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Indoor air —

Part 18: **Detection and enumeration of moulds — Sampling by impaction**

Air intérieur —

Partie 18: Détection et dénombrement des moisissures — Échantillonnage par impaction

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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 16000-18 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*:

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— *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)*

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— *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 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*

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— *Part 26: Sampling strategy for carbon dioxide* (CO_2)

— *Part 28: Determination of odour emissions from building products using test chambers*

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The following parts are under preparation:

— *Part 21: Detection and enumeration of moulds — Sampling from materials*

— *Part 27: Determination of settled fibrous dust on surfaces by SEM (scanning electron microscopy) (direct method)*

— *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 ester*

— *Part 32: Investigation of constructions on pollutants and other injurious factors — Inspections*

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Introduction

Mould is a common name for filamentous fungi from different taxonomic groups (Ascomycetes, Zygomycetes, and their anamorphic states formerly known as deuteromycetes or fungi imperfecti). They form a mycelium and spores by which they become visible macroscopically. Most spores are in the size range 2 µm to 10 µm, while some go 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 or embedded in a slime matrix (e.g. *Stachybotrys*, *Fusarium*) and are 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, should be considered a hygienic problem because epidemiological studies have revealed that dampness or mould growth in homes and health impairment of 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 taking any measurements, a measurement strategy is required.

This part of ISO 16000 specifies a method for active short-term sampling (1 min to 10 min) whereas an active long-term sampling procedure (0,5 h to several hours) is specified in ISO 16000-16.

This part of ISO 16000 is based on parts of VDI 4300 Part 10:2008[11].

ISO 16017[8][9] and ISO 12219[3]–[7] also focus on volatile organic compound (VOC) measurements.

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Indoor air —

Part 18:
Detection and enumeration of moulds — Sampling by impaction

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 short-term (1 min to 10 min) sampling of moulds in indoor air by impaction on solid agar media. Following the instructions given, a sample is obtained for subsequent detection of moulds by cultivation in accordance with ISO 16000-17.

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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.

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ISO 16000-16, *Indoor air — Part 16: Detection and enumeration of moulds — Sampling by filtration*

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

3 Terms and definitions

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

3.1
aerodynamic diameter
diameter of a sphere of density 1 g/cm^3 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], 2.2.

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[10]]

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3.3
colony forming unit
cfu
unit by which the culturable number of microorganisms is expressed

[EN 13098:2000[10]]

NOTE 1 One colony can originate from one 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.

3.4
cut-off value
particle size (aerodynamic diameter) for which the sampling efficiency is 50 %

3.5
cultivation
⟨air quality⟩ growing of microorganisms on culture media

[ISO 16000-16:2008, 3.6]

3.6
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.

[ISO 16000-16:2008, 3.3]

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3.7
impaction
sampling of particles suspended in air by inertial separation on a solid surface

NOTE For the purposes of this part of ISO 16000, the solid surface consists of agar (see also ISO 4225:1994[1], 3.18, 3.49 which define devices using impaction).

3.8
microorganism
any microbial entity, cellular or non-cellular, capable of replication or of transferring of genetic material, or entities that have lost these properties

[EN 13098:2000[10]]

3.9
mould
⟨air quality⟩ filamentous fungi from several taxonomic groups, namely Ascomycetes, Zygomycetes, and their anamorphic states formerly known as deuteromycetes or 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]

3.10
physical sampling efficiency
capacity of the sampler to collect particles with specific sizes suspended in air

[EN 13098:2000[10]]

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3.11
total sampling efficiency
product of the physical sampling efficiency and the biological preservation efficiency

[EN 13098:2000[10]]

4 Principle

A defined quantity of air is drawn through an impactor containing one or several plates with agar medium (DG18 and malt-extract or potato dextrose agar). The particles in the air stream impact on the agar surface due to their inertia when the direction of the air flow is diverted to bypass the solid surface.

Airborne moulds are thereby collected directly on the agar plates.

The sampling device is constructed for the detection of particles in the size of mould spores (>1 µm to ~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 µm.

NOTE Two main types of impactors are widely used and available commercially: a) slit samplers; b) sieve samplers. In slit samplers, air is drawn through a narrow slit and particles are impacted on to a rotating agar plate. In sieve samplers, air is drawn through a perforated plate (sieve) with holes of a defined diameter and particles are impacted on to an agar plate fixed below. Sieve samplers can be operated as stacks with different sieves leading to different flow velocities to collect different particle size fractions (i.e. the six-stage Andersen impactor). Validation data are only provided for single stage sieve impactors (mainly with ≥300 holes) using agar plates with a diameter of 9 cm.

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After sampling, the mould spores are cultivated and resulting colonies counted in accordance with the procedure specified in ISO 16000-17.

