INTERNATIONAL STANDARD

ISO 16000-19

First edition
2012-06-01

Indoor air —

Part 19: Sampling strategy for moulds

Air intérieur —

Partie 19: Stratégie d'échantillonnage des moisissures

Reference number
ISO 16000-19:2012(E)

ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

Published in Switzerland

Contents

Page

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 16000-19 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 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 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 28: Determination of odour emissions from building products using test chambers*

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*

Introduction

Mould spores and metabolites can be inhaled via the air and cause allergic and irritating reactions and/or complex symptoms in humans. Moreover, mould growth can be associated with severe odour nuisances. In rare cases, some mould species can cause infections (so-called mycoses) in certain risk groups.[14][18][19]

There is sufficient epidemiological evidence that damp and mouldy buildings increase the risk of respiratory symptoms, respiratory infections and enhances asthma symptoms of the occupants.[8] In addition, there is some evidence for increased risk of development of allergic rhinitis and asthma. Furthermore, there is clinical evidence for rare symptoms like allergic alveolitis, chronic rhinosinusitis and allergic sinusitis. Toxicological studies *in vivo* and *in vitro* show irritating and toxic reactions of microorganisms (including spores, cell components and metabolites) from damp buildings.[8]

Growth of microorganisms in damp buildings can lead to increased concentrations of spores, cell fragments, allergens, mycotoxins, endotoxins, β-glucanes and MVOC (microbial volatile organic compounds). From the studies conducted so far it is not clear which compounds are the causative agents of the health effects observed. Nevertheless, increased concentrations of each of these compounds are considered a potential health risk[8][18] and growth of mould in buildings should, therefore, be avoided.

The prime objective of this part of ISO 16000 is to provide assistance in identifying mould sources in indoor environments.

Indoor air —

Part 19: Sampling strategy for moulds

1 Scope

This part of ISO 16000 describes the measurement strategy for the detection of fungi in indoor environments.

It describes suitable sampling and analysis methods together with a description of the applicability and the interpretation of the measurement results to maximize the comparability of the measured data obtained for a given measurement objective. It does not include details on recording building characteristics or field inspections by qualified professionals which have to take place prior to any microbiological measurement.

This part of ISO 16000 is not applicable to a detailed description of the building physics- and building-engineering-related procedures applicable to field inspections. The methods and procedures presented do not allow quantitative exposure assessment with regard to the room occupants.

The application of this part of ISO 16000 presupposes the knowledge of ISO 16000-1.

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 16000-16, *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*

3 Terms and definitions

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

3.1
pre-existing mouldy condition
desiccated “old” mould growth, where additional biomass growth no longer occurs and the indoor air mould spore concentration gradually decreases with time

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

[SOURCE: EN 13098:2000[6]]

NOTE The biological collection efficiency considers the sampling stress occurring during sampling and analysis in addition to the physical collection efficiency.

3.3
identification of moulds
assignment of moulds to spore types or groups on the basis of defined properties (e.g. morphological, biochemical, molecular-biological properties)

NOTE The term “differentiation” is frequently used instead of identification. The term “differentiation” is, however, misleading because the intention is not to merely differentiate the moulds but to identify them, i.e. to assign them, e.g. to genera or species.

3.4
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.

3.5
filtration
collection of particles suspended in a gas or liquid by flow through a porous medium

[SOURCE: 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.

3.6
total spore count
number of (culturable and non-culturable) spores that are collected and enumerated under the microscope

NOTE For the term “spores”, see 3.19, Note 2.

3.7
yeast
unicellular fungus that does not normally produce a mycelium and reproduce by budding (budding fungi) as against moulds, which reproduce by sporulation

3.8
impaction
sampling of particles suspended in air by inertial separation on a solid surface (culture medium or adhesive-coated slides)

NOTE 1 See 16000-18.

NOTE 2 Sampling is carried out using either round-hole or slit impactors, for instance. As the air passes through the orifices, it is accelerated and the particles are impacted on the medium located directly behind the nozzles as a result of their inertia, while the air flows around the culture medium and exits the sampler. Impaction samples are only suitable for direct analysis without further resuspension of the sample.

