SLOVENSKI STANDARD
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Zrak na delovnem mestu - Navodilo za merjenje lebdečih mikroorganizmov in endotoksinov

Workplace atmosphere - Guidelines for measurement of airborne micro-organisms and endotoxin

Arbeitsplatzatmosphäre - Leitlinien für die Messung von Mikroorganismen und Endotoxin in der Luft

Atmospheres des lieux de travail - Regles pour le mesurage de micro-organismes et d'endotoxine en suspension dans l'air

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Workplace atmosphere - Guidelines for measurement of airborne micro-organisms and endotoxin

Atmosphères des lieux de travail - Règles pour le mesurage de micro-organismes et d'endotoxine en suspension dans l'air

Arbeitsplatzatmosphäre - Leitlinien für die Messung von Mikroorganismen und Endotoxin in der Luft

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Foreword

This European Standard 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of an identical text or by endorsement, at the latest by March 2001, and conflicting national standards shall be withdrawn at the latest by March 2001.

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, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

This European Standard has been prepared by Technical Committee CEN/TC 137 "Assessment of workplace exposure", the secretariat of which is held by DIN.

Annexes A, B, C and D are informative.

This standard includes a bibliography.

Introduction

Assessing occupational exposure to airborne microbial contaminants in a representative way is a challenging task. It is necessary however that information can be gathered to evaluate and to minimise exposure to biological agents. The sampling equipment used often introduces its own critical limitations, as in the assessment of aerosol fractions. Some sampling equipment may be capable only of measuring culturable micro-organisms, while others allow the characterisation of both the total number of organisms and the culturable fraction. Analytical procedures may add further to the difficulties and the uncertainties, e.g. the method used may not allow the identification of the biological agents present, or may cause unwanted interference between different biological agents. However, by adhering to the principles outlined in this standard for choice of sampling and analytical procedures, these uncertainties can be reduced and controlled, allowing comparable and representative measurements to be made.

1 Scope

The European Standard provides guidelines for the assessment of workplace exposure to airborne micro-organisms including the determination of total number and culturable number of micro-organisms in the workplace atmosphere. The standard also provides methods for measurement of airborne endotoxin in the work environment.

The European Standard does not apply to viruses, specific pathogenic micro-organisms and toxins other than endotoxin, although some of the measurement principles may be the same.

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. 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, *Workplace atmospheres - Size fraction definitions for measurement of airborne particles*

EN 482, *Workplace atmospheres - General requirements for the performance of procedures for the measurement of chemical agents*

EN 689, *Workplace atmospheres - Guidance for the assessment of exposure by inhalation to chemical agents for comparison with limit values and measurement strategy*

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*

ISO 7218, *Microbiology of food and animal feeding stuffs - General rules for microbiological examinations*

3 Terms and definitions

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

3.1
actinomycetes
varied group of rod-shaped to filamentous Gram-positive bacteria.

NOTE Filamentous actinomycetes form a branching network of thin filaments called a mycelium. Most actinomycetes replicate by conidia-like spores which can easily be made airborne.

3.2
bacteria
large group of prokaryotic micro-organisms with one chromosome in a nuclear region and which replicate only asexually by cell division.

NOTE Different cell-wall chemistry is used for the classification of Gram-positive and Gram-negative bacteria. Morphological criteria divide into spheres (cocci) and rods. Some species produce endospores as survival units.

3.3
bioaerosol
airborne particles with biological origin

NOTE 1 In this standard, micro-organisms and endotoxin are considered.

NOTE 2 The majority of bioaerosol particles are complex as far as size, shape and constituent elements are concerned

3.4
biological preservation efficiency
the capacity of the sampler to maintain the viability of the airborne micro-organisms during collection and also to keep the microbial products intact.

3.5
colony forming unit
the unit by which the culturable number is expressed.

NOTE 1 One colony forming unit can originate from one single micro-organism, an aggregate of many micro-organisms or from one or many micro-organisms attached to one particle.

NOTE 2 The number of outgrown colonies may depend on cultivation conditions.

3.6
culturable number
the number of micro-organisms, single cells or aggregates able to form colonies on a solid nutrient medium.

NOTE The viable number (see 3.21) includes all potentially metabolic active micro-organisms. Some micro-organisms are viable but are not necessarily culturable

3.7
elevated level
a level above normal background level of micro-organisms in a specified environment.

