INTERNATIONAL STANDARD

ISO 16000-17

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Indoor air —

Part 17: Detection and enumeration of moulds — Culture-based method

Air intérieur —

Partie 17: Détection et dénombrement des moisissures — Méthode par culture

Reference number
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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 16000-17 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 (NO_2)*

— *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 to10 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 4253-2 [5] and VDI 4300-10 [6].

Indoor air —

Part 17:
Detection and enumeration of moulds — Culture-based method

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 a method for the detection and enumeration of moulds by cultivation after sampling by impaction according to ISO 16000-18 or by filtration according to ISO 16000-16. It is also suitable for cultivation of moulds from material suspensions or from direct plating.

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 8199, *Water quality — General guidance on the enumeration of micro-organisms by culture*

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

ISO 16000-18, *Indoor air — Part 18: Detection and enumeration of moulds — Sampling by impaction* [1)]

3 Definitions

For the purpose of this part of ISO 16000, the following terms and definitions apply:

3.1
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]

1) To be published.

3.2
filtration
collection of particles suspended in gas or liquid by flow through a porous medium

[EN 13098:2000 [4]]

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.

3.3
indirect method
⟨air quality⟩ resuspension of deposited microorganisms with subsequent plating of aliquots on a suitable culture medium, incubation and counting of colonies growing under the conditions selected

3.4
colony forming unit
cfu
unit by which the culturable number of microorganisms is expressed

[EN 13098:2000 [4]]

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 one particle.

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

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

[ISO 16000-16:2008]

3.6
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 [4]]

3.7
moisture indicator
⟨air quality⟩ mould in indoor environments preferring relatively high humidity for growth and therefore indicating moisture problems when present

3.8
secondary colony
colony which does not originate from the “primary” sampling of airborne spores but from a spore released from a colony growing on the agar plates

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.

[ISO 16000-16:2008]

4 Principle

Agar plates (DG18 agar and malt-extract agar or potato dextrose agar) obtained from sampling by impaction are incubated directly at (25 ± 3) °C.

Filters obtained from sampling by filtration are re-suspended in saline solution (0,9 % mass fraction NaCl) with 0,01 % polysorbate 80 [2]. Decimal dilutions of the suspension are prepared and aliquots spread on DG18 agar as well as on malt-extract agar or potato dextrose agar (indirect method). Agar plates are incubated at (25 ± 3) °C. For special purposes plates can be incubated at (36 ± 2) °C (e.g. thermotolerant *Aspergillus* spp.) or (45 ± 2) °C (*Aspergillus fumigatus*).

After incubation, mould colonies are identified and counted. The extent of identification depends on the objective of the investigation.

5 Apparatus

Usual microbiological laboratory equipment, and in particular the following.

5.1 **Incubator**, vibration free, thermostatically controlled at (25 ± 3) °C.

5.2 **Incubator**, vibration free, thermostatically controlled at (36 ± 2) °C.

5.3 **Incubator**, vibration free, thermostatically controlled at (45 ± 2) °C.

5.4 **Refrigerator**, thermostatically controlled at (5 ± 3) °C.

5.5 **pH meter**, with an accuracy of ± 0,1 pH unit.

5.6 **Microbiological safety cabinet**, Class II, for user and product protection.

5.7 **Water bath**, capable of being maintained at 35 °C to 40 °C with shaker.

5.8 **Test tube shaker**, e.g. Vortex shaker [3].

5.9 **Petri dishes**, vented, sterile, of diameter ~90 mm.

5.10 **Autoclave**, capable of operating at (115 ± 3) °C and at (121 ± 3) °C.

6 Culture media and diluents

Use reagents of recognized analytical grade, unless other grades can be shown to lead to similar results, and only distilled or deionised water or water of equivalent purity.

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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2) Polysorbate 80 is equivalent to polyoxyethylenesorbitan monooleate or polyethylene glycol sorbitan monooleate. Tween is an example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

3) Example of a suitable product available commercially. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product. Equivalent products may be used if they can be shown to produce similar results.

6.1 Dicloran 18 % glycerol agar (DG18 agar)

The components are listed in Table 1.

Table 1 — Composition of dicloran 18 % glycerol agar (DG18 agar)

Component	Quantity
Peptone [4)]	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
Dicloran (2,6-dichloro-4-nitroaniline) 0,2 % volume fraction in ethanol	1,0 ml [a]
Chloramphenicol	0,1 g
Glycerol	220 g [b]
Agar	15,0 g
Water	1 000 ml

[a] Final concentration in medium: 0,002 g/l.

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

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

Plates of DG18 agar in bags will keep for up to one week at (5 ± 3) °C in the dark.

DG18 agar has a defined reduced water activity. Take care to avoid further reduction in water activity by desiccation because this may prevent fungi from growing on this agar.

NOTE DG18 agar is suitable for the detection of a wide spectrum of xerophilic (i.e. preferring dryness) fungi. Glycerol reduces the water activity, a_{H_2O}, to 0,95. Chloramphenicol inhibits bacteria, especially gram-negative bacteria. Dicloran inhibits the spread of fast-growing mould colonies and thus prevents overgrowing of slow-growing colonies.

6.2 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

4) Different peptones are used by different manufacturers (e.g. casein peptone, mycological peptone). This does usually not influence the quantitative results of the measurements, but may 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.

NOTE The addition of Chloramphenicol (0,05 g/l) may be necessary if samples contain high concentrations of bacteria. This is usually not the case for samples of indoor air but bacteria may be present in high numbers in material and dust samples.

Add ingredients and agar in the water and dissolve by boiling. Sterilise in an autoclave at (115 ± 3) °C for (10 ± 1) min. After sterilisation, 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 one month at (5 ± 3) °C in the dark.

Many commercial malt-extract agars with different compositions are available. Ensure that the ingredients correspond to the composition given above.

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

Add ingredients and agar to the water and dissolve by boiling. Sterilise in an autoclave at (115 ± 3) °C for (10 ± 1) min. After sterilisation, the pH shall correspond to 5,6 ± 0,2 at 25 °C. Dispense aliquots of about 20 ml into Petri dishes.

Plates of potato dextrose agar in bags will keep for up to one month at (5 ± 3) °C in the dark.

6.4 Saline solution

The components are listed in Table 4.

Table 4 — Composition of saline solution

Component	Quantity
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml

Dissolve the NaCl in the water and sterilise in an autoclave at (121 ± 3) °C for (15 ± 1) min.

6.5 Saline solution with polysorbate 80

The components are listed in Table 5.