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Indoor air —

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

Air intérieur —

Partie 21: Détection et dénombrement des moisissures — Échantillonnage à partir de matériaux

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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.

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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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_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 ester*

— *Part 32: Investigation of buildings for pollutants and other injurious factors — Inspections*

The following parts are under preparation:

— *Part 33: Determination of phthalates with gas chromatography/mass spectrometry (GC/MS)*

— *Part 34: Strategies for the measurement of airborne particles (PM 2,5 fraction)*

— *Part 35: Measurement of polybrominated diphenylether, hexabromocyclododecane and hexabromobenzene*

A test method for the reduction rate of airborne bacteria by air purifiers using a test chamber will form a future part 36.

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 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, should be considered as 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 sampling of moulds from building materials.

This part of ISO 16000 is based on parts of VDI 4300 Part 10.

Indoor air —

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

1 Scope

This part of ISO 16000 specifies requirements for sampling of moulds from building materials. Following the instructions given, samples are obtained for microscopy or for subsequent detection of moulds by cultivation according to ISO 16000-17.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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
colony forming unit
cfu
unit by which the culturable number of microorganisms is expressed

[SOURCE: EN 13098:2000]

Note 1 to entry: One colony 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 to entry: The number of colonies can depend on the cultivation conditions.

3.2
cultivation
<air quality> growing of microorganisms on culture media

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

3.3
filamentous fungus
fungus growing in the form of filaments of cells known as hyphae

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

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

3.4
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]

3.5
mould
<air quality> filamentous fungi from several taxonomic groups, namely ascomycetes, zygomycetes, and their anamorphic states formerly known as deuteromycetes or fungi imperfecti

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

3.6
mycelium
branched hyphae network

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

4 Principle of method

Mould-infested materials are examined either by surface sampling (see 7.1) or bulk sampling (see 7.2), i.e. examination of the complete material or defined deeper material layers. The methods used depend on the investigation objective as described in ISO 16000-19. Surfaces are sampled using the contact plate (see 7.1.2), tape-lift (see 7.1.3), or swab method (see 7.1.4). After sampling, the mould spores can be analysed by direct microscopy (see 7.4) or processed and cultured using the suspension method (see 7.5). The cultivation procedure is described in ISO 16000-17.

5 Apparatus and materials

Usual microbiological laboratory equipment, and in particular:

5.1 Equipment for sampling

5.1.1 Agar plates or flexible plastic stripes, containing DG-18 agar and malt extract or potato dextrose agar (see Clause 6) with the culture medium slightly projecting over the edge.

5.1.2 Cotton swabs, sterile, to take swab samples.

5.1.3 Containers to protect the agar plates and material samples during transport, e.g. plastic bags.

5.1.4 Disinfectant, e.g. iso-propanol or ethanol (70 % volume fraction) to disinfect sampling tools.

5.1.5 Drill, disinfected, with a diameter of at least 3 cm, preferably 5 cm, to take defined cores from the material.

5.1.6 Insulated/refrigerated container, for transport of agar plates and material samples below 25 °C.

5.1.7 Sampling tools, sterile, to take bulk samples of materials in different depths, e.g. spatula, spoons, knives, drilling equipment.

5.2 Equipment for preparing the agar plates

5.2.1 Autoclave, at (121 ± 3) °C and (115 ± 3) °C.

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

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

5.3 Equipment for processing the bulk samples

5.3.1 Aluminium container, to weigh material samples.

5.3.2 Analytical balance, with an accuracy of ±0,01 g.

5.3.3 Glass flask, baffled flask, sterile, 250 ml.

5.3.4 Shaking dish, horizontal, 200 rpm.

5.3.5 Test tube shaker, e.g. Vortex shaker.

6 Culture media and reagents

6.1 General

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. These dehydrated substrates shall be prepared according to the instructions from the manufacturer. For surface sampling, agar plates or flexible plastic stripes containing agar medium are also commercially available.

6.2 Dichlorane 18 % glycerol agar (DG-18)

The components are listed in Table 1.

Table 1 — Composition of dichlorane 18 % glycerol agar (DG-18 agar)

Component	Quantity
Peptone[c]	5,0 g
Glucose	10,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,0 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0,5 g
Dichlorane (2,6-dichloro-4-nitroaniline) 0,2 % volume fraction in ethanol (100 %)	1,0 ml[a]
Chloramphenicol	0,1 g
Glycerol	220 g[b]

[a] Final concentrate in medium: 0,002 g/1.

[b] 18 % mass fraction of approximately 1 220 g final mass = approximately 220 g.

[c] 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 comparisons of recovery and of morphological appearance of the colonies are, therefore, important.