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

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

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Foreword

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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 documents 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:

— [*to be completed*]

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 (1) ionic dissolution, (2) corona formation or (3) 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.

The rigorous characterization of the working suspension prior and during in vitro toxicity assays is essential to exclude the in vitro experimental artefacts. 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. Therefore, it is important to carefully assess and describe the characteristics of the suspension of nano-objects being tested.

ISO 19337 describes the essential characteristics and applicable measurement methods of working suspensions that contain nano-object samples for in vitro toxicity assays. The intention is that reliable test results on nano-object toxicity could be shared and communicated among stakeholders of nano-objects, such as regulators, general public, manufacturers and end users. This ISO 19337 does not describe a procedure for validation of working suspension.

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

1 Scope

This ISO 19337 describes 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.

NOTE This ISO19337 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*

ISO 29701, *Nanotechnologies — Endotoxin test on nanomaterial samples for in vitro systems — Limulus amebocyte lysate (LAL) test*

3 Terms and definitions

For the purposes of this document, the terms and definitions contained 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 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
MAT	monocyte activation test
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 might 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. This clause 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

— Contamination

Measurements of those characteristics shall be made for each dose of working suspensions. See Annex A for the normative flow of measurements.

5.2 Stability of working suspensions

5.2.1 General

Stability of working suspension is a key characteristic as it directly impacts the in vitro assay conditions in terms of the dose of the nano-objects to the cells.[2],[3],[4] Aggregation/agglomeration and gravitational settling of the nano-objects are major issues that may affect the stability of the suspended nano-objects. The suspension stability shall be evaluated for the two characteristics, i.e. the relative change of representative size of secondary particles of nano-objects and the relative change of the concentration of nano-objects in the working suspension. The change in size of secondary particles of nano-objects can result from agglomeration of smaller particles in culture media. The relative change of nano-object concentration can result from gravitational settling during an in vitro toxicity assay by considering experimental duration required for the in vitro toxicity assay. Evaluation results of the stability shall be expressed in the unit of per cent (%) over the timescale for in vitro toxicity assay.

NOTE ISO/TR 13097 [5] is recommended as a comprehensive guidance for stability of working suspension.

5.2.2 Representative size change of secondary particles of nano-objects

An appropriate method shall be selected to directly measure the representative size change of secondary particles of nano-objects from among dynamic light scattering (DLS),[3],[6] laser diffraction (LD) [7] and static light scattering (SLS).[8] Other methods not listed in this document can be used and reported in accordance with 'Optional methods' in 6.6.

See Annex B (informative) for measurements.

5.2.3 Concentration change of nano-objects

An appropriate method shall be selected to measure the concentration change of nano-objects suspended in the biological media from among the static light scattering,[3],[6],[8] inductively coupled plasma mass spectrometry (ICP-MS),[9],[10],[11] ultraviolet-visible (UV-Vis) absorption, X-ray transmission [12] and the total organic carbon analysis.[13] Other methods not listed in this document can be used and reported in accordance with 'Optional methods' in 6.6.

See Annex B (informative) for measurements.

5.3 Concentration of metal ions

Metal ions, produced as a result of nano-object test sample dissolution, can contribute to observed cell toxicity. The concentration of metal ions in the working suspension shall be measured after separation of particulate matter. Particulate matter can be separated from the ionic fraction by ultra-filtration (U/F), ultra-filtration assisted by centrifugation (C-U/F) tangential flow filtration (TFF) or centrifugation. The measurement shall be made for all metallic elements that are included in the nano-object sample. An appropriate method shall be selected to measure the metal ion concentrations from among inductively coupled plasma-atomic emission spectrometry (ICP-AES), ICP-MS, atomic absorption spectrometry (AAS) and the colorimetric method. It shall be noted that many constituents in culture media such as Na and Cl can interfere with metals analysis for some spectrometry techniques, especially ICP-MS.[14-16] Other methods not listed in this document can be used and reported in accordance with 'Optional methods' in 6.6. Measurement results of concentrations shall be expressed in the unit of molarity, mass/mass or mass/volume. The measurements can be omitted when a toxic effect is not observed to the cells in the working suspensions. See Annex A for an example of flow of measurements.