3.9
colony forming unit
cfu
⟨air quality⟩ unit by which the culturable number of microorganisms is expressed

[SOURCE: EN 13098:2000[6]]

NOTE 1 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 The number of colonies can depend on the cultivation conditions.

3.10
colony morphology type
group of colonies which due to their morphological appearance seem to belong to a specific species

3.11
colony count
⟨air quality⟩ number of all microorganism colonies visible on a culture medium after incubation under the selected cultivation conditions

3.12
culturable mould
mould that can be cultured under the selected cultivation conditions

NOTE Parameters governing the culturability are, for instance, the type of culture medium and the incubation temperature.

3.13
cultivation
growing of microorganisms on culture media

3.14
mycotoxin
secondary metabolites of moulds which are toxic to humans and animals

3.15
mycelium
total of fungal hyphae

3.16
non-culturable mould
mould that cannot be cultured under the selected cultivation conditions

3.17
physical sampling efficiency
capacity of the sampler to collect particles with specific aerodynamic diameters suspended in air

[SOURCE: EN 13098:2000,[6] modified — "aerodynamic diameters" has replaced "sizes".]

3.18
sampling stress
damage suffered by the microorganisms during sampling (e.g. through mechanical and chemical effects or through water deprivation)

3.19
mould
filamentous fungi from several taxonomic groups, namely *Ascomycetes*, *Zygomycetes*, and their anamorphic states formerly known as *Deuteromycetes* or fungi imperfecti

NOTE 1 Taxonomically, moulds do not represent a uniform group.

NOTE 2 Moulds form different types of spores depending on the taxonomic group they belong to, namely conidiospores (conidia), sporangiospores or ascospores. In practice, all these reproductive stages are summarized under the term “spores”.

3.20
mould damage
damage caused to building materials and surfaces by mould growth

NOTE Mould damage can result in loss in value, health risks and restrict the occupancy of the affected sites.

3.21
secondary colony
colony that does not originate from the “primary” sampling of airborne spores but from a spore released from a colony growing on the agar plates

3.22
secondary contamination
mould contamination of surfaces not caused by mould growth but originating from a (contaminated) primary source after aerial dispersion

3.23
cut-off value
particle size (aerodynamic diameter) for which the sampling efficiency is 50 %

3.24
total sampling efficiency
product of the physical sampling efficiency and the biological preservation efficiency

[SOURCE: EN 13098:2000[6]]

4 Properties, origin and occurrence of moulds in indoor environments

Moulds are ubiquitous on our planet. They are involved in the decomposition of organic material and, therefore, play an important role in the natural carbon cycle. Their concentration in the ambient air depends, *inter alia*, on location, climate, time of the day and season. Airborne mould concentrations are subject to great variability.[9][10][11] This is due to the following reasons.

The mould concentration in local ambient air is mainly determined by the location relative to the respective mould sources, wind direction and wind force. Mould spores are frequently released by specific sources such as decaying material. Both natural processes and production processes, such as composting, recycling, animal production facilities, grain and food processing plants as well as horticulture facilities, can be sources of mould dispersion.

Sporulation, i.e. the production of mould spores occurs discontinuously. It is governed, *inter alia*, by the mould lifecycle phase, the environmental conditions, stress factors, humidity as well as substrate composition and availability.

Factors governing the dispersion of spores, most of which have aerodynamic diameters in the range of 2 µm to 40 µm, are mechanically or thermally induced air movements, drying phases (leading e.g. to de-agglomeration of deposited dust) and the capability of air dispersal of the mould spores.[12][13][14]

Due to the ubiquitous nature of moulds, it can be assumed that they are always present in indoor air. The presence of moulds in indoor air can be due to spores originating from ambient air on the one hand and to recent active mould growth, pre-existing mouldy conditions or mould deposits (settled spores) on the other. To distinguish between sources, it is, therefore, important to perform ambient air measurements for reference whenever conducting indoor air measurements for moulds.[14][15] In addition, the collection of a control sample from a suitable reference room may be helpful.

