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NATIONAL STANDARD OF THE PEOPLE'S REPUBLIC OF CHINA

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Nanotechnologies - Endotoxin test on nanomaterial samples for in vitrosystems - Limulus amebocyte lysate (LAL) test

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foreword

This document is in accordance with the provisions of GB/T 1.1-2020 "Guidelines for Standardization Work Part 1.Structure and Drafting Rules of Standardization Documents"

drafted.

This document is equivalent to ISO 29701.2010 "Limulus Reagent Method for In Vitro Endotoxin Testing of Nanomaterials in Nanotechnology".

This document adds a chapter "Normative References".

Please note that some content of this document may be patented. The issuing agency of this document assumes no responsibility for identifying patents.

Introduction

Endotoxins (lipopolysaccharide LPS) are gram-negative bacteria such as Escherichia coli, Salmonella, Shigella, Pseudomonas, Neisseria and

Part of the outer membrane of cell walls such as Haemophilus. Endotoxins can cause various systemic reactions in mammals, including humans, such as fever,

Disseminated intravascular coagulation, hypotension, shock, and death. These responses are mediated by a variety of cytokines, complement system and coagulation cascade activation, etc.

guide. Endotoxins are also present in the everyday environment. In the in vitro and in vivo testing systems of nanomaterials, most of the tests have to go through a variety of preparations.

process; special care needs to be taken during preparation, otherwise endotoxins are likely to contaminate the nanomaterials.

For toxicity screening of nanomaterials, biocompatibility testing, and study of toxicity

mechanisms caused by nanomaterials, a variety of substrates have been developed and used.

In vitro test systems and animal models for cells. Among them, macrophages and other related mammalian cells are the main surveillance cells in vivo.

cells, often used as test cells, these cells are highly reactive to endotoxin and it is difficult to distinguish whether the response is caused by endotoxin or nanomaterials

of. Therefore, endotoxin contamination can interfere with the results of in vitro tests.

Following proper precautions when preparing samples to be tested can reduce endotoxin contamination. To minimize endotoxin contamination or to determine

If there is no significant endotoxin in the test sample, preliminary detection of endotoxin is required. In order to fully interpret the data obtained in the in vitro biological test system

It is also important to quantify endotoxin levels.

Endotoxins may also contaminate medical devices and drugs applied by parenteral routes, so quantitative and semi-quantitative assays have been developed,

Detection of endotoxins in vivo and in vitro for regulation and standardization of laboratory procedures related to nanomaterials (see References

[6]). Detection of bacterial endotoxin using Limulus Reagent (LAL) has been developed as an in vitro assay to test for the presence of endotoxin contamination;

As an alternative method for the detection of rabbit pyrogens, the Limulus reagent method has been described in the pharmacopoeia of many countries.

This document provides considerations for the detection of nanomaterial samples using Limulus Reagent (LAL) for in vitro bioassays.

Endotoxin in nanotechnology nanomaterials

In vitro test Limulus reagent method

1 Scope

This document describes the application of the Limulus assay (LAL) to evaluate the endotoxin levels of nanomaterials for in vitro biosystem applications. The test party

The method is suitable for the detection of nanomaterials dispersed in aqueous solutions such as water, serum or reaction solution, and these media can be incubated with nanomaterials at 37 °C

a certain time.

This document is applicable to the detection of in vitro samples, and these methods are

also applicable to the application of nanomaterials to animals by parenteral routes.

Material.

2 Normative references

There are no normative references in this document.

3 Terms and Definitions

The following terms and definitions apply to this document.

3.1

coagulogen

The coagulable protein in the LAL reagent plays a primary role in gel formation caused by endotoxin.

3.2

coagulin

Coagulation protein is digested by coagulation protease in LAL reagent.

47-Phenylalanine 175) (see reference [7]).

3.3

endotoxin

Part of the outer component of the cell wall of Gram-negative bacteria.

NOTE. The main active ingredient is lipopolysaccharide (LPS).

3.4

endotoxinunit

EU

Standard unit of endotoxin activity.

Note 1.According to the definition of endotoxin unit formulated by the World Health Organization (WHO) Expert Committee on Biological Standardization (ECBS) in.1996, 0.1ng of Escherichia coli

The 0113.HK10.K(-)-derived WHO Reference Standard for Endotoxin (RSE) has an activity of 10 EU/ng (see reference [8]).

Note 2.EU is equivalent to the International Unit (IU) of endotoxin.

3.5

lambda