
Indoor air —

Part 16:

**Detection and enumeration of moulds —
Sampling by filtration**

Air intérieur —

*Partie 16: Détection et dénombrement des moisissures —
Échantillonnage par filtration*

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 16000-16 was prepared by Technical Committee 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 — 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/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 (NO₂)*
- *Part 16: Detection and enumeration of moulds — Sampling by filtration*
- *Part 17: Detection and enumeration of moulds — Culture-based method*
- *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 compounds and carbonyl compounds without formaldehyde concentrations by sorptive building materials*

The following parts are under preparation:

- *Part 18: Detection and enumeration of moulds — Sampling by impaction*
- *Part 19: Sampling strategy for moulds*
- *Part 25: Determination of the emission of semi-volatile organic compounds by building products — Micro-chamber method*
- *Part 28: Sensory evaluation of emissions from building materials and products*

The following parts are planned:

- *Part 20: Detection and enumeration of moulds — Sampling from house dust*
- *Part 21: Detection and enumeration of moulds — Sampling from materials*

- *Part 22: Detection and enumeration of moulds — Molecular methods*
- *Part 27: Standard method for the quantitative analysis of asbestos fibres in settled dust*

Furthermore,

- *ISO 12219-1 (under preparation), Indoor air — Road vehicles — Part 1: Whole vehicle test chamber — Specification and method for the determination of volatile organic compounds in car interiors,*
- *ISO 16017-1, Indoor, ambient and workplace air — Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography — Part 1: Pumped sampling, and*
- *ISO 16017-2, Indoor, ambient and workplace air — Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography — Part 2: Diffusive sampling*

focus on volatile organic compound (VOC) measurements.

Introduction

Mould is a common name for filamentous fungi from different taxonomic groups (Zygomycetes, Ascomycetes [Ascomycota], 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 2 µm to 10 µm, some up to 30 µm and a very 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 (*Stachybotrys*, *Fusarium*) and less mobile.

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

Standardised methods for sampling, detection and enumeration of moulds including standards for sampling strategies are important for comparative assessment of mould problems indoors. Before taking any measurements, a measurement strategy is required.

The procedure specified in this part of ISO 16000 is based on VDI 4252-2^[7], which is widely used for detection and enumeration of fungi in ambient air and was adapted to be suitable also for indoor environments.

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Indoor air —

Part 16:

Detection and enumeration of moulds — Sampling by filtration

WARNING — The use of this part of ISO 16000 may involve hazardous materials, operations and equipment. This part of ISO 16000 does not purport to address any safety problems associated with its use. It is the responsibility of the user of this part of ISO 16000 to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 16000 specifies requirements for long-term (0,5 h to several hours) sampling of moulds in indoor air by filtration. Following the instructions given, a sample is obtained for subsequent detection of moulds by cultivation after suspension according to ISO 16000-17, which is part of the complete measurement procedure.

This part of ISO 16000 is not suitable for personal sampling.

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.

ISO 16000-17, *Indoor air — Part 17: Detection and enumeration of moulds — Culture-based method*

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 relative density 1 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

NOTE Adapted from ISO 7708:1995, 2.2.

3.2

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 ^[6]]

3.3

filamentous fungus

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

NOTE 1 Hyphae aggregated in bundles are called mycelia.

NOTE 2 The term “filamentous fungi” differentiates fungi with hyphal growth from yeasts.

3.4

filtration

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

[EN 13098:2000 ^[6]]

NOTE In this part of ISO 16000, filtration is understood as the separation of microorganisms or moulds from a defined volume of air by means of filters.

3.5

colony forming unit

cfu

unit by which the culturable number of microorganisms is expressed

[EN 13098:2000 ^[6]]

NOTE 1 One colony forming unit can originate from one single microorganism, from aggregates of many microorganisms as well as from one or many microorganisms attached to a particle.

NOTE 2 The number of colonies can depend on the cultivation conditions.

3.6

cultivation

⟨air quality⟩ growing of microorganisms on culture media

3.7

field blank

⟨air quality⟩ sample taken in an identical manner as the real sample, but without sucking air through the sampling apparatus

NOTE 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 (see ISO 16000-17).

3.8

microorganism

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

[EN 13098:2000 ^[6]]

3.9

mould

⟨air quality⟩ 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.

