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Nanoteknologi – Endotoxintest på nanomaterialprov för in-vitrosystem – Limulus amebocytlysats (LAL) test (ISO 29701:2010)

Nanotechnologies – Endotoxin test on nanomaterial samples for in vitro systems – Limulus amoebocyte lysate (LAL) test (ISO 29701:2010)

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EUROPEAN STANDARD

EN ISO 29701

NORME EUROPÉENNE

EUROPÄISCHE NORM

September 2010

ICS 11.100.10; 07.030

English Version

**Nanotechnologies - Endotoxin test on nanomaterial samples for
in vitro systems - Limulus amoebocyte lysate (LAL) test (ISO
29701:2010)**

Nanotechnologies - Essai de détection d'endotoxines sur
des échantillons de nanomatériaux pour des systèmes in
vitro - Essai au lysat d'améboocyte 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)

This European Standard was approved by CEN on 22 August 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of an identical text or by endorsement, at the latest by March 2011, and conflicting national standards shall be withdrawn at the latest by March 2011.

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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* amoebocyte 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* amoebocyte lysate (LAL) test

1 Scope

This International Standard describes the application of a test using *Limulus* amoebocyte 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.