



# SLOVENSKI STANDARD

## SIST ISO 16000-20:2015

01-april-2015

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### Notranji zrak - 20. del: Ugotavljanje prisotnosti in števila gliv/plesni - Ugotavljanje skupnega števila spor

Indoor air - Part 20: Detection and enumeration of moulds - Determination of total spore count

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Air intérieur - Partie 20: Détection et dénombrement des moisissures - Détermination du nombre total de spores

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**Indoor air —**

Part 20:

**Detection and enumeration of moulds  
— Determination of total spore count**

*Air intérieur —*

*Partie 20: Détection et dénombrement des moisissures —  
Détermination du nombre total de spores*

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## ISO 16000-20:2014(E)

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see [www.iso.org/directives](http://www.iso.org/directives)).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: [Foreword - Supplementary information](#)

The committee responsible for this document is ISO/TC 146, *Air quality*, Subcommittee SC 6, *Indoor air*.

ISO 16000 consists of the following parts, under the general title *Indoor air*:

- *Part 1: General aspects of sampling strategy*
- *Part 2: Sampling strategy for formaldehyde*
- *Part 3: Determination of formaldehyde and other carbonyl compounds in indoor air and test chamber air — Active sampling method*
- *Part 4: Determination of formaldehyde — Diffusive sampling method*
- *Part 5: Sampling strategy for volatile organic compounds (VOCs)*
- *Part 6: Determination of volatile organic compounds in indoor and test chamber air by active sampling on Tenax TA sorbent, thermal desorption and gas chromatography using MS or MS-FID*
- *Part 7: Sampling strategy for determination of airborne asbestos fibre concentrations*
- *Part 8: Determination of local mean ages of air in buildings for characterizing ventilation conditions*
- *Part 9: Determination of the emission of volatile organic compounds from building products and furnishing — Emission test chamber method*
- *Part 10: Determination of the emission of volatile organic compounds from building products and furnishing — Emission test cell method*
- *Part 11: Determination of the emission of volatile organic compounds from building products and furnishing — Sampling, storage of samples and preparation of test specimens*
- *Part 12: Sampling strategy for polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polycyclic aromatic hydrocarbons (PAHs)*

- Part 13: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDDs/PCDFs) — Collection on sorbent-backed filters
- Part 14: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-p-dioxins/dibenzofurans (PCDDs/PCDFs) — Extraction, clean-up and analysis by high-resolution gas chromatography and mass spectrometry
- Part 15: Sampling strategy for nitrogen dioxide (NO<sub>2</sub>)
- Part 16: Detection and enumeration of moulds — Sampling by filtration
- Part 17: Detection and enumeration of moulds — Culture-based method
- Part 18: Detection and enumeration of moulds — Sampling by impaction
- Part 19: Sampling strategy for moulds
- Part 20: Detection and enumeration of moulds — Determination of total spore count
- Part 21: Detection and enumeration of moulds — Sampling from materials
- Part 23: Performance test for evaluating the reduction of formaldehyde concentrations by sorptive building materials
- Part 24: Performance test for evaluating the reduction of volatile organic compound (except formaldehyde) concentrations by sorptive building materials
- Part 25: Determination of the emission of semi-volatile organic compounds by building products — Micro-chamber method
- Part 26: Sampling strategy for carbon dioxide (CO<sub>2</sub>)
- Part 27: Determination of settled fibrous dust on surfaces by SEM (scanning electron microscopy) (direct method)
- Part 28: Determination of odour emissions from building products using test chambers
- Part 29: Test methods for VOC detectors
- Part 30: Sensory testing of indoor air
- Part 31: Measurement of flame retardants and plasticizers based on organophosphorus compounds — Phosphoric acid esters
- Part 32: Investigation of constructions on pollutants and other injurious factors — Inspection

The following parts are under preparation:

- Part 33: Determination of phthalates with GC-MS
- Part 34: Strategies for the measurement of airborne particles (PM 2,5 fraction)
- Part 35: Measurement of polybrominated diphenylether, hexabromocyclododecane and hexabromobenzene
- Part 36: Test method for the reduction rate of airborne bacteria by air purifiers using a test chamber

**ISO 16000-20:2014(E)****Introduction**

Mould is a common name for filamentous fungi from different taxonomic groups (Ascomycota, Zygomycota, and their anamorphic states former known as Deuteromycota or fungi imperfecti). They form a mycelium and spores 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.

Mould spores are widely distributed in the outdoor environment and, therefore, occur in varying concentrations also indoors. Growth of moulds in indoor environments, however, has to be considered a hygienic problem because epidemiological studies have revealed that dampness and/or mould growth in homes and health problems affecting the occupants are closely related.

Harmonized methods for sampling, detection and enumeration of moulds including standards for sampling strategies are important for comparative assessment of mould problems indoors. Before doing any measurements a plan for the measurement strategy should be made.

This part of ISO 16000 describes methods for air sampling of mould spores for subsequent microscopic analysis.

This part of ISO 16000 is based on parts of VDI 4300 Part 10.<sup>[6]</sup>

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## Indoor air —

### Part 20:

## Detection and enumeration of moulds — Determination of total spore count

### 1 Scope

This part of ISO 16000 specifies requirements for sampling of moulds from air. Following the instructions given, samples are obtained for microscopy to determine the total concentration of spores.

