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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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Foreword

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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).

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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_2)*

— *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_2)*

— *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*

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.[6]

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.

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 [7].

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 µm to ca. 30 µm). To achieve this, the cut-off value of the sampling device should preferably be 1 µm or less and shall not be more than 2,6 µm.

NOTE Three main types of impactors are widely used and available commercially: samplers with replaceable slides and air velocity of ca. 30 L/min, e.g. PS 30 and MBASS30, samplers[1)] with replaceable slides and air velocity of ca. 15 L/min, e.g. Allergenco MK3[2)], and samplers with disposable cassettes and air velocity of ca. 15 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).

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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.

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

through the impactor without slides for several minutes at the new sampling point prior to the sampling with the slide in place.

For disposable samplers, follow the instructions of the manufacturer.

Fill in a sampling protocol (see Clause 9 and Annex B).

Transport samples to the laboratory (see 6.4) and analyse by direct microscopy (see 6.2).

6.2 Direct microscopy

The spores on the sticky surface are stained with e.g. lactophenol blue solution (see 5.2) or cotton blue in lactic acid and evaluated under the microscope at 400× and 1000× magnification.

NOTE 1 To get an overview of the sample trace, it can be useful to make an initial view at 100× or 200× magnification.

If different volumes have been sampled, choose the most appropriate (enough spores, not overloaded) for counting.

NOTE 2 Counting of the different spore types under the microscope is a difficult task which can only be performed by skilled and well trained personnel.

NOTE 3 Lactophenol blue solution is toxic and has to be avoided whenever possible. Staining of spores with alternative staining solution has to be anticipated. Cotton blue in lactic acid does, however, not work with the PS 30 with MBASS30[3)] which was used for determining the performance characteristics (see Clause 10).

The slit impaction sampling method produces a sample trace approx. 1,6 cm long and approx. 1 mm wide on the glass slide which is evaluated by microscopy.

Normally, the slides are evaluated for the following spore types: Basidiospores, Ascospores, *Cladosporium*, type *Aspergillus/Penicillium*, *Stachybotrys*, *Chaetomium*, type *Alternaria/Ulocladium*, type *Helminthosporium*, *Epicoccum*, other spores, and mycelial fragments. Additional spore types which occur in unusual concentrations and can be assigned to a morphological type are likewise reported.

Basidiospores, ascospores, spores of the types *Alternaria/Ulocladium*, *Helminthosporium,* and *Epicoccum* do not usually originate from indoor sources and therefore provide an indication as to what extent the sample has been influenced by outdoor air (e.g. due to leaks around windows, mechanical disturbance of settled outdoor spores).

Large, readily recognizable spores, e.g. *Stachybotrys* or *Chaetomium* spores, are counted on the whole surface at 400× magnification in longitudinal direction of the sample trace. In this way, the complete loaded impaction surface can be evaluated within a reasonable time scale and hence, few spores determined per sample volume. The theoretical detection limit is one spore in the volume sampled.

For small spores of the type, e.g. *Aspergillus/Penicillium,* an additional detailed evaluation is made at 1000× magnification in the direction perpendicular to the sample trace. Detailed evaluations are very time-consuming so that only a small portion of the sample (usually approx. 10 % to 30 % of the total impaction surface, see Clause 10) can be evaluated. Consequently, the detection limit for small spores is higher than that for large spores. For a sample volume of 200 L and the evaluation of 10 transverse traverses, the theoretical detection limit is approx. 50 spores/m^3 (one spore present in the 10 traverses).

For quantification, preferably, 10 or more spores should be present in the microscopic area counted.

NOTE 4 Particles like skin scales and other (mineral and organic) particles are not counted, but might be indicated in categories like low, medium, relatively high and high to have an indication of the influence of corresponding activities in the room.

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

6.3 Calculation and expression of results

The quantitative result is reported as concentration of the enumerated spore types and mycelial fragments per m^3 of air. The total spore concentration is obtained by summating the concentrations of the individual spore types.

NOTE Spores present in aggregates are enumerated individually. It is, however, helpful to document the presence of aggregates in the analysis report so as to provide information on how many individual spores and how many aggregate spores were present, which might be needed for specific investigation objectives.

In the 400× evaluation (see 6.2), which is performed in the longitudinal direction of the sample trace, the entire sample volume is evaluated. For the calculation of the spore concentration per m^3 of air, the result per sample volume is multiplied by a corresponding factor (e.g. $F = 5$ for a sample volume of 200 L, see example 1).

In the detailed 1 000× evaluation, which is performed in the direction perpendicular to the sample trace (see 6.2), parameters like the slit geometry, the size of the objective visual field and the number of transverse traverses evaluated enter into the calculation. Results are rounded to two decimal numbers.

The concentration can be calculated according to Formula (1).

$$C_L = \frac{L}{B} \cdot \frac{1}{V_G} \cdot Z \quad (1)$$

where

C_L is the concentration of the air sample in spores/m^3;

L is the total length of the sample trace in mm;

B is the length of the evaluated area of the sample trace in mm;

V_G is the sample volume in m^3;

Z is the total spore count.

B is calculated according to Formula (2).

$$B = D \times Z_Q \quad (2)$$

where

D is the diameter of the visual field in mm;

Z_Q is the number of evaluated transverse traverses.

EXAMPLE 1 20 spores of *Stachybotrys* were detected on the whole sampling surface. The sample volume was 200 L.

Result: 20 × 5 = 100 spores/m^3 air = 1,0 × 10^2 spores/m^3 air

EXAMPLE 2 A sample was collected using a slit impactor (16 mm long, 1,1 mm wide) and evaluated at 1 000× magnification. The visual field of the microscope and objective used has a diameter of 175 µm. The sample volume was 200 L. 20 transverse traverses were evaluated with a count of 60 spores of the type *Aspergillus/Penicillium*.

$$C_L = \frac{16}{0{,}175 \cdot 20} \cdot \frac{1}{0{,}2} \cdot 60 = 1\,371 \text{ spores/m}^3 \text{ air}$$

Result: 1,4 × 10^3 spores/m^3 air