Possible causes of indoor mould sources are surface moisture on building materials or moisture in the building structure, but also rotting food, potted plants, biowaste collection, source separation of waste, deposited dust due to poor cleaning as well as the keeping of animals in residential settings. Moisture damage can be attributable to building defects, inappropriate ventilation and heating or unfavourable arrangement of furniture as well as water damage (e.g. plumbing leaks or flooding events). Elevated mould levels in indoor environments and the occurrence of certain mould species (see Annex A) are indicative of excessive moisture. When residential environments or occupational settings are infested with moulds, the mould source shall be located to be able to plan remedial measures.

Main factors affecting the intensity of mould growth and the mould species developing are moisture, temperature, nutrient supply and the pH. If environmental conditions are favourable, a great variety of moulds can develop. Once environmental conditions become less favourable, the species best adapted to the given conditions will predominate.[16]

Mould sources can release spores, mycelial fragments, but also cell components and metabolic products such as β-glucans (polysaccharides contained in the cell wall of fungi), ergosterol (steroid compound contained in the cell membrane of fungi), toxins and MVOCs (microbial volatile organic compounds such as certain aldehydes, alcohols, esters, ketones). On cultivation, colonies can grow not only from spores, but also from mycelial fragments.

The number and airborne dissemination of spores released vary with the type of mould damage. For an assessment of indoor mould sources, it is, therefore, important to differentiate the individual mould species by their type of spore dispersal. Experience has shown that even minor mould contamination of materials can result in elevated indoor air mould levels if the species involved have dry spores with good air dispersal capabilities (e.g. *Penicillium* and *Aspergillus).* By contrast, airborne spore concentrations are much lower when materials are colonized, for instance, by moulds of the genera *Acremonium*, *Fusarium* or the species *Stachybotrys chartarum* that have relatively large spores embedded in slimy substances and, therefore, have poor air dispersal capabilities.

Furthermore, it should be taken into account that mould spores are not necessarily present as individual spores in the air or settled dust, but also occur in the form of spore aggregates or are particle-borne. Depending on the analysis method, they are determined individually or as spore aggregate. Materials, indoor air and house dust contain not only culturable but also non-culturable mould spores, some of which can have the same allergenic and toxic effects as culturable spores. For this reason, techniques have been developed that allow the microscopic determination of both culturable and non-culturable moulds.

Mould detection and identification are performed either after cultivation based on morphological criteria, biochemical reactions and/or molecular techniques or by direct microscopic examination. Identification based on the morphological structure (macroscopic examination, stereo-microscopy and microscopy) either after prior cultivation or by direct microscopy is still the most prevalent approach for the detection of moulds.

Besides, there are other analytical methods based on the determination of cell components and metabolites of moulds such as β-glucans, ergosterol, toxins and MVOCs.[17] The determination of these compounds serves, however, only as supplementary information.

The sampling methods employed for detection of moulds are determined by the objective of the investigation. Depending on the sampling method, the moulds suffer a sampling stress during sample collection and preparation, which can lead to their drying-out or dying. Factors affecting the culturability of mould spores are their physiological state as well as the culture medium employed. Some mould species cannot be cultured at all under laboratory conditions.

NOTE The genera *Stachybotrys* and *Chaetomium* hardly grow and sporulate only poorly, if at all, on DG18 agar. The use of this culture medium for culture-based analysis of these genera is therefore not recommended (see ISO 16000-17).

For a literature summary see References [8]–[10], [12], and [14]–[18].

5 Sampling and detection methods

Depending on the objective of the investigation, materials (see ISO 16000-21, in preparation), air (see ISO 16000-16 and ISO 16000-18) and house dust may be sampled and analysed for culturable moulds (see ISO 16000-17). Moulds can also be quantified and, to some extent, differentiated without prior cultivation. For this purpose, airborne mould spores are collected on filters or directly on an adhesive-coated microscope slide, followed by staining and subsequent direct microscopy.

Annex B gives an overview on the most common devices for total spore count measurements as well as for sampling devices for filtration and impaction and the respective analysis methods.