### 2 Terms and definitions

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

#### 2.1

##### **cultivation**

<air quality> growing of microorganisms on culture media

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

#### 2.2

##### **cut-off value**

particle size (aerodynamic diameter) for which the sampling efficiency is 50 %

#### 2.3

##### **filamentous fungus**

fungus growing in the form of filaments of cells known as hyphae

[SOURCE: ISO 16000-16:2008, 3.3]

Note 1 to entry: The term “filamentous fungi” differentiates fungi with hyphal growth from yeasts.

#### 2.4

##### **impaction**

sampling of particles suspended in air by inertial separation on a solid surface

#### 2.5

##### **microorganism**

any microbial entity, cellular or non-cellular, capable of replication or of transferring of genetic material or entities that have lost these properties

[SOURCE: EN 13098:2000]

#### 2.6

##### **mould**

<air quality> filamentous fungi from several taxonomic groups; namely Ascomycota, Zygomycota, and their anamorphic states former known as Deuteromycota or fungi imperfecti

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

Note 1 to entry: Moulds form different types of spores depending on the taxonomic group they belong to, namely conidiospores (conidia), sporangiospores, or ascospores.

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### 2.7

#### **mycelium**

branched hyphae network

[SOURCE: ISO/TS 10832:2009, 3.5]

### 2.8

#### **physical sampling efficiency**

capacity of the sampler to collect particles with specific sizes suspended in air

Note 1 to entry: See Reference [Z].

## 3 Principle of method

A defined air quantity is drawn through an impactor containing a sticky solid surface which can subsequently be used for microscopy. The particles in the air stream impact on the surface, due to their inertia, when the air flows bend to bypass the solid surface.

Airborne moulds are thereby collected directly on the sticky surface.

Physical sampling efficiency is influenced by the geometry of the slit, air velocity, and the adhesion capability of the surface.

The sampling device is constructed for the detection of particles in the size of mould spores ( $>1 \mu\text{m}$  to ca.  $30 \mu\text{m}$ ). To achieve this, the cut-off value of the sampling device should preferably be  $1 \mu\text{m}$  or less and shall not be more than  $2,6 \mu\text{m}$ .

NOTE Three main types of impactors are widely used and available commercially: samplers with replaceable slides and air velocity of ca.  $30 \text{ L/min}$ , e.g. PS 30 and MBASS30, samplers<sup>1)</sup> with replaceable slides and air velocity of ca.  $15 \text{ L/min}$ , e.g. Allergenco MK3<sup>2)</sup>, and samplers with disposable cassettes and air velocity of ca.  $15 \text{ L/min}$  (see Annex A).

After sampling, the mould spores are counted under a microscope. No cultivation is performed. Therefore, the total spore concentration, including culturable and non-culturable spores can be determined.

## 4 Apparatus and materials

Usual microbiological laboratory equipment, and in particular:

- 5.1 **Stand**, for positioning the impactor at the sampling height needed.
- 5.2 **Impactor**, with disposable slides or cassettes.
- 5.3 **Vacuum pump**, for ensuring a constant flow rate during continuous operation.
- 5.4 **Gas volume meter**, for determining the gas volume sucked at the sampling head, in operating cubic meters.
- 5.5 **Timer**, for presetting the time and duration of sampling.
- 5.6 **Protective housing**, for protecting the impactor from harmful environmental conditions (optional, mainly for outdoor use).

1) PS 30 and MBASS30 are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

2) Allergenco MK3 is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

5.7 **Microscope**, equipped with 40× and 100× objectives for ca. 400× and 1000× magnification.

## 5 Reagents

### 5.1 General

All reagents and chemicals shall be of recognized quality “for microbiology” or better. Water used shall be distilled or of equivalent quality.

### 5.2 Lactophenol blue solution

The components of the staining solution are listed in [Table 1](#).

**WARNING — Lactophenol blue solution is toxic and can lead to adverse health reactions. Exposure through direct contact or inhalation has to be avoided.**

**Table 1 — Composition of staining solution**

| Component                               | Quantity |
|---|----------|
| Cotton blue                             | 0,5 g    |
| Lactic acid (% by mass of 80 % to 85 %) | 4,0 g    |
| Phenol                                  | 4,0 g    |
| Glycerol                                | 8,0 g    |
| Distilled water                         | 100 ml   |

Add ingredients in 100 ml water and dissolve.

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## 6 Measurement procedure

### 6.1 Sampling

Sampling is usually conducted at a height of 0,75 m to 1,5 m above ground. For special questions, other heights might be applicable. Take care when sampling at low heights that no settled house dust is sucked in the sampling device.

Prepare the required number of impactors and slides or cassettes in accordance with the measurement task and the measurement strategy.

**NOTE** If the concentration of spores cannot be anticipated, several volumes (e.g. 50 L, 100 L, and 200 L) can be sampled and the most suitable sampling (enough spores, not overloaded) can then be used counting.

Check the equipment for completeness and functionality with a check list. Perform function control at regular intervals. Function control implies primarily the volumetric flow control (see [Clause 8](#)).

Use a sterile device containing the slides or cassettes for each measurement point. Alternatively, clean the slit with ethanol or isopropanol (70 %; volume fraction) and dry it afterwards (e.g. with compressed air).

Place the slides or cassettes in the impactors. Take care to avoid contamination.

Start the sampling device in accordance with the manufacturer’s operating instructions. Multiple measurements using different sampling volumes are recommended. This is especially important when the level of the anticipated concentration of moulds is not known.

After sampling, remove the slides or cassettes from the sampling apparatus and pack them in sterile containers and/or plastic bags in order to avoid any secondary contamination. Subsequently, draw air