SLOVENSKI STANDARD
SIST-TS CEN/TS 16115:2011

01-december-2011

Kakovost zunanjega zraka - Meritve bioaerosolov - 1. del: Določevanje gliv z uporabo sistemov vzorčenja s filtri in analizatorji, temelječimi na kulturi

Ambient air quality - Measurement of bioaerosols - Part 1: Determination of moulds using filter sampling systems and cultivation based analyses

Luftbeschaffenheit - Messen von Bioaerosolen - Teil 1: Bestimmung von Schimmelpilzen mittels Probenahme auf Filtern und kulturellem Nachweis

Qualité de l'air ambiant - Mesurage de bioaérosols - Dosage des moisissures à l'aide de systèmes de prélèvement sur filtres et d'analyses de cultures

Ta slovenski standard je istoveten z: **CEN/TS 16115-1:2011**

ICS:

13.040.20 Kakovost okoljskega zraka Ambient atmospheres

SIST-TS CEN/TS 16115:2011 **en,fr,de**

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 16115-1

April 2011

ICS 13.040.20

English Version

Ambient air quality - Measurement of bioaerosols - Part 1: Determination of moulds using filter sampling systems and culture-based analyses

Qualité de l'air ambiant - Mesurage de bioaérosols - Partie 1: Dosage des moisissures à l'aide de systèmes de prélèvement sur filtres et d'analyses de cultures

Luftbeschaffenheit - Messen von Bioaerosolen - Teil 1: Bestimmung von Schimmelpilzen mittels Probenahme auf Filtern und kulturellem Nachweis

This Technical Specification (CEN/TS) was approved by CEN on 4 October 2010 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

Foreword

This document (CEN/TS 16115-1:2011) has been prepared by Technical Committee CEN/TC 264 “Air quality”, the secretariat of which is held by DIN.

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

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to announce this Technical Specification: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

Airborne particles of biological origin are called bioaerosols. Depending on the emission source bioaerosols vary in composition; one component of ambient bioaerosols with possible ecological and health relevance can be moulds. Natural and anthropogenic sources for mould spores are widely distributed in the environment. Anthropogenic sources can for example be agriculture and construction activities or waste treatment.

Mould is a common name for filamentous fungi from different taxonomic groups (Zygomycetes, Ascomycetes, Deuteromycetes). They form a mycelium (hyphae) and spores – namely conidiospores (conidia), sporangiospores or ascospores – by which they become visible macroscopically. Most spores are in the size range of 2 µm to 10 µm, some up to 30 µm and only few up to 100 µm. Spores of some mould genera are small and become airborne very easily (e.g., *Aspergillus, Penicillium*) while others are bigger and/or embedded in a slime matrix (e.g., *Stachybotrys, Fusarium*) and less mobile.

The procedure described in this document is based on VDI 4252 Part 2 [1], VDI 4253 Part 2 [2] and is related to the ISO standards on indoor air ISO 16000-16 [3] and ISO 16000-17 [4].

1 Scope

This Technical Specification describes the measurement of moulds in ambient air in order to identify, quantify and characterize bioaerosol pollution in ambient air resulting from emissions from different sources.

The method described specifies the sampling of moulds as part of the suspended particulate matter (SPM, here particles with aerodynamic diameter up to ca. 30 µm) using a filter sampling system with gelatine/polycarbonate filter combination followed by the culture-based analyses on DG18 agar. The sampling duration can be varied between 10 min to 24 h. The health effect of bioaerosols is not limited to any particle fraction, therefore, this document describes the sampling of moulds as part of the suspended particulate matter as a convention method.

NOTE The sampling method described in this document in principle is likely to be appropriate for the sampling of actinomycetes and other spore-forming bacteria (resistant to desiccation). For these species a special analytical procedure using different culture media should be applied, but this is not within the scope of this document.

The standard method set out in this Technical Specification is accepted by convention as reference method. The measured quantity, here the number of colony forming units per cubic meter (CFU/m^3), is determined by the inlet design of the sampling head, the associated operational parameters and the analytical procedure.

Standardized methods for sampling, detection and enumeration of moulds including standards for sampling strategies are important for comparative assessment of moulds in ambient air. Before doing any measurements a plan for the measurement strategy is necessary (see CEN/TS 16115-2 [5]).