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5 Apparatus and materials

5.1 Sampling device

A detailed example of a single stage sieve impactor is given in Annex A. Impactors with several stages are only used for special purposes when a size fractioning of the particles is required.

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

5.1.2 Impactor, slit or sieve type.

5.1.3 Agar plates, diameter 9 cm, containing DG18 agar and malt-extract or potato dextrose agar (see Clause 6).

5.1.4 Vacuum pump ensuring a constant flow rate during continuous operation.

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

5.1.6 Timer for presetting the time and duration of sampling.

5.1.7 Protective housing (optional, mainly for outdoor use) to protect the impactor from harmful environmental conditions.

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5.2 Equipment for preparing the agar plates

Usual microbiological laboratory equipment, and in particular the following.

5.2.1 pH meter with an accuracy of ±0,1.

5.2.2 Petri dishes vented, sterile, diameter ~9 cm.

5.2.3 Autoclave capable of being operated at (121 ± 3) °C and (115 ± 3) °C.

5.3 Equipment for sampling

5.3.1 Plastic bags to protect the agar plates during transport.

5.3.2 Insulated and refrigerated container for transport of agar plates below 25 °C.

5.3.3 Disinfectant, e.g. isopropanol or ethanol (70 % volume fraction).

5.3.4 Compressed air (oil-free, optional) for drying the equipment after disinfection.

6 Culture media and reagents

6.1 General

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

Use of commercially available, dehydrated substrates is encouraged, provided they comply with the descriptions given. They shall be prepared according to the instructions from the manufacturer.

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6.2 Dichloran 18 % glycerol agar (DG18)

The components are listed in Table 1.

Table 1 — Composition of dichloran 18 % mass fraction glycerol agar (DG18 agar)

Component	Quantity
Peptone[a]	5,0 g
Glucose	10,0 g
Potassium dihydrogenphosphate (KH_2PO_4)	1,0 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0,5 g
Dichloran (2,6-dichloro-4-nitroaniline) 0,2 % mass fraction in ethanol (100 %)	1,0 ml
Chloramphenicol	0,1 g
Glycerol	220 g[b]
Agar	15,0 g
Water	1 000 ml

[a] Different peptones are used by different manufacturers (e.g. casein peptone, mycological peptone). This does not usually influence the quantitative results of the measurements, but can have an influence on the appearance of the colonies. Positive controls for comparison of recovery and of morphological appearance of the colonies are, therefore, important.

[b] 18 % mass fraction of ~1 220 g gives a final mass of ~220 g.

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Add minor ingredients and agar to ~800 ml water and dissolve by boiling. Make up to 1 000 ml and add 220 g glycerol. Sterilize in an autoclave at (121 ± 3) °C for (15 ± 1) min. After sterilization, the pH shall correspond to 5,6 ± 0,2 at 25 °C. Dispense aliquots of about 20 ml into Petri dishes.

Plates of DG18 agar in bags may be kept for up to 1 week at (15 ± 3) °C in the dark.

NOTE 1 Dependent on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful, providing they have been shown not to influence the test results.

NOTE 2 DG18 agar is suitable for the detection of a wide spectrum of xerophilic fungi (i.e. preferring dryness). Glycerol reduces the water activity, a_a to 0,95. Chloramphenicol inhibits bacteria, especially gram-negative bacteria. Dichloran inhibits the spreading of fast-growing mould colonies and thus prevents overgrowing of slow-growing colonies.

6.3 Malt-extract agar

The components are listed in Table 2.

Table 2 — Composition of malt-extract agar

Component	Quantity
Malt-extract	30,0 g
Peptone from soy	3,0 g
Agar	15,0 g
Water	1 000 ml

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NOTE 1 The addition of chloramphenicol (0,05 g/l) can be necessary if samples contain high concentrations of bacteria.

NOTE 2 Dependent on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful providing they have been shown not to influence the test results.

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Add ingredients and agar to the water and dissolve by boiling. Sterilize in an autoclave at (115 ± 3) °C for (10 ± 1) min. After sterilization, the pH shall correspond to 5,5 ± 0,2 at 25 °C. Dispense aliquots of about 20 ml into Petri dishes.

Plates of malt-extract agar in bags will keep for up to 1 month at (5 ± 3) °C in the dark.

IMPORTANT — Many commercial malt-extract agars with different compositions are available. Check that the ingredients correspond to the composition given in Table 2.

6.4 Potato dextrose agar

The components are listed in Table 3.

Table 3 — Composition of potato dextrose agar

Component	Quantity
Potato extract	4,0 g
Glucose	20,0 g
Agar	15,0 g
Water	1 000 ml

NOTE 1 The addition of chloramphenicol (0,05 g/l) can be necessary if samples contain high concentrations of bacteria.

NOTE 2 Dependent on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful, providing they have been shown not to influence the test results.

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