See Annex C (informative) for measurements.

5.4 Concentration of culture medium components

5.4.1 General

A nano-object sample added to a culture medium to generate a working suspension may adsorb components of the culture medium.[1] This adsorption can result in starvation stress to the test cells. The concentration of protein components and calcium, as surrogates for the nutritional components in the solvent shall be measured after allowing enough time after the addition of nano-object sample to the culture medium. If culture medium components other than protein and calcium that may significantly affect the stability of working suspension for in vitro toxicity assays are known, the concentration of those components shall be measured as well. The measurements can be omitted when a toxic effect is not observed to the cells in the working suspensions. See Annex A for an example of flow of measurements.

NOTE Nano-object sample in culture medium shall be incubated with the same conditions of in vitro test. Nano-objects may affect pH, osmolality, and other essential characteristics in the culture medium.

5.4.2 Proteins

An appropriate method shall be chosen for the protein concentration measurement from among bicinchoninic acid (BCA), Bradford, Lowry, and ultraviolet, refractive index and SLS methods coupled with the asymmetrical-flow field-flow fractionation (AF4).[17,18] When BCA,[19] Bradford [20] or Lowry [21] method are chosen, the protein concentration in the solvent shall be measured after separation of particulate matter from the working suspension. Results of protein concentration measurement shall be expressed in the unit of mass/volume

See Annex D (informative) for measurements.

5.4.3 Calcium

An appropriate measurement method shall be chosen for the calcium concentration measurement from among ICP-AES, ICP-MS, AAS and the colorimetric method. Results of calcium concentration measurement shall be expressed in the unit of molarity, mass/mass or mass/volume.

See Annex D (informative) for measurements.

5.5 Contamination

Contamination can be a source of additional toxic action. Endotoxin and mycoplasma shall be determined with appropriate methods.

Endotoxin measurement are available with Limulus amebocyte lysate (LAL) test [ISO 29701:2010], the chromogenic-based LAL assays,[22] the monocyte activation test (MAT),[23], recombinant Factor C test,[24] and high performance liquid chromatography coupled with mass spectrometry methods[25].

Mycoplasma contamination is one of the major problems in vitro assay. Mycoplasma can be detected by PCR based methods,[26] culture methods,[27] and fluorescence microscopy method [28].

NOTE Nano-object sample shall be treated aseptically and shall be confirmed that there is no history of contamination except described here.

See Annex E (informative) for measurements.

6 Reporting

6.1 General

Measurement and evaluation results obtained according to this International standard shall be reported describing the source and the constituents of the nano-objects, culture medium and serum, as described in the following subclauses.

6.2 Name of nano-objects and manufacturing information

Name, catalogue, and lot/batch number of nano-objects and manufacturer information including name, address and contact information.

6.3 Composition and metallic elements included in the nano-object sample

Define principal and accessory materials, coating materials, catalytic materials and impurities, including their known or estimated quantity.

6.4 Culture medium and serum

Name, manufacturer, and lot/batch number of the medium, type and concentration of added serum (v/v %), pH values of original medium and pH values during assessment, and type and concentration of other additives, if any.

6.5 Measurement results

The following are required to report for individual doses of working suspensions and measurement timeframe. However, the results of the test for contaminants can be reported for the stock of nano-objects instead of individual doses.

Reporting of metal ions, culture medium components, and contaminants are not required when toxicity was not observed for the individual doses of working suspension.

— Stability of working suspension

  a) Representative size change and concentration change

  b) Date of measurement

  c) Employed measurement methods for representative size change and concentration change

  d) Performing institution and data reliability information

  e) Supporting information on preparation method of working suspension

  f) Other special supporting information if any

— Metal ions

  a) Names of metal ions and their concentrations

  b) Date of measurement

  c) Employed measurement method

  d) Performing institution and data reliability information

  e) Other special supporting information if any

— Culture medium components