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Izpostavljenost na delovnem mestu - Kvantitativno določevanje lebdečih endotoksinov

Workplace exposure - Quantitative measurement of airborne endotoxins

Exposition am Arbeitsplatz - Quantitative Messung von luftgetragenen Endotoxinen

Exposition sur les lieux de travail - Mesure quantitative des endotoxines aéroportées

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Workplace exposure - Quantitative measurement of airborne endotoxins

Exposition sur les lieux de travail - Mesure quantitative des endotoxines aéroportées

Exposition am Arbeitsplatz - Quantitative Messung von luftgetragenen Endotoxinen

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European foreword

This document (EN 14031:2021) has been prepared by Technical Committee CEN/TC 137 “Assessment of workplace exposure to chemical and biological agents”, 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of an identical text or by endorsement, at the latest by January 2022, and conflicting national standards shall be withdrawn at the latest by January 2022.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes EN 14031:2003.

The major technical changes between this document and the previous edition are as follows:

a) document title adjusted to the wording used in the Scope;

b) terms and definitions taken over from EN 13098:2019, where appropriate;

c) Clause 4 restructured;

d) requirements for sample storage at the laboratory rewritten;

e) recommendations for extraction of samples revised;

f) storage conditions described more precisely;

g) further requirements on documentation of extraction added;

h reference given to the recombinant Factor C (rFC) method as Note to 6.1;

i) new Table A.1 with examples on working areas with exposure to endotoxin added;

j) new Annex C on deposition of endotoxins on inner sampler walls added;

k) whole document editorially revised.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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Introduction

The term 'endotoxin' refers to lipopolysaccharides that are present in the outer membrane of the cell walls of Gram-negative bacteria. These lipopolysaccharides are 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.

Endotoxins play an important role in the development of organic dust-related symptoms (for example, inflammatory reactions in the airways and/or systemic reactions) among workers exposed via inhalation.

To investigate ill health resulting from occupational exposure to airborne endotoxins it is important therefore to measure their presence in workplace air accurately. At present, different methods are used to measure airborne endotoxins. Standardization with respect to sampling, transportation, extraction, analytical methods and storage of samples or extracts is important in order to obtain accurate and comparable results and reduce uncertainties in exposure assessment.

1 Scope

This document specifies methods for the quantitative measurement of airborne endotoxins and gives general requirements for sampling on filters, transportation, storage as well as the analysis of samples.

This document provides also guidelines for the assessment of workplace exposure to airborne endotoxins.

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.

EN 481, *Workplace atmospheres — Size fraction definitions for measurement of airborne particles*

EN 1540, *Workplace exposure — Terminology*

EN ISO 13137, *Workplace atmospheres — Pumps for personal sampling of chemical and biological agents — Requirements and test methods (ISO 13137)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 1540 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

Note 1 to entry: In particular, the following terms used in this document are defined in EN 1540: aerosol, biological agent, bioaerosol, exposure (by inhalation), inhalable fraction, measuring procedure, personal sampling, static sampling and workplace.

3.1
control standard endotoxin
CSE
standard that is traceable to the reference standard endotoxin (RSE)

3.2
endotoxin
constituent of the external membrane of Gram-negative bacteria (lipopolysaccharide), consisting of a complex lipid, lipid A, which is covalently bound to a polysaccharide

Note 1 to entry: “Free endotoxin” is liberated after cell death and by budding from living cells. Lipid A is the active (toxic) part and is a potent pro-inflammatory substance and can induce febrile, bronchial and other symptoms in exposed workers. The composition and the toxicity of endotoxin differ between bacteria species.

Note 2 to entry: A brief overview with respect to physical and chemical properties of endotoxins and sources of occupational exposure is given in Annex A.

[SOURCE: EN 13098:2019, 3.9 – modified, Note 2 to entry added]

3.3
endotoxinfree liquid
water or solution containing no measurable endotoxin

Note 1 to entry: Endotoxinfree and pyrogenfree are used interchangeably in the literature.

Note 2 to entry: In practise, less than 0,2 endotoxin units per millilitre is often used as threshold value for endotoxinfree.