WARNING — The use of this Technical Specification may involve hazardous materials, operations and equipment. This Technical Specification does not purport to address all the safety problems associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

2 Normative references

The following referenced documents are indispensable for the application 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 ISO 8199:2007*, Water quality — General guidance on the enumeration of micro-organisms by culture (ISO 8199:2005)*

3 Terms and definitions

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

3.1
aerodynamic diameter
diameter of a sphere of density 1 g/cm³ with the same terminal velocity due to gravitational force in calm air as the particle, under the prevailing conditions of temperature, pressure and relative humidity

[ISO 7708:1995, 2.2 [6]]

3.2
ambient air
outdoor air in the lower troposphere excluding workplace air

[EN 14907:2005, 3.1.1 [7]]

3.3
analytical blank value
value determined by a blank sample covering the analytical procedure to ensure that no significant contamination occurs during the complete analytical procedure including autoclaving, agar preparation, suspension and extraction of the filters, dilution, incubation, counting, etc.

3.4
bioaerosol
airborne particles of biological origin

[EN 13098:2000, 3.3 [8]].

NOTE Bioaerosols in the sense of this document are all aggregations of particles in the atmosphere to which fungi (spores, conidia, fragments of hyphae), bacteria, viruses and/or pollen as well as their cell membrane components and metabolites (e.g. endotoxins, mycotoxins) are attached or that consist of the above mentioned components.

3.5
biological sampling efficiency
biological preservation efficiency
capacity of the sampler to maintain the viability of the airborne microorganisms during collection and also to keep the microbial products intact

[EN 13098:2000, 3.4 [8]]

NOTE The biological sampling efficiency considers the sampling stress occurring during sampling and analysis in addition to the physical sampling efficiency. It refers to the proportion (in percent) of collected organisms which have not lost the ability to be cultured subsequently. It is strain- and species specific.

3.6
colony count
number of all visible colonies of microorganisms on a culture medium after incubation under the selected conditions

3.7
Colony Forming Unit
CFU
unit by which the culturable number of microorganisms is expressed

[EN 13098:2000, 3.5 [8]].

NOTE 1 One Colony Forming Unit can originate from one single microorganism, an aggregate of many microorganisms or from one or many microorganisms attached to one particle.

NOTE 2 The number of outgrowing colonies depends on cultivation conditions.

3.8
culture-based analyses
cultivation
growing of microorganisms on culture media

[ISO 16000-16:2008, 3.6 [3]]

NOTE The prerequisites for the detection are the abilities to grow and propagate.

3.9
face velocity
air flow rate divided by the face area

NOTE 1 The face velocity is expressed in metres per second.

[Adapted from EN 779:2002, 3.11 [9]]

NOTE 2 In this document, the face velocity is defined as the volume flow rate divided by the effective filter area.

3.10
field blank value
value determined by a blank sample covering the complete measurement procedure including preparation, sampling, transport and analyses to ensure that no significant contamination has occurred during all steps of measurement and to check that the operator can achieve a quantification level adapted to the task

NOTE A field blank sample is a sample taken in an identical manner as the real sample, but without sucking air through the sampling device. The resulting blank represents the number of CFU entering the sample simply by handling the filter during sampling. The results of the field blanks are not used for correction of measurement results but to detect sampling errors.

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

[EN 13098:2000, 3.11 [8]]

NOTE In this document, filtration is understood as the separation of moulds from a defined volume of air by means of filters.

3.12
indirect method
suspension of deposited microorganisms with subsequent plating of aliquots on a suitable culture medium, incubation and counting of colonies growing under the conditions selected

[Adapted from ISO 16000-17:2008, 3.3 [4]]

3.13
microbial air pollution
concentrations of airborne microorganisms that exceed natural concentrations or differ in type from the naturally occurring mircoorganisms

3.14
microorganism
microbial entity, either cellular or non cellular, that is capable of multiplication or transfer of genetic material, or entities that have lost these properties

[EN 13098:2000, 3.16 [8]]

3.15
mould
filamentous fungi from several taxonomic groups namely Zygomycetes, Ascomycetes (Ascomycota) and Deuteromycetes (fungi imperfecti)

NOTE Moulds form different types of spores depending on the taxonomic group they belong to, namely conidiospores (conidia), sporangiospores or ascospores.