6 Measurement strategy

6.1 General aspects

There is no standard procedure for measurement and assessment of mould damage. The type and amount of measurements as well as the analytical methods employed are determined by the circumstances triggering the investigation and the investigation objectives. A visual field inspection (walk through) by technically qualified professionals prior to sampling is a key prerequisite for detecting and assessing mould sources in indoor environments. Besides a good knowledge of building engineering and building physics, the professionals conducting the inspection should have a sufficient background in indoor air hygiene and microbiology.

Investigations are conducted with the objective of locating mould sources in indoor environments. To support findings from visual observations and confirm suspected mould growth, professionals can draw on a variety of sampling and analysis methods. These include methods for determining mould concentrations in or on materials, procedures for the measurement of mould concentrations in indoor air as well as procedures for determining mould concentrations in house dust. An example for a report accompanying sampling is attached as Annex C.

Circumstances triggering a microbiological investigation of indoor environments may include the following (see also Table 1):

— visible mould damage;

— material dampness without presence of visible mould growth;

— structural or non-structural building anomalies without presence of visible mould growth;

— health problems without presence of visible mould growth;

— odour problems without presence of visible mould growth;

— verification measurements during and after remediation.

In the case of visible mould damage with known source, the remediation and elimination of the underlying causes should be addressed as a priority. In many cases, microbiological investigations is not necessary.

If mould damage is suspected without the presence of visible sources, the indoor environment can be examined for the presence of elevated mould concentrations. Depending on the circumstances triggering the investigation, the following media may be sampled and analysed:

a) materials and their surfaces (see 6.1.1);

b) indoor air in comparison with ambient air (see 6.1.2);

c) house dust (see 6.1.3).

The results of the measurements described in the following sections provide only indications on the damage stage. Assessing the actual age of the mould growth is not feasible as the state of mould growth can change drastically within very short time intervals.

The inspection of HVAC systems for lack of hygiene is not the subject of this part of ISO 16000.

In planning and performing measurements, the specific field conditions and influencing factors having a major impact on the investigation results shall be taken into account and documented.

6.1.1 Analysis of surfaces or materials

For the selection of a suitable sampling method and the definition of the sampling locations, the following questions have to be clarified.

— Is mould growth or secondary contamination expected on the surface or the material?

— Is a surface colonization or a colonization of deeper layers expected?

— Are the moulds expected or under study culturable?

— Are criteria available for an assessment of the analysis result?

Criteria for differentiating between active mould growth and mould deposits on material surfaces or in wall cavities originating from natural sedimentation are the mould concentration and evidence of mould structures, e.g. mycelium or spore carriers, in the material or on its surface. The mould concentration in the material or on the material surface varies with the type of material, especially the density of the material, and the mould species. Different mould species grow on or in a material depending on moisture, temperature and nutrient source. Suspected mould contamination of surfaces may be confirmed by surface sampling using the tape-lift and contact plate methods. The contact plate method presupposes that the moulds are culturable. If the surface has already been disinfected or if contamination with *Stachybotrys chartarum* is suspected, the contact plate method is not applicable. In such cases, it is necessary that tape-lift samples of the surfaces be examined by direct microscopy.

Surface sampling (contact plate and tape-lift methods) has limited suitability for materials with rough surfaces (e.g. plaster, insulation materials). Usually, the samples are suspended in a buffer followed by determination of the mould concentration by cultivation or direct microscopy.[25]

Where no empirical threshold levels exist for classifying a material as “contaminated” or “not contaminated”, materials displaying no visible mould growth are sampled as controls for comparison.

6.1.2 Analysis of indoor air

The objective of indoor air sampling and analysis is to determine the concentration of moulds in a representative air sample in order to assess the likelihood of mould sources in the indoor environment. Depending on the investigation objective, this requires a more or less complete identification of the moulds (see 6.2). In analysing air samples, special attention is given to differences in the species spectrum present in the indoor compared to ambient air. Moreover, the presence of moisture-indicator species (see Table A.1) should be taken into account. At high mould spore concentrations in the ambient air due to the specific weather conditions, the concentration of ambient air species in the indoor air can be many times higher than the concentrations of the moisture-indicator species of interest. If the concentration of typical ambient air species exceeds that of indoor-environment-specific moisture-indicators by a factor of more than 10, indoor air sampling allows no conclusions as to the presence of a potential mould source because moisture indicators may be overgrown by fungi from ambient air.

