS	atomic absorption spectrometry
BCA	bicinchoninate acid
BSA	Bovine serum albumin
C-U/F	ultrafiltration assisted by centrifugation
DLS	dynamic light scattering
AF4	asymmetrical-flow field-flow fractionation
ICP-AES	inductively coupled plasma-atomic emission spectrometry
ICP-MS	inductively coupled plasma mass spectrometry
LAL	limulus amebocyte lysate
LD	laser diffraction
LPS	liposaccharides
MAT	monocyte activation test
PCR	polymerase chain reaction
ppt	parts per trillion
SLS	static light scattering
TFF	tangential flow filtration
TOC	total organic carbon
U/F	ultrafiltration
UV-Vis	ultraviolet-visible

5 Characteristics and measurement methods

5.1 General

To characterize the working suspension for in vitro toxicity assays, it is necessary to identify certain characteristics that can impact the biological system tested. Working suspensions for individual dose shall be divided into two samples, one for toxicity assay and another for characteristics measurements. Clause 5 specifies essential characteristics of the working suspension, listed below, and measurement methods that are applicable to them:

— stability of working suspensions;

— concentration of metal ions;

— concentration of culture medium components;