[ISO 16000-16:2008, 3.9 [3]]

3.16
physical sampling efficiency
capacity of the sampling device to collect particles with specific sizes suspended in ambient air

[Adapted from EN 13098:2000, 3.17 [8]]

3.17
PM2,5
fraction of suspended particulate matter which passes through a size-selective inlet with a 50 % cut-off efficiency at 2,5 µm aerodynamic diameter

[EN 14907:2005, 3.1.5 [7]]

NOTE By convention, the size-selective standard inlet design prescribed in EN 14907:2005, 5.1.2, used at the flow rate given in EN 14907:2005, 5.1.5, possesses the required characteristics in order to sample the $PM_{2,5}$ fraction in ambient air.

3.18
PM10
fraction of suspended particulate matter which passes through a size-selective inlet with a 50 % cut-off efficiency at 10 µm aerodynamic diameter

NOTE Definition in analogy to PM2,5; adapted from EN 14907:2005, 3.1.5 [7]]

3.19
Suspended Particulate Matter
SPM
notion of all particles surrounded by air in a given, undisturbed volume of air

[EN 14907:2005, 3.1.6 [7]]

NOTE The bioaerosol sampling head shows a mean cut-off value of 30 µm aerodynamic diameter without a rigid upper separation limit due to its construction design and the specified flow rate, both determining the face velocity at the filter.

3.20
total sampling efficiency
product of the physical sampling efficiency and the biological preservation efficiency

[EN 13098:2000, 3.19 [8]]

4 Symbols and abbreviations

Not applicable.

5 Basic principle of the method

5.1 Sampling

In this document the measurement object are the airborne moulds as part of the suspended particulate matter (SPM, here particles with aerodynamic diameter up to approximately 30 µm).

During filtration, a defined air quantity is sucked through a filter – on or in which separation of the suspended particles occurs (see Annex A). Airborne moulds are collected on gelatine filters resulting in a high total sampling efficiency. Polycarbonate filters are used below the gelatine filters as supporting and protective filters to enhance stability (see Annex B).

Ambient air sampling devices validated for different fractions of particles are commercially available and are widely used for ambient air particle sampling according to national guidelines and European Standards:

— VDI 2463 Part 7 [11] and VDI 2463 Part 8 [12] for suspended particulate matter;

— EN 12341:1998 for PM10 [10];

— EN 14907:2005 for PM2,5 [7].

For sampling of bioaerosols an adapted bioaerosol sampling head, e.g. with larger filter diameters, has been developed resulting in changes of the face velocity at the filter compared with the standards given above.

NOTE 1 The sampling head was modified in respect of the reference method described in the national guideline VDI 2463 Part 8 [12]. The applicability of this adapted sampling head for bioaerosols has been confirmed by comparison measurements in 2001 (see Annex A), whereas the reference sampling head according to VDI 2463 Part 8 was validated in 1987/88 during an international WRAC validation campaign (WRAC = Wide range aerosol classifier) [13; 14].

The reason for the modification of the standardised sampling head for suspended particulate matter was the reduction of the face velocity in order to decrease the sampling stress. Additionally, the modifications enable the use of disposable or sterilizable filter holders ensuring aseptic conditions during the handling of filter and sampling head and avoiding carry-over effects and contaminations.

In a comparison measurement campaign it was shown that the adapted bioaerosol sampling head described in A.1 using a filter with a diameter of 8 cm (effective filter diameter of 7 cm) resulting in a face velocity of approximately 20 cm/s (19,5 cm/s to 23,8 cm/s depending on the flow rate of 2,7 m^3/h to 3,3 m^3/h) gave comparable results to the non-modified standardised sampling head for sampling SPM (here up to 30 µm) with regard to the *physical sampling efficiency*. Additionally a validation trial using this bioaerosol sampling head for the detection of moulds in ambient air under real conditions including sampling and analyses was performed (see A.2) showing comparable *biological sampling efficiency*. The performance characteristics and minimum requirements of the bioaerosol sampling head are given in Clause 8.