To avoid interferences, rooms in which the lowest airborne concentrations are expected should be sampled first.

The sampling methods in accordance with ISO 16000-16 (filtration) and ISO 16000-18 (impaction) are based on different measurement principles and do not produce the same results for all measurement tasks. For the selection of the sampling procedure and the determination of the required number of sampling locations and the sampling duration, the influencing parameters and conditions prevailing in the specific situation shall be established by a prior field inspection.

For this purpose, the following questions shall be clarified.

— Is a largely constant mould concentration expected in the room?

— Are air movements present that reflect a normal activity in the room?

— Are major fluctuations in the mould concentration expected as a result of short-term influences (e.g. occupant influences, convection or downward air flows)?

When no major occupancy-related air movements are expected and no influences leading regularly to major variations in the airborne mould levels are evident in the rooms being assessed (e.g. residential rooms), both short-term sampling (sampling period 1 min to 10 min) and long-term sampling (sampling period > 30 min) are appropriate methods. In practice, sampling by impaction is the preferred procedure for short-term sampling. This sampling method requires a prior estimate of the expected mould concentration. Different air volumes are sampled at each sampling location to be able to cover a broader concentration range. This is accomplished by collecting impaction samples over different sampling durations. The detection of indoor-relevant moulds presupposes that particles with a diameter greater than 2 µm can be quantitatively collected on the culture medium or adhesive-coated slide (for the determination of the total spore count). This presupposes that the impactors are designed for a cut-off d_{50} < 2 µm (see Table B.2). All short-term measurements should be conducted over a minimum period of 1 min. The sample volume should not be less than 50 l. In unoccupied rooms, short-time measurements may be performed without occupancy simulation, since experience has shown that especially the installation of the sampling equipments as well as their operation usually results in air movements at the sampling location that are comparable to those during normal conditions of use.

In rooms with major “old” dust deposits, sampling can cause unintentional disturbance of settled dust, which can lead to false positive results.

If a sampling device generates major exit air flows resulting in the disturbance of deposited dust, the exit air stream should be conducted of the room being investigated and/or care should be taken to ensure that it is not directed at potential mould sources, such as the floor or dusty or mouldy materials.

Filtration methods are the sole applicable option for long-term measurements. Filtration sampling is the method of choice when sampling is carried out during normal activities in the room and when major air movements and fluctuating mould concentrations are expected. Filtration sampling is also the preferred method for culturable sampling when airborne mould concentrations are expected to exceed 2 000 cfu/m^3. At sampling durations of 1 h and longer in unoccupied rooms, additional occupancy simulations are needed during the sampling period. The occupancy simulation should reflect the usual occupancy of the room.

Regardless of the sampling method, the windows and doors of the room shall be closed approx. 8 h before commencing sampling and kept closed during the sampling process. Samples should preferably be collected in the centre of the room with a minimum distance of 1 m from enclosing walls and at a height of approx. 0,75 m to 1,5 m. In all cases, an ambient air sample shall be collected for reference. Moreover, the collection of air samples in an appropriate reference room can be useful. Indoor and ambient air samples shall be collected on the same day with as short a time interval in-between as possible.

NOTE In buildings with air-conditioning, shorter time intervals (2 h) between closing the windows and sampling can be sufficient. Measurement of fungi in ambient air might not be necessary in buildings with filtered incoming air and no windows that can be opened by the occupants.

The specific conditions at the sampling location and the climatic conditions during sampling are documented in a sampling report (see Annex C).

6.1.3 Analysis of house dust

House dust analyses are normally only conducted to complement the results from indoor air measurements. As there are currently no suitable procedures for the determination of non-culturable moulds in house dust, the analysis is limited to the detection of culturable moulds. House dust analyses are a useful tool to check the results from indoor air measurements for plausibility. Before commencing sampling, it should be clarified whether suitable sampling locations with sufficient quantities of settled dust exist.[26][27]

Reference data used for the assessment of the analysis results shall have been obtained by the same sampling, sample preparation and analysis methods. Results differ greatly if total house dust or fine dust of a certain size fraction is being used.