3.10

physical sampling efficiency

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

[EN 13098:2000 ^[6]]

3.11

total sampling efficiency

product of the physical sampling efficiency and the biological preservation efficiency

[EN 13098:2000 ^[6]]

4 Principle

During filtration, a defined air quantity is sucked through a filter — on or in which separation of the suspended particles occurs.

Airborne moulds are collected on gelatine filters resulting in a high total sampling efficiency (see Annex A). Polycarbonate filters are used below the gelatine filters to enhance stability (see Annex A). Filters other than those of gelatine may be used provided they have been shown to have a relative recovery of at least 90 % of the mass recovered on the gelatine type.

The sampling device is constructed for the detection of particles of the size of mould spores ($> 1 \mu\text{m}$ to $\sim 30 \mu\text{m}$). To achieve this, the flow velocity of the filter shall be in the range 100 mm/s to 250 mm/s.

NOTE 1 If a filter with a diameter of 80 mm is used, this flow velocity is achieved by a flow rate of about 1,5 m³/h to 3,3 m³/h (25 l/min to 55 l/min).

NOTE 2 This method has been validated for a flow velocity of 217 mm/s. The physical sampling efficiency for other velocities may be lower.

NOTE 3 Particles $> 30 \mu\text{m}$ are also retained by the filters. If the filter holder is operated in a hanging position (e.g. outdoor measurements with strong winds or rain) it is nonetheless possible that bigger particles may not reach the filter holder due to their inertia.

After sampling, the mould spores are cultivated and counted. This procedure is specified in ISO 16000-17.

5 Apparatus and materials ISO 16000-16:2008

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5.1 Sampling device

The following components are needed.

5.1.1 Stand, to position the sampling head at the sampling height needed.

5.1.2 Sampling head, to position the filter holder with the inserted filters in a hanging position, if necessary.

A bent pipe or hose connection can be used to connect the sampling head to the sampling apparatus. The inner diameter of the pipe or hose shall be 8 mm to 10 mm.

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

5.1.4 Filters, of gelatine ¹⁾, sterile, of pore size 3 μm , and of polycarbonate, sterile, of pore size 0,8 μm (see Annex A).

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

The flow rate has to be adjusted to produce a flow velocity at the filter in the range 100 mm/s to 250 mm/s (see Clause 4).

1) Sartorius Stedim Biotech, Göttingen, is an example of a suitable commercial supplier. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this supplier.

5.1.6 Gas volume meter, to determine the volume, in operating cubic metres, of gas sucked in at the sampling head.

5.1.7 Timer, for presetting time and duration of sampling.

5.1.8 Protective housing, to protect the sampling device from harmful environmental conditions (optional, mainly for outdoor use).

The distance between the upper edge of the protective housing and the lower edge of the sampling head should be at least 400 mm.

5.2 Materials

5.2.1 Sterile container, for filter containment during transport; e.g. Petri dishes.

5.2.2 Insulated container, for sample transport.

5.2.3 Protective gloves, preferably sterile disposable gloves or disinfected.

5.2.4 Disinfectant, e.g. *iso*-propanol or ethanol (70 % volume fraction).

5.2.5 Sterile tweezers, to handle the filters.

6 Measurement procedure

6.1 Preparation for sampling

Assemble the sampling equipment according to Figure 1.

A detailed example of a suitable sampling device is given in Annex B.

Connect the gas volume meter between the pump and the sampling head in order to determine the sampling air volume without any interference caused by the leakage flow rate of the pump. The volume of the sampled air is displayed in operating cubic metres to an accuracy of 0,01 m³. The temperature and pressure within the gas volume meter and in the air are continuously monitored.

The sampling air volume in operating cubic metres referenced to air conditions, V_B , is calculated electronically using Equation (1):

$$V_B = V_G \frac{T_A p_G}{T_G p_A} \quad (1)$$

where

p_A is the air pressure;

p_G is the air pressure within the gas volume meter;

T_A is the air temperature;

T_G is the temperature within the gas volume meter;

V_G is the sampling air volume measured by the gas volume meter.

Prepare the required number of sampling devices in accordance with the measurement task and the measurement strategy. The air flow rate of the sampling apparatus should not exceed 10 % of the air change rate of the room in question. If the air change rate is unknown, the air volume sampled per hour shall not exceed 10 % of the volume of the room.