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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*



Reference number  
ISO 16000-21:2013(E)

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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](http://www.iso.org/directives)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

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<sub>2</sub>)*
- *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<sub>2</sub>)*
- *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  $(121 \pm 3) ^\circ\text{C}$  and  $(115 \pm 3) ^\circ\text{C}$ .
- 5.2.2 **Petri dishes**, vented, sterile, diameter approximately 9 cm.
- 5.2.3 **pH meter**, with an accuracy of  $\pm 0,1$ .

## 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  $\pm 0,01$  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 <sup>c</sup>	5,0 g
Glucose	10,0 g
Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	1,0 g
Magnesium sulfate heptahydrate ( $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ )	0,5 g
Dichlorane (2,6-dichloro-4-nitroaniline) 0,2 % volume fraction in ethanol (100 %)	1,0 ml <sup>a</sup>
Chloramphenicol	0,1 g
Glycerol	220 g <sup>b</sup>

<sup>a</sup> Final concentrate in medium: 0,002 g/l.

<sup>b</sup> 18 % mass fraction of approximately 1 220 g final mass = approximately 220 g.

<sup>c</sup> 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.

**Table 1** (continued)

Component	Quantity
Agar	15,0 g
Distilled water	1 000 ml
<p>a Final concentrate in medium: 0,002 g/l.</p> <p>b 18 % mass fraction of approximately 1 220 g final mass = approximately 220 g.</p> <p>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.</p>	

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

Plates of DG-18 agar in bags can be kept for up to 1 mo at  $(5 \pm 3)^\circ\text{C}$  in the dark.

NOTE 1 DG-18 agar is suitable for the detection of a wide spectrum of xerophilic fungi (i.e. preferring dryness). Glycerol reduces the water activity,  $a_w$ , to 0,95. Chloramphenicol inhibits bacteria, especially gram-negative bacteria. Dichlorane inhibits the spreading of fast-growing mould colonies and thus prevents overgrowing of slow-growing colonies.

NOTE 2 Depending on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful providing they have been shown to not influence the test results. In case of using streptomycin or ampicillin, these antibiotics should be added to the sterilized DG-18 agar just before dispensation.

### 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
Distilled 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 Depending on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful providing they have been shown to not influence the test results. In case of using streptomycin or ampicillin, these antibiotics should be added to the sterilized malt extract agar just before dispensation.

Add ingredients and agar in the water and dissolve by boiling. Sterilize in an autoclave at  $(115 \pm 3)^\circ\text{C}$  for  $(10 \pm 1)$  min. After sterilization, the pH shall correspond to  $5,5 \pm 0,2$  at  $25^\circ\text{C}$ . Dispense aliquots of about 20 ml in Petri dishes.

Plates of malt extract agar in bags will keep for up to 1 mo at  $(5 \pm 3)^\circ\text{C}$  in the dark.

NOTE 3 Many commercial malt extract agars with different compositions are available. It is important to check that the ingredients correspond to the composition given above.

### 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
Distilled 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 Depending on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful providing they have been shown to not influence the test results. In case of using streptomycin or ampicillin, these antibiotics should be added to the sterilized potato dextrose agar just before dispensation.

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

Plates of potato dextrose agar in bags will keep for up to 1 mo at  $(5 \pm 3)$  °C in the dark.

## 6.5 Dilution buffer

The components are listed in [Table 4](#). The dilution buffer contains a phosphate buffer to compensate acid or alkaline conditions in the material samples.

**Table 4 — Composition of dilution buffer**

Component	Quantity
Potassium dihydrogen phosphate ( $\text{KH}_2\text{PO}_4$ )	3,52 g
Disodium hydrogen phosphate dehydrate ( $\text{Na}_2\text{HPO}_4 \times 2\text{H}_2\text{O}$ )	7,27 g
Sodium chloride (NaCl)	4,30 g
Tween <sup>®a</sup> 80/(volume fraction 0,01 %)	0,1 ml
Distilled water	1 000 ml

<sup>a</sup> Tween<sup>®</sup> 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.

Add ingredients in approximately 900 ml water and dissolve. pH shall correspond to  $7,0 \pm 0,2$  at 25 °C. Check pH and adjust if necessary. Make up to 1 000 ml and dispense in appropriate aliquots in flasks and 9 ml aliquots in tubes. Sterilize in an autoclave at  $(121 \pm 3)$  °C for  $(15 \pm 1)$  min.

## 6.6 Staining solution

The components of the staining solution are listed in [Table 5](#).

