SLOVENSKI STANDARD
SIST EN 14031:2003

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Zrak na delovnem mestu - Določ evanje lebdeč ih endotoksinov

Workplace atmospheres - Determination of airborne endotoxins

Arbeitsplatzatmosphäre - Bestimmung von luftgetragenen Endotoxinen

Atmospheres des lieux de travail - Détermination des endotoxines en suspension dans l'air

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Workplace atmospheres - Determination of airborne endotoxins

Atmosphères des lieux de travail - Détermination des endotoxines en suspension dans l'air

Arbeitsplatzatmosphäre - Bestimmung von luftgetragenen Endotoxinen

This European Standard was approved by CEN on 21 November 2002.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Contents

Foreword

This document (EN 14031:2003) has been prepared by Technical Committee CEN/TC 137 "Assessment of workplace exposure", the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by July 2003, and conflicting national standards shall be withdrawn at the latest by July 2003.

Annexes A and B are informative.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

Endotoxins are integral components of the outer membrane of gramnegative bacteria which are composed of proteins, lipids, and lipopolysaccharides. The term 'endotoxin' refers to the toxin as present on the bacterial cell wall. Lipopolysaccharides of gram negative bacteria refer to a class of pure lipid carbohydrate molecules (free of protein and other cell wall components) that are held responsible for most of the biological properties characteristic of bacterial endotoxins. In annex A a brief overview is given with respect to physical and chemical properties of endotoxins and sources of exposure in the occupational environment.

Endotoxins are believed to play an important role in the development of organic dust related diseases in exposed workers.

It is important to assess occupational exposure to airborne endotoxins in a representative way to evaluate the exposure. There are, however, at present no generally accepted procedures for the measurement of environmental endotoxins, thus different practices continue to exist. Rigorous standardization with respect to sampling media, extraction media, analytical methods and storage conditions for endotoxins are needed to obtain results that are comparable between studies. By adhering to the recommendations outlined in this standard for choice of sampling, storage of samples, extraction and analytical procedures uncertainties in exposure assessment can be reduced and controlled, allowing comparable and representative measurements to be made.

1 Scope

This European Standard provides guidelines for the assessment of workplace exposure to airborne bacterial endotoxins. The standard provides methods for sampling, transportation, and storage of samples and determination of endotoxins.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate place in the text, and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references, the latest edition of the publication referred to applies (including amendments).

EN 481:1993, *Workplace atmospheres – Size fraction definitions for measurement of airborne particles.*

EN 1232, *Workplace atmospheres – Pumps for personal sampling of chemical agents – Requirements and test methods.*

EN 12919, *Workplace atmospheres – Pumps for the sampling of chemical agents with a volume flow rate of over 5 l/min - Requirements and test methods.*

3 Terms and definitions

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

3.1
bioaerosol
airborne particles with biological origin

NOTE See EN 13098:2000, 3.3, notes.

3.2
control standard endotoxin
standard that is traceable to the RSE

3.3
endotoxin
see EN 13098:2000.

3.4
endotoxinfree liquids
water and solutions with <0,2 EU/ml

NOTE Endotoxinfree and pyrogenfree are used interchangeable in the literature.

3.5
endotoxin unit
see EN 13098:2000

NOTE Endotoxin concentrations in samples are referenced to a defined reference standard endotoxin (RSE) and subsequently expressed in units based on the activity of the reference material in a LAL assay.

3.6
exposure (by inhalation)
situation in which a chemical or biological agent is present in air which is inhaled by a person

[EN 1540:1998]

3.7
inhalable particle size fraction
inhalable fraction which constitutes the mass fraction of total airborne particles which is inhaled through the nose and mouth

[EN 481:1993]

3.8
LAL-assay
functional assay to measure endotoxin concentrations

NOTE The method is highly sensitive and based on the activation of a clotting enzyme present in the lysate of hemolymph of the *Limulus polyphemus* (Horseshoe crab).

3.9
lipopolysaccharides
LPS present in gramnegative bacteria are water-soluble and stable molecules composed of lipid and polysaccharide

NOTE The lipid part of LPS is termed 'lipid A' and is essential for the toxic properties of LPS. The terms *'endotoxins'* and *'lipopolysaccharides'* are often used interchangeably in the scientific literature.