In general, any sampling head can be used, that assures a comparable physical sampling efficiency of the SPM fraction SPM (here up to 30 µm) and a comparable biological sampling efficiency with regard to the bioaerosol sampling head described in this document. Additionally, aseptic handling of the filter shall be assured und contaminations shall be avoided.

NOTE 2 If only the inhalable fraction is of interest, PM10 sampling can be performed. In this case fractions of bioaerosols which are not inhalable but can cause irritation by contact e.g. to mucous membranes are missed. When using the filter combination gelatine/polycarbonate filters with $PM_{2,5}$ resp. PM_{10} sampling heads, the physical requirements for particle sampling (cut-off efficiency, flow rate, etc., see EN 14907 [7] resp. EN 12341 [10]) apply.

After sampling the mould spores are cultured and counted according Clause 7.

5.2 Analyses

With the methods described here, mesophilic and thermotolerant moulds are quantified by culture-based analyses of the viable and culturable propagules on selective agar. The quantitative determination of the mould concentration is performed by counting the visually recognisable colonies. The density of the colonies grown on the culture medium shall always allow proper enumeration of the colonies. The density of the colonies results from the number of dilution steps after suspension of the cells from the filter. Therefore, in principal several dilution steps need to be plated out.

6 Sampling

6.1 Sampling equipment

The following components are needed:

6.1.1 Stand, to position the bioaerosol sampling head at the sampling height needed.

6.1.2 Bioaerosol sampling head[1)], to position the filter holder with the inserted filters in a hanging position, if necessary.

[1)] Sampling heads for bioaerosols as well as complete sampling devices are commercially available from several manufacturers, e.g. Leckel, Berlin/Germany; Derenda, Stahnsdorf/Germany; Digitel, Hegnau/Switzerland. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named.

A bent pipe or hose connection can be used to connect the bioaerosol sampling head to the sampling device. If using the bioaerosol sampling head described here, the inner diameter of the pipe or hose shall be 8 mm to 10 mm. The length of the connecting hose should not exceed 1,5 m. If using other sampling heads, these dimensions shall be adapted accordingly in order to ensure the physical requirements (e.g, face velocity, flow rate, see Table 3).

6.1.3 Filter holder, sterile (disposable or sterilizable), to insert the filters.

6.1.4 Filter, gelatine filter [2], sterile, pore size 3 µm, and polycarbonate filter [3], sterile, pore size 0,8 µm (see Annex B).

The physical sampling efficiency of both gelatine and polycarbonate filters shall be > 95 % for moulds resp. particles with an aerodynamic diameter range of > 1 µm, using a flow velocity at the filter of v = 21,7 cm/s ± 10 %. The combination of the two filters ensures a sampling efficiency of 99 % (Annex B).

6.1.5 Vacuum pump, ensuring a constant flow rate during continuous operation.

The flow rate has to be adapted in order to achieve a face velocity at the filter of 22 cm/s ± 10 %.

NOTE 1 If a filter with a diameter of 8 cm (effective diameter of 7 cm) is used, this face velocity is achieved by a flow rate of about 3 m^3/h ± 10 %. During the sampling duration the filter resistance may increase therefore is recommended to use a pump with a capacity of approximately 6 m^3/h.

NOTE 2 This method has been validated for a face velocity at the filter of 21,7 cm/s (see Annex A).

6.1.6 Gas volume meter, to determine the gas volume sucked at the bioaerosol sampling head, in operating cubic meters.

Display accuracy of the flow rate: 0,01 m^3/h

NOTE The use of volumetric measuring systems should take into account the manufacturer's specifications with regard to the prevailing conditions during sampling, e.g. difference pressure between ambient and operating conditions, temperature, humidity.

6.1.7 Timer, for presetting time and duration of sampling.

6.1.8 Protective housing, to protect the sampling device from harmful environmental conditions (optional).

The distance between the upper edge of the protective housing and the lower edge of the bioaerosol sampling head should be at least 40 cm.

6.1.9 Commonly used devices for measuring ambient air conditions and operating conditions, e.g. temperature, humidity, pressure.