6.2 Selection of appropriate procedure

6.2.1 Field inspection

The first step of a mould assessment in indoor environments is a field inspection in order to take an inventory. On this occasion, the circumstances triggering the investigation and details of the condition of the building, room furnishings, etc. are assembled.

Table 1 — Recommendations to help decision-making for sampling after a field inspection

Finding and objective			Matter being examined			Further procedure
			Material	Indoor air	House dust	
1	Visible mould damage		A[a]	B[b]	B[b]	Identify and, if applicable, eliminate the moisture source
2.1	Suspected mould damage	Material dampness	A	B	B	Identify and, if applicable, eliminate the moisture source
2.2		Non-structural / structural anomalies	—	A	B	Check anomalies, identify source, if applicable, and remedy
2.3		Health problems	—	A	B	Identify and remedy source
2.4		Odour problems	—	A	B	Identify odour source
3	Remediation monitoring		A	A	B	—
A Suitable examination to answer the questions of interest B Supplementary examination to answer the questions of interest (optional)						
[a] Material sampling can be useful to answer specific questions concerning major mould damage (see 6.2.2.1). [b] If it is necessary to analyse a dispersion of the contamination.						

The building structure, especially the surfaces of critical building components, is visually examined during the inspection. Moreover, information on potential causes of mould growth should be collected. For this purpose, relevant physical parameters such as temperature and humidity in the room and on materials (e.g. condensate) are recorded. If the findings give no clear picture, further non-structural building investigations (e.g. moisture measurements, thermography, Blower-Door test) may be performed. The detailed description of the procedures used during inspection is not covered by this part of ISO 16000.

Qualified professionals normally recognize visible mould growth without the need for any elaborate sampling and analysis methods. If sampling is required, information on the sampling location can be gathered using the sampling report in Annex C. Table 1 lists possible options for further analysis depending on the findings of the field inspection. The specific procedures are described in detail in 6.2.2 to 6.2.6.

When performing air and house-dust sampling after field inspection, it is necessary to take into account that any intrusive inspections into the building structure carried out during the field inspection can have released additional moulds to the indoor environment that are not attributable to the presence of mould growth.

6.2.2 Investigations prompted by questions related to visible mould damage

6.2.2.1 General aspects

For major visible mould damage, materials may be investigated to answer the following questions:

— confirmation of mould contamination or growth (see 6.2.2.2);

— establishing the damage extent and potential secondary contamination (see 6.2.2.3);

— contamination assessment (see 6.2.2.4);

— establishing prerequisites for remediation monitoring (see 6.2.2.5);

— investigations prompted on orders of a physician due to health problems (see 6.2.2.6).

For special investigation objectives (e.g. establishing the dispersion of mould contamination originating from a primary source), sampling of house dust and indoor air can provide useful supplementary information.

Moreover, it cannot be ruled out that there are hidden sources in addition to the visible ones (see 6.2.2.2 to 6.2.2.6). It is necessary to take into account building design-related hidden mould sources (curtain walls, wall cavities) in the further procedure adopted.

Investigation objectives for which further examinations are advisable are described below together with the recommended procedure and suitable measurement methods.

6.2.2.2 Confirmation of mould contamination or growth

The question as to whether discolorations or large areas of efflorescence observed during the visual inspection is attributable to mould growth or other causes (particle deposits, blackening, salt efflorescence) can be answered by microscopic examinations (tape-lift samples or direct material microscopy).

With these methods, it can also be established whether mould has grown on the material, i.e. produced a mycelium and sporulation structures, or whether only spores have sedimented on the material. Contact plate/swab samples are unsuitable for this purpose, since in many cases they do not enable a reliable distinction between mould growth and dust deposits with sedimented spores. Moreover, the contact plate/swab method detects only culturable moulds. If the site investigated has been treated with fungicides, this can lead to false negative results.

6.2.2.3 Establishing the damage extent and potential secondary contamination

For an assessment of the mould damage as well as for remediation planning, the extent of the damage, both surface and interior damage, shall be established. For the damage assessment, the colonization of surfaces has implications other than mould growth that has penetrated into the plaster or other materials.

