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

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A list of organizations represented on this committee can be obtained on request to its secretary.

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Nanotechnologies - Essai de détection d'endotoxines sur des échantillons de nanomatériaux pour des systèmes in vitro - Essai au lysat d'amébocyte de Limule (LAL) (ISO 29701:2010)

Nanotechnologien - Endotoxinprüfung an Proben aus nanomaterial für In-vitro-Systeme - Limulus-Amoebozyten-Lysat-Prüfung (LAL-Prüfung) (ISO 29701:2010)

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Foreword

The text of ISO 29701:2010 has been prepared by Technical Committee ISO/TC 229 “Nanotechnologies” of the International Organization for Standardization (ISO) and has been taken over as EN ISO 29701:2010 by Technical Committee CEN/TC 352 “Nanotechnologies” the secretariat of which is held by BSI.

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of an identical text or by endorsement, at the latest by March 2011, and conflicting national standards shall be withdrawn at the latest by March 2011.

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Endorsement notice

The text of ISO 29701:2010 has been approved by CEN as a EN ISO 29701:2010 without any modification.

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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 2.

The main task of technical committees is to prepare International Standards. 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.

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ISO 29701 was prepared by Technical Committee ISO/TC 229, *Nanotechnologies*.

Introduction

Endotoxins (lipopolysaccharides LPS) are part of the outer membrane of the cell wall of Gram-negative bacteria such as *E. coli*, *Salmonella*, *Shigella*, *Pseudomonas*, *Neisseria*, *Haemophilus*. Endotoxins can cause a variety of systemic reactions in mammals, including humans, such as fever, disseminated intravascular coagulation, hypotension, shock and death: the responses are mediated by production of various kinds of cytokines, activation of the complement cascade, activation of the coagulation cascade, etc. Endotoxins are present in the ordinary environment. Since most test samples of nanomaterials intended for *in vitro* and *in vivo* test systems require various preparation procedures, endotoxins might contaminate the test nanomaterials if the samples are prepared without special care.

For the purpose of toxicity screening or biocompatibility testing of nanomaterials, or mechanism studies on the possible toxicity induced by nanomaterials, various cell-based *in vitro* test systems and *in vivo* animal models are being developed and employed. In *in vitro* test systems, macrophages and other relevant mammalian cells are frequently used as the test cells especially for nanomaterials because they are primarily the responsible surveillance cells in the body. However, these cells are highly reactive to endotoxins; therefore it is difficult to distinguish the response to endotoxins from that to nanomaterials. Consequently, contamination by endotoxins would confound the result of tests *in vitro*.

Contamination by endotoxins of test samples may be reduced if appropriate precautions are followed in preparation of the test sample. Therefore the preliminary detection of endotoxins is required to minimize the contamination by endotoxins or confirm the insignificant levels of endotoxins in the test sample. It is also important to quantify endotoxin levels for the adequate interpretation of data obtained by *in vitro* biological test systems.

Since endotoxins may contaminate medical devices and medicines for parenteral use, quantitative and semi-quantitative assay methods to test for endotoxins both *in vivo* and *in vitro* have been developed and used for regulatory purposes as well as laboratory standard operational procedures for nanomaterials (see Reference [6]). The bacterial endotoxin test using *Limulus* amebocyte lysate (LAL) reagent has been developed as an *in vitro* assay method to test for the presence of endotoxin contamination as an alternative to the pyrogenicity test using rabbits, and methods are described in the pharmacopoeia of many countries.

This International Standard provides considerations for the application of the LAL test to nanomaterial samples intended for *in vitro* biological tests.

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

1 Scope

This International Standard describes the application of a test using *Limulus* amebocyte lysate (LAL) reagent for the evaluation of nanomaterials intended for cell-based *in vitro* biological test systems. The test is suitable for use with nanomaterial samples dispersed in aqueous media, e.g. water, serum or reaction medium, and to such media incubated with nanomaterials for an appropriate duration at 37 °C.

This International Standard is restricted to test samples for *in vitro* systems, but the methods can also be adapted to nanomaterials to be administered to animals by parenteral routes.

2 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

2.1
coagulogen
clottable protein in LAL which is known to play a central role in gel-clot formation by endotoxins

NOTE Coagulogen derived from Japanese horseshoe crab (*Tachypleus tridentatus*) consists of a total 175 amino acids with the molecular weight of 19,723 (see Reference [7]).

2.2
coagulin
resulting fragments of coagulogen after limited proteolysis of clotting enzyme in LAL

NOTE A coagulin derived from Japanese horseshoe crab (*Tachypleus tridentatus*) consists of the N-terminal fragment peptides (Ala1 – Arg18) and the C-terminal fragment peptides (Gly47 – Phe175) (see Reference [7]).

2.3
endotoxin
part of the outer membrane of the cell envelope of Gram-negative bacteria

NOTE The main active ingredient is lipopolysaccharides (LPS).

2.4
endotoxin unit
EU
standard unit of endotoxin activity

NOTE 1 The endotoxin unit was defined by the World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) in 1996, relative to the activity of 0,1 ng of WHO reference standard endotoxin (RSE) from *Escherichia coli* 0113:HK10:K(-) or 10 EU/ng (see Reference [8]).

NOTE 2 EU is equal to international unit (IU) of endotoxin.