3.4
endotoxin unit
EU
unit standardized against the defined reference material, reference standard endotoxin

[SOURCE: EN 13098:2019, 3.10]

3.5
Limulus Amoebocyte Lysate
LAL
enzymes extracted from the blood cells of the horse shoe crab (*Limulus polyphemus*) that are activated by endotoxin and other molecules (glucans etc.)

[SOURCE: EN 13098:2019, 3.16]

3.6
Limulus Amoebocyte Lysate-assay
LAL-assay
functional assay to measure endotoxin concentrations

Note 1 to entry: The method is highly sensitive and based on the activation of a clotting enzyme present in the lysate of hemolymph of the horseshoe crab (*Limulus polyphemus*).

3.7
lipopolysaccharide
LPS
water-soluble and stable molecule composed of lipid and polysaccharide present in Gram-negative bacteria

Note 1 to entry: The lipid part of LPS is termed 'lipid A' and is essential for the toxic properties of LPS.

Note 2 to entry: The terms *'endotoxin'* and *'lipopolysaccharide'* are often used interchangeably in scientific literature regarding occupational health.

3.8
reference standard endotoxin
RSE
purified lipopolysaccharide from *Escherichia coli* that serves as an international reference standard

4 Requirements

4.1 Skills needed for sampling

The person who takes the sample shall be trained in aseptic techniques to avoid contamination of the sample during any measurement phase. This includes knowledge about sampling equipment and how to perform the sampling.

4.2 Sampling

4.2.1 General

Measurement of airborne endotoxins should preferably be performed by sampling of the inhalable fraction of aerosols. Aerosols shall be sampled on a filter using a pump to draw air through the filter.

Personal sampling shall be used for risk evaluation including comparison with exposure guidelines.

Static sampling can be used to identify sources of exposure and to measure background concentrations of airborne endotoxins.

NOTE Other comparable sampling methods can be used for evaluation of other specific parameters, for example, to measure endotoxins in different size distributions or particle size fractions, or for short- or long-term exposure measurement.

4.2.2 Equipment

The pumps used for personal sampling and static sampling shall fulfil the requirements specified in EN ISO 13137. Personal samplers shall collect the inhalable fraction of aerosols as specified in EN 481.

The filter holder or cassette should be rendered endotoxinfree. Heat-resistant reusuable filter holder/transportation jars shall be made endotoxinfree by heating up according to 4.5. Transportation jars that do not meet these requirements shall be cleaned thoroughly so that contamination of the samples is avoided. Cleaning agents shall be removed by subsequent washing in endotoxinfree water. Disposable filter holder do not need to be sterilized when it is assumed that they are endotoxinfree due to the manufacturing process. Possible contamination shall be controlled by examination of blank samples (see 4.7).

4.2.3 Filters

Binderfree glass fibre filters should be used for sampling of airborne endotoxins because these filters perform very well regarding their collection efficiency, blank values and recovery. If any other filter is used the performance of these parameters shall be comparable.

NOTE 1 The type of filter used when sampling endotoxins from the air has a strong influence on the measurement results. Several studies report higher measured concentrations when glass fibre filters were used. However, in other studies, concentrations measured with the use of PVC, PC or PTFE filters were equivalent to or even higher than those measured with glass fibre filters. The effect of the nature of the filter seems to be multifactorial and probably involves parameters as diverse as the filter material itself (possibility of adsorption of endotoxins on the filter, interference during analysis), the sampling method, the extraction protocol etc.. In the current state of knowledge, it is difficult to give precise recommendations concerning the choice of filters and further studies are still necessary for that purpose.

NOTE 2 Several filter materials (for example, polyvinylchloride, polytetrafluoroethylene and polycarbonate) are commonly used but some materials adsorb endotoxins and thereby give false low values.

4.3 Sample transport

After sampling, filters shall be prepared for transport by sealing in a container or sampler cassette. The sample shall be transported in conditions that prevent large variations in temperature and the gaining of moisture. If transport time is above 24 h, or there is risk of large temperature variations, actions to prevent moisture shall be taken, either by using an isolating container or by including a dehumidifier.