For long term measurements a data logger may be necessary.

6.2 Materials

6.2.1 Container, sterile, for filter containment during transport, e.g. Petri dishes.

6.2.2 Container, insulated, for sample transport.

6.2.3 Disposable protective gloves, to avoid contamination and ensure occupational safety.

6.2.4 Disinfectant, e.g. iso-propanol or ethanol (70 %, volume content).

---

[2] Gelatine filters are commercially manufactured by Sartorius Stedim Biotech GmbH, Goettingen, Germany, however, they are available from many suppliers. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named.

[3] Polycarbonate filters are available from many manufacturers.

6.2.5 Tweezers, sterile, to handle the filters.

6.2.6 Thermometer, pressure gauge, hygrometer, to measure ambient air conditions during sampling and transportation.

6.3 Sampling procedure

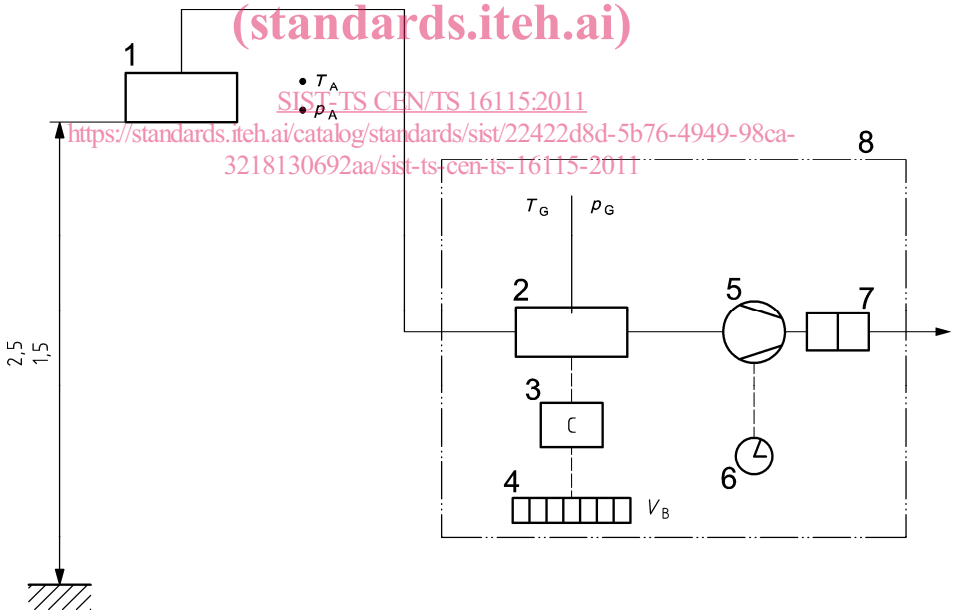
6.3.1 Preparation for sampling

The required number of measurement devices with accessories or other equipment shall be prepared in accordance with the measurement task and the measurement strategy resulting thereof. It is recommended to check the equipment for completeness and functionality using a check list.

The calibration validity of the sampling device shall be verified; otherwise new calibration shall be conducted prior to the beginning of the measurements (see 6.5). The correct function of the sampling equipment shall be documented in the sampling report.

Exclusively sterile filters and sterile filter holders shall be used for the measurements. If factory sterile disposable filters with filter holders are not available, then the sterilized filters shall be inserted in the filter holder at the laboratories safety cabinet and packed in sterility. For this purpose, first the polycarbonate filter and afterwards the gelatine filter shall be placed in the filter holder. Thereby, special attention shall be paid that the filters are tightly inserted into the filter holder. Filter sterility shall be guaranteed up to the moment of sampling. During transport, the filters shall be protected from dust, heat and strong vibrations.

Assemble the sampling device according to Figure 1. A detailed example of a suitable sampling device is given in Annex A.



Key

1 Bioaerosol sampling head
2 Gas volume meter (e.g. orifice plate, thermal mass flow rate meter)
3 Electronic circuit for conversion into operating cubic metres
4 Display for sampling volume V_B in operating cubic metres
5 Vacuum pump
6 Timer
7 Filter for abraded material
8 Protective housing

Figure 1 — Schematic set-up of the sampling device