**Table 5 — Composition of staining solution**

Component	Quantity
Cotton blue	0,5 g
Lactic acid (80 % to 85 %)	4,0 g
Glycerol	8,0 g
Distilled water	100 ml

Add ingredients in 100 ml water and dissolve.

## 7 Measurement procedure

Depending on the measurement task, different methods for sampling and analysis of materials can be applied as specified in ISO 16000-19.

### 7.1 Sampling from surfaces

#### 7.1.1 General

Direct contact plates (see [7.1.2](#)) and the tape-lift method (see [7.1.3](#)) are used to sample surfaces of materials. In addition, sterile swabs can be used for surface sampling on surfaces that are not accessible to agar plates [e.g. corners, chinks (see [7.1.4](#))]. These surface sampling methods provide only semiquantitative results. Presenting the measurement result in terms of “colony-forming units (cfu) per unit area” is not recommended, as a dense mould layer will normally form on surface samples of infested materials and some moulds can overgrow or inhibit competing species. A more appropriate approach is the description of the population density on the culture medium (e.g. sporadic, high, dense layer). Identification of mould species provides more information compared to mould quantification. For very clean surfaces (sterility testing), quantification can be anticipated by the direct plate method since no or only very few colonies are expected to grow on the agar surface.

#### 7.1.2 Contact plate method

A specialized Petri dish (e.g. RODAC<sup>®</sup><sup>1)</sup> or a specialized flexible plastic bag filled such that the growing medium slightly projects over its edge is pressed against the material at the place to be examined. DG-18 agar and malt extract agar or potato dextrose agar are used concurrently as culture media. Other culture media can be needed, depending on the question to be answered.

Transport the plates to the laboratory (see [7.3](#)) and incubate and analyse according to ISO 16000-17.

#### 7.1.3 Tape-lift method

The tape-lift method transfers the moulds from the material surface to a transparent adhesive film. For this purpose, press the adhesive tape carefully against the material surface to be sampled and then pull it away. Stick the tape with the adhering moulds to a document plastic folder with grained surface, place it into a clean transport bag, and ship it to the laboratory for analysis. Alternatively, stick the adhesive tape to a microscope slide or a clean transparent plastic bag.

NOTE 1 In case of only few mould or surface material adhering to the tape, the use of microscope slides or clean transparent plastic bags is not convenient because it will be difficult to remove the tape without warping or breaking it.

Samples are transported to the laboratory (see [7.3](#)) and analysed by microscopy (see [7.4](#)).

The tape-lift method and direct material microscopy offer the advantage that suspected mould growth on the material can be confirmed by the detection of mycelium.

#### 7.1.4 Swab samples

Depending on the problem to be investigated, a sample is collected from the material surface with a dry or moist sterile swab and streaked out on DG-18 agar and malt extract agar or potato dextrose agar. Other culture media can be needed, depending on the question to be answered. Only qualitative or semiquantitative (if a sampling method is defined) results are obtained by this method. When high concentrations are to be expected, the swab sample can be processed using the suspension method (see [7.5](#)), giving an indication of the concentration of fungi present on the surface area sampled. Compared

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1) RODAC<sup>®</sup> (Replicate Organism Detection and Counting) is the trade name of a product commercially available from a variety of sources. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

to the direct contact plate method, this sampling procedure offers the advantage that the sample can be plated on different agars in parallel. Moreover, it allows the sampling of surfaces that are not accessible to agar plates (e.g. corners, chinks).

## 7.2 Bulk sampling

Depending on the investigation objective, bulk material samples are analysed from the complete material or from defined material layers. The material to be examined is removed in a suitable manner using sterilized tools, and packed into a sterile container or bag.

Ideally, samples from different depths can be obtained by taking a drill core sample of at least 3 cm, preferably 5 cm, in diameter. Samples from a defined depth can subsequently be removed aseptically and analysed in the laboratory.

Samples are transported to the laboratory (see 7.3) and analysed either by direct microscopy (see 7.4) or by the suspension method (see 7.5) followed by cultivation as described in ISO 16000-17.

## 7.3 Transport and storage

Pack material samples into sterile containers or bags and agar plates with the sampling surface up in closed containers. Protect material samples and agar plates from disturbing impacts (sunshine, humidity or desiccation, heat and dust, etc.) and transport them to the laboratory immediately after sampling. Transport temperature shall not exceed the incubation temperature of  $(25 \pm 3) ^\circ\text{C}$ . Cool samples during transport, if necessary. Take care not to freeze them and avoid very low temperatures because of condensation problems. Document conditions during transport (temperature, duration). Process samples, preferably within 24 h but not later than 48 h after the end of the sampling period. Until further processing, the samples should be stored in a refrigerator at  $(5 \pm 3) ^\circ\text{C}$ .