Conditions during transport shall be documented, including transport time and temperature log.

If the temperature is above 30 °C the filters shall be placed in cooler conditions (for example, by using a cooling box).

4.4 Sample storage at the laboratory

If the sample is not extracted within two weeks after arrival at the laboratory the sample shall be stored in conditions that prevent large variations in temperature and gain of moisture. Alternatively, for storage of more than two weeks they should be frozen at a temperature of about – 20 °C or below, because endotoxins are very stable when frozen. However, samples shall not be frozen and thawed more than once, as this can affect the detectable endotoxin content of them.

4.5 Glassware

Glassware shall be rendered endotoxinfree, for example, by heating to at least 180 °C for about 4 h.

4.6 Extraction liquids, water and detergents

All liquids used shall be endotoxinfree.

4.7 Test for contamination

To test for contamination at least two filters shall be included in each sampling run as blank samples. Except for the actual sampling, the filters used as blank samples shall be treated in the same way as the other filters that are used for sampling.

NOTE If not more than four samples are collected, one blank sample can be seen as sufficient.

4.8 Sampling documentation

The sampling operations carried out shall be documented to obtain comparable and reliable concentration values of endotoxins.

The sampling documentation shall include at least the following information:

a) name of the organization and person performing the sampling;

b) date of sampling;

c) purpose of sampling;

d) a unique identifier code for the sample and sampling device (for example, sampling pump, used);

e) name and address of the company where the sampling was carried out, or a unique identifier to preserve confidentiality;

f) workplace description including bioaerosol generating activities;

g) type and name of sampler used;

h) type and name of filter used;

i) 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, placement of sampling equipment;

j) positioning of sampling equipment;

k) location of sampling inlet and orientation relative to air movement, where applicable;

l) start and end time of sampling and its duration;

m) flow rate, in litres per minute, at least before and after sampling period;

n) sampled volume;

o) sampled health-related fraction (see EN 481);

p) details about sample transport and sample storage;

q) environmental conditions during sampling (temperature, relative humidity, outdoor weather conditions);

r) other relevant observations.

5 Extraction

5.1 General

Samples should not be extracted unless they can be analyzed immediately. If this is not possible, extracts may be stored refrigerated overnight. For long-term storage, extracts may be frozen as specified in 5.4.

5.2 and 5.3 specify the equipment to be used in a recommended method for the extraction of endotoxins from filters as well as the extraction procedure. If other equipment and methods are used they shall be demonstrably equivalent in performance to this recommended method.

NOTE For deposition of endotoxins in inner sampler walls see Annex C.

5.2 Equipment

5.2.1 Laboratory shaker, able to shake the sample vigorously in extraction liquid.

5.2.2 Vortex shaker.

5.2.3 Centrifuge, able to centrifuge at 1 000 × *G.*

5.2.4 Tubes or containers, made of glass or polypropylene, endotoxinfree.

5.3 Procedure

The loaded filter shall be transferred aseptically into a tube or container. Extraction shall be performed in endotoxinfree water using a method validated to maximize extraction.

NOTE 1 Due to lack of data no recommendation can be given for use of detergents in the extraction procedure.

For filters with a diameter of less than 50 mm a minimum of 5 ml of an extraction liquid should be used, and a minimum of 10 ml for filters with a diameter of 50 mm or more. In order to obtain maximum extraction of endotoxin into suspension, samples are rocked/shaken for about 1 h at a speed of at least 2 000 min^{-1} using the laboratory shaker at room temperature.

Before extraction the pH value should be measured in a subsample of the extraction liquid to ensure compatibility with the performance of the LAL-assay and to comply with the recommendations given by the manufacturer of the LAL-assay kit used.

NOTE 2 The pH value of bioaerol extracts is normally in the range from 6 to 8 which usually complies with the recommendations given by manufacturers of LAL-assay kits.

Samples shall be centrifuged for about 15 min at about 1 000 × *G*. Supernatant shall be collected, well mixed and divided into aliquots of extracts.