3.10
reference standard endotoxin
purified LPS from *Escherichia coli* that serves as an international reference standard

4 Abbreviations

CSE control standard endotoxin

EU endotoxin units

LAL limulus amoebocyte lysate

LPS lipopolysaccharides

RSE reference standard endotoxin

5 Overall demands in endotoxin work

5.1 General

Environmental monitoring of endotoxins is performed by sampling inhalable aerosols. Aerosols are sampled on a filter using a pump to draw air through the filter. For assessing occupational exposure personal sampling shall be used for example when comparing with exposure guidelines. Area sampling can be used to identify sources of exposure.

5.2 Demands on operator

The operator that performs the sampling shall be trained in aseptic work conditions, have knowledge of sampling equipment and know how to carry out the sampling. The operator shall avoid contamination of the sample during all phases of sampling.

5.3 Demands on equipment

The pumps shall fulfil the requirements of the standards for pumps for personal and static sampling (EN 1232 and EN 12919). Personal samplers shall collect the inhalable aerosol fraction as defined by EN 481.

The filter holder or cassette should be rendered endotoxinfree. Prior to sampling, reusable samplers should be cleaned thoroughly so that contamination of the samples are avoided. Cleaning agents shall be removed by subsequent washing in endotoxinfree water. Disposable samplers do not need to be sterilised when one can assume the manufacturing process to be fairly endotoxinfree. Possible contamination shall be controlled by using blank filters (see 5.9).

5.4 Demands on glassware

All other materials used during sampling, transportation and storage shall be rendered endotoxinfree. Glassware can be rendered endotoxinfree by heating at least at 180 °C for 4 h.

5.5 Demands on extraction liquids, water and detergents

All liquids used shall be endotoxinfree.

5.6 Demands on filters

Binderfree glass fibre filters should be used for endotoxin sampling while these filters usually give the highest recoveries. Other types of filters can be used. If any other filter is used it shall be validated toward filters of glass fibre.

NOTE Several types of filters (cellulose esters, polyvinylchloride, polytetrafluoroethylene and polycarbonate) are commonly used but some material adsorb endotoxins and thereby give false low values.

5.7 Transportation of samples

After sampling filters shall be prepared for transport by sealing in a container or sampler cassette. The sample shall be transported in dry conditions, preferably with a dehumidifier. Samples can be transported at room- or outside temperature but conditions for transport shall be documented and include transportation time, temperature etc. If transportation time is over 24 h the samples shall be transported in a condition that prevents the filters from getting moist during transportation, i.e. with a dehumidifier or frozen.

5.8 Storage of samples at the laboratory

Samples shall never be repeatedly frozen and thawed, as this may effect the detectable endotoxin content of the sample. Endotoxins are very stable when frozen. If the sample is not extracted within a few days after arrival to the laboratory the sample shall be stored at approximately –20 °C or below.

5.9 Test for contamination

To test for contamination a series of at least 2 blank filters shall be included in each sampling session. Except for the actual sampling, blank filters shall be treated equal to the other filters that are used for sampling.

5.10 Sampling documentation

A list of sampling operations shall be documented to obtain comparable and reliable concentration values of endotoxins. The sampling document shall include at least the following parameters:

- name of the organization and operator performing the sampling;
- date of sampling;
- a unique identifier code for the sample;
- name and address of the plant where sampling was carried out, or a unique identifier to preserve confidentiality;
- type of sampling (personal or static) and in case of personal sampling: name and function of the person who was involved in the sampling, or a unique identifier to preserve confidentiality; or in case of static sampling: location of sampling;
- type of sampler and type of filter used;
- start and end time of sampling;
- flow rate before and after sampling period;
- sampled volume;
- details about sample transportation and storage (temperature, duration, humidity);
- other relevant observations from the operator.

6 Extraction

6.1 General

The following paragraphs describe a standard method for the extraction of endotoxins from filters. If other equipment and methods are used they shall be validated against this described method.

6.2 Equipment

A standard shaker apparatus able to vigorously shake the sample in extraction fluid should be used. In addition a standard table centrifuge is needed that is able to centrifuge at 1 000 $\times$ *G*. Endotoxinfree glass or polypropylene tubes shall be used throughout the extraction procedure.