## 7.4 Direct microscopy

Tape-lift samples are directly used for microscopy. Bulk material samples can be analysed by direct microscopy using preparations produced from the material samples (e.g. disintegrated material pieces, cross sections, wash-out preparations). The microscopic analysis offers the advantage that suspected mould growth on/in the material can be confirmed by detection of mycelium.

The samples are stained with cotton blue in lactic acid (see 6.6) and evaluated under the microscope at up to  $1\,000\times$  magnification. With lime-containing materials (e.g. plaster), evaluation after staining with cotton blue in lactic acid is not reasonably practicable because of the gas bubbles forming as a result of the reaction of the lactic acid with the carbonates. In this case, an alternative staining agent (e.g. aniline blue) shall be used.

When analysing surface or bulk samples of materials, due attention should be given to whether only spores are present or also mould mycelium. The presence of mycelium is indicative of mould growth on/in the materials while spores can also originate from other sources.

The microscopic evaluation provides only semiquantitative results. The result is reported as spore types and mycelial fragments identified in the order of their frequency of occurrence.

## 7.5 Suspension of material and swab samples

The suspension method uses homogenized material which is suspended in a buffer solution (see 6.5) to liberate the moulds from the material or the cotton swabs (see 7.1.4). The cotton swab is transferred to a tube with a defined volume of buffer (see 6.5) and a suspension with different dilution steps is produced. Subsequently, aliquots of the suspension are transferred to agar plates for cultivation (DG-18 agar and malt extract agar or potato dextrose agar) as described in ISO 16000-17.

The material sample is weighed, measured, and described in terms of moisture and other properties. Subsequently, cut/crush the sample to pieces of  $<5$  mm. Depending on the material, transfer 1 g to 10 g to a sterile baffled flask. Add 50 ml to 100 ml dilution buffer. The buffer serves to adjust a pH at which

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the majority of the moulds will suffer minimum damage. Tween 80® is used to lower the surface tension and facilitate suspension. Take care that the material sample is completely covered by buffer.

Shake the flask at 200 rpm for 15 min to liberate the spores from the material.

**NOTE** Stomachers have been successfully used to liberate spores from material samples. Preliminary results from comparative studies have shown a slightly higher recovery with this method compared to the shaking method described in [7.5](#).

Based on the original suspension, set up a serial dilution series. Immediately prior to dilution, shake the suspension for  $3 \times 3$  s on a test tube shaker. Transfer 1 ml of the suspension to 9 ml dilution buffer (see [6.5](#)) using a sterile disposable pipette or a cotton-stuffed glass pipette. In the same way, carry out two further dilution steps resulting in dilutions 1:10, 1:100, and 1:1 000.

The number of dilution steps and the dilution intervals should be adapted to the expected mould concentration and the specific measurement task. It might be necessary to set up additional dilution steps.

Subsequent plating of aliquots from the original suspension and from the dilutions as well as cultivation and further analysis is carried out according to ISO 16000-17.

## 8 Quality assurance

The laboratory shall implement quality assurance measures to be documented and made available any time.

## 9 Sampling protocol

The samples shall be uniquely identified and labelled accordingly.

A sampling protocol shall be filled in for each sample before (or just after) sampling.

The protocol shall, as minimum, indicate

- a) a reference to this part of ISO 16000,
- b) the name and address of the client,
- c) the measurement task,
- d) the type of material sampled,
- e) the type of sampling device used, and
- f) the sampling date, hour, location of sampling.

## 10 Performance characteristics

The suitability of the suspension method was tested by comparative measurements with DG-18 agar and malt extract agar (see [Annex A](#)).