3.8
endotoxin
a 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 "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 may induce febrile, bronchial and other symptoms in exposed workers. The composition and the toxicity of endotoxin differs between species.

3.9
endotoxin unit
a unit standardized against the defined reference material (Reference standard endotoxin).

3.10
exposure (by inhalation)
a situation in which a chemical or biological agent is present in air which is inhaled by a person (see EN 482).

3.11
filtration
collection of particles suspended in gas or liquid by flow through a porous medium.

3.12
fungi
a diverse group of eukaryotic micro-organisms with membrane-bound nucleus comprising several chromosomes.

NOTE Multiplication is mainly asexual but several groups replicate also by sexual spores. Filamentous fungi (moulds) grow in lengthy hyphae and form compact tufts called mycelia. Asexual spores (conidia) are easily made airborne. Yeasts are usually unicellular, of spherical shape and their cells multiply sexually or asexually by budding.

3.13
impaction
collection of airborne particles accelerated through a nozzle or orifice on a surface by inertia effect.

3.14
impingement
combination of impaction onto a surface and subsequent dispersion into a liquid medium.

3.15
Limulus Amoebocyte Lysate
enzymes extracted from the blood cells of the horse shoe crab (Limulus polyphemus) that are activated by endotoxin.

3.16
micro-organisms
any microbiological entity, cellular or non cellular, capable of replication or of transferring genetic material, or entities that have lost these properties.

NOTE The term micro-organism covers the term of biological agent, according to the Directive 90/679/EEC: micro-organisms, including those which have been genetically modified, cell cultures and human endoparasites which may be able to provoke any infection, allergy or toxicity.

3.17
physical sampling efficiency
the capacity of the sampler to collect particles with specific sizes suspended in workplace air.

3.18
sieve sampler
multi-orifice impactor.

3.19
total sampling efficiency
the product of the physical sampling efficiency and the biological preservation efficiency.

3.20
total number of micro-organisms
the number of micro-organisms determined as single organisms (or a corresponding measure).

NOTE Both the viable and the non-viable micro-organisms are included.

3.21
viable number of micro-organisms
the number of micro-organisms having a potential for metabolic activity.

NOTE A viable micro-organism is not necessarily culturable, which means that the number of culturable micro-organisms may underestimate the viable number.

3.22
work pattern
the definable series of activities from the periods under consideration (see EN 689).

3.23
workplace
the workplace is the defined area or areas in which the work activities are carried out (see EN 689).

4 Symbols and abbreviations

ATP Adenosine triphosphate

CFU Colony forming unit

CV Coefficient of variation

DNA Deoxyribonucleic acid

EU Endotoxin unit

GSD Geometric standard deviation

LAL Limulus Amoebocyte Lysate

LPS Lipopolysaccharide (see 3.8)

SEM Scanning electron microscope

5 Measurement of micro-organisms and endotoxin

5.1 Biological agents and biological properties

Bioaerosols may contain different micro-organisms and/or different components originating from these. Micro-organisms may be classified in different taxonomic groups like Gram-positive and Gram-negative bacteria, actinomycetes, fungi, protozoa, algae and viruses. These may be further classified to genus or species level. Immunologic reactions e.g. allergic and/or toxic reactions can result from exposure to micro-organisms irrespective of their viability.

NOTE Species identification may be useful in studies of specific allergic and toxic reactions.

5.2 Aim of measurement

The measurement of micro-organisms and endotoxin in the workplace air has two objectives:

- to assess workers´ exposure; and/or
- to assess the biological characteristics of air at different locations and/or at different times and over different intervals of time.

It is essential to state the purpose of the measurement and how the results will be interpreted.

NOTE Measurement tasks could be to locate the sources emitting micro-organisms, to measure a worker´s daily or work shift exposure, to identify peaks in exposure, to test the efficiency of control measures, or to control actions taken to diminish the exposure. It is essential to verify that the sampling and analysis corresponds to the assessment objectives and to the search for the targeted component of the bioaerosol (see 5.1). It can be appropriate to be able to measure both viable and non-viable bio-aerosols.

5.3 Measurement options

The following can be used to measure micro-organisms and endotoxin:

- microbial cells by direct counting (the total number);
- microbial cells and cell aggregates by culturing on agar media (the culturable number);
- cellular components of micro-organisms, from viable, non-viable or disintegrated micro-organisms, e.g. constituents of cell structure which may also have inflammatory properties, such as endotoxin and glucans;
- primary metabolites (e.g. ATP) which may serve as markers for micro-organisms or of their vital activity;
- secondary metabolites (e.g. mycotoxins) which may be found in the micro-organisms and other particles in the aerosol.

5.4 Uncertainty of the measurements

The uncertainty in measurements originates from the sampling method and the method of analysis. Sampling and analytical methods shall be validated. Reproducibility shall be determined.

NOTE Validation of methods for measurements of micro-organisms is limited by lack of reference materials and/or reference methods.

5.5 Variability of exposure level

The variability in exposure levels of micro-organisms can be very high and much higher than the precision of measurement methods.

NOTE Geometric standard deviations (GSD) of 4 to 6, for a measurement with a duration of 8 h are not unusual. As a consequence, the uncertainty in the estimation of long term exposure from a single measurement is high.

For example, if the geometric mean is $4 \cdot 10^5$ m^{-3} micro-organisms and the GSD is 5, then the 95 % confidence interval of one measurement is $1,6 \cdot 10^4$ m^{-3} to 10^7 m^{-3}. Shorter sampling periods will further increase the uncertainty.

Identification of the causes of the variability can help to reduce the measurement effort by stratified sampling of special tasks or exposure situations.

6 Sampling

6.1 Principles of sampling

Sampling of aerosols of microbiological origin should be made in accordance with the principles of sampling to assess workers' exposure to other substances hazardous to health. Static or personal exposure to bioaerosols can be intermittent and of short duration, and be related to specific work activities. Nevertheless bioaerosols can be capable of eliciting a health effect if the exposure is of sufficient intensity. At other times during work, exposure to bioaerosols can be minimal. To obtain a relative profile of exposure, it is recommended that several consecutive measurements are performed according to a specific strategy. This is also consistent with other sampling procedures for bioaerosol measurement, such as direct collection onto agar media, which dictate that the sampling period should be of short duration.

Measurements of micro-organisms should follow sampling methodology guidelines under EN 689.

6.2 Samplers available

A wide range of bioaerosol sampling devices are available. However, they broadly fall into three categories according to their principles of collection, as follows:

- impaction onto a solid or a semi-solid surface such as agar medium;
- impingement into liquid;
- filtration.

Some methods are appropriate only for static sampling, while others can also be used for personal sampling from workers' breathing zones. It is necessary to be aware of the principles of collection and of their advantages and limitations, as some will be better than others for sampling in some work environments. The method of sampling will often determine the subsequent analytical procedures.

NOTE For recommendations for selection of measurement methods see annex A.

6.3 Requirements of the sampler

The sampler shall be fit for the purpose of the measurement to be done and shall be compatible with the analysis requirements.

The sampler used shall have a known and documented sampling efficiency, e.g. capable of sampling total micro-organisms, viable micro-organisms or microbial components. The physical sampling efficiency of the sampler shall be known and documented and related to the aerodynamic diameter of collected particles. The sampler should be able to collect precise aerosol fractions, as defined by EN 481. To determine the viable number/culturable number of micro-organisms, the sampler shall be tested with relevant micro-organisms to establish the preservation efficiency. The ability of sampling medium to maintain the integrity of the sampled organisms shall be known.

6.4 Pumps

The pumps shall fulfil the requirements of the standards for pumps for personal and static sampling (EN 1232 and EN 12919) respectively.

6.5 Demands on the operator

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

6.6 Sampling recommendations

Before carrying out any sampling operation at the workplace, it is necessary to examine the technical and plant specific parameters in the working area and to consider the operating procedures which may influence the worker's exposure to micro-organisms, in order to set up a sampling strategy.

When a sampling programme has been drawn up, sampling operations shall follow the applicable parts of the sampling strategy in EN 689 and the sampling protocol (see 6.7).

6.7 Sampling documentation

A list of sampling operations shall be documented to obtain comparable and reliable concentration values of micro-organisms or endotoxin.

The sampling documentation shall include at least the following parameters:

- name of the organisation and person performing the sampling;
- date of sampling;
- a unique identifier code for the sample;
- name and address of the company where the sampling was carried out, or a unique identifier to preserve confidentiality;
- location of sampling;
- type and name of sampler and type and name of collection substrate used including volume of collection medium used for liquid samplers;
- type of sampling (personal or static);
- location of sampling equipment;
- location of sampling inlet and the orientation relative to air movement;
- sampling start and end time and duration;
- flow rate (L/min);
- sampled volume;