## Annex A (informative)

### Sample exchange for method validation

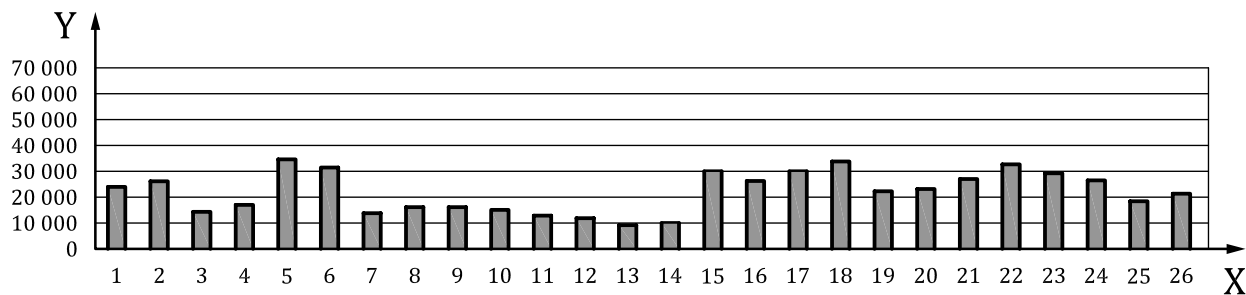
The suitability of the sampling method described in this part of ISO 16000 was tested by sending samples from a homogenized, moisture-damaged material to eight to 10 different laboratories. One or two laboratories were asked to analyse five samples in parallel to check for the distribution of fungi in the material. All other laboratories analysed two samples in parallel.

One sample exchange was performed for plaster with six laboratories. Total concentration of culturable fungi, calculated according to ISO 16000-17, was relatively low [mean 502 cfu/g (see [Table A.1](#))]. Measurement uncertainty for five parallel samples in one laboratory was 25 % and for results of all laboratories, 33 %.

Several sample exchange trials were conducted with polystyrene and mineral fibre. Laboratories were asked to present results separately for DG-18 and malt extract agar. Typical results are presented in [Figure A.1](#) for DG-18 agar and in [Figure A.2](#) for malt extract agar. Measurement uncertainty for parallel samples in one laboratory were in the range of 10 % to 20 % and for results of all laboratories, around 30 %.

**Table A.1 — Results of sample exchange using plaster**

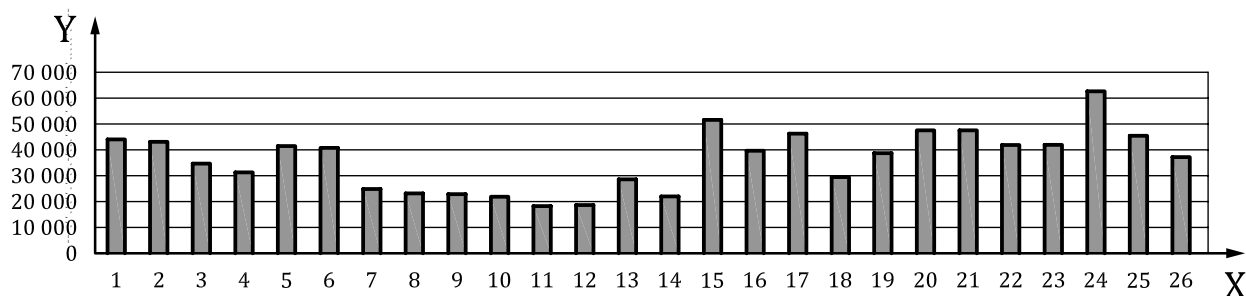
Number of laboratory	Concentration fungi cfu/g material (combined result of DG-18 and malt extract agar according to ISO 16000-17)
1	478, 353, 360, 327, 231 350 (mean) SD 25 %
2	463
3	355
4	430
5	720
6	695
Mean of lab 1 to 6	502
Standard deviation (SD) lab 1 to 6	33 %



**Key**

- X number of laboratory
- Y colony forming units (cfu) per gram dichlorane glycerol agar (DG-18)

**Figure A.1 — Results of concentrations of fungi (cfu/g DG-18) in polystyrene during sample exchange (numbers 1 to 26 indicate laboratories and parallel samples)**



**Key**

- X number of laboratory
- Y colony forming units (cfu) per gram malt extract agar (MEA)

**Figure A.2 — Results of concentrations of fungi (cfu/g MEA) in polystyrene during sample exchange (numbers 1 to 26 indicate laboratories and parallel samples)**



## Bibliography

- [1] ISO 16000-16, *Indoor air — Part 16: Detection and enumeration of moulds — Sampling by filtration*
- [2] VDI 4300 Part 10, *Messen von Innenraumluftverunreinigungen — Messstrategien bei der Untersuchung von Schimmelpilzen im Innenraum* [Measurement of indoor air pollution — Measurement strategies for determination of mould fungi in indoor environment]
- [3] EN 13098, *Workplace atmospheres — Guidelines for measurement of airborne micro-organisms and endotoxin*

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