To establish how deeply the mould growth has penetrated into the material, core samples or material collected layer by layer are examined.

The extent of surface mould growth is determined by collecting samples at different distances from the damage centre. Methods recommended for this purpose are the suspension technique and, if the material is suitable, microscopic examination (tape-lift sample or material microscopy). Microscopy provides information as to whether the damage involves fresh, active mould growth or a dried-up, pre-existing mouldy condition.

If other rooms are being examined for contamination in addition to the areas of visible mould damage, air sampling (see ISO 16000-16 and ISO 16000-18) and, additionally, house dust sampling are suitable methods. Secondary contamination of objects (furniture, textiles, garments) can be detected by contact plate/swab sampling.

If hidden mould growth is suspected in addition to the visible mould damage, the procedure described in 6.2.3 to 6.2.6 should be adopted.

6.2.2.4 Contamination assessment

Apart from the damage extent, the distinction between fresh active mould growth and a pre-existing mouldy condition is an important factor for the damage assessment. With fresh active mould growth, it is likely that high concentrations of spores are released to the indoor air and the composition of the mould species can change relatively fast. In contrast, a pre-existing mould growth can already have reached a state where major spore dispersion no longer occurs.

Microscopic evaluation of tape-lift samples normally provides indications as to whether the mould growth is fresh, or desiccated and historic. In pre-existing mould growth, the mycelial structures are frequently no longer intact or only fragments can be detected due to mite activity.

Contact plate/swab samples are only conditionally suited to assess whether a mouldy condition involves fresh active or inactive pre-existing mould growth or secondary contamination only. If the concentration of culturable moulds is in strong contradiction with the spores identified by microscopy or the visual impression of mould growth, it can be assumed that the mould is no longer active. This presupposes, however, that no disinfection with fungicides has been carried out.

In addition to the actual examination of the mould growth, moisture measurements on/in materials should always be conducted in order to assess whether a dispersion of the mould contamination is expected due to elevated moisture levels.

The above examinations provide information only on the damage state. A determination of the mould age as such is not feasible as the state of a mould growth can change tremendously within very short time intervals.

6.2.2.5 Prerequisites for remediation monitoring

Even if the mould damage is confined and hazards are predictable without further examination, it can be useful to differentiate the moulds in order to obtain data on the mould species present in the damage for subsequent remediation monitoring.

Mould growth on the surface can be examined by collecting tape-lift samples and contact plate/swab samples while suspension samples are required if the mould has penetrated into the material interior. Air sampling is not normally required as a prerequisite for remediation monitoring.

6.2.2.6 Investigations prompted on orders of a physician due to health problems

If, from a medical viewpoint, there are strong indications for a link between health problems and mould damage, a comprehensive survey of the mould species present should be conducted.

The mould growth or the material should be examined by contact plate/swab samples or the suspension method complemented by a tape-lift sample.

If additional, hidden mould growth is suspected, the procedure described in 6.2.5 is applied.

6.2.3 Investigations prompted by suspected moisture-induced mould damage

6.2.3.1 General aspects

Material dampness is a key prerequisite for mould growth and might not always be recognized by the naked eye. Material dampness is caused by water disasters, building defects, new building dampness or problematic occupant lifestyle habits.

In selecting the strategy for mould sampling and analysis, it is necessary to take the following aspects into account:

— cause of damage;

— age of damage;

— type of damp material;

— type of dampness: surface dampness (e.g. heat bridge) or penetrating dampness (e.g. defective horizontal sealing);

— in the case of water damage: was the water of good hygienic quality (e.g. drinking water) or is a microbial contamination expected (e.g. waste water).

Objectives of an investigation prompted by the presence of damp materials may include

a) confirmation of suspected mould contamination (see 6.2.3.2);

b) establishing the damage extent (see 6.2.3.3);

c) contamination assessment (see 6.2.3.4);

d) prerequisites for remediation monitoring (see 6.2.3.4);

e) investigations prompted on orders of a physician due to health problems (see 6.2.3.4).

6.2.3.2 Confirmation of suspected mould contamination

To confirm that material dampness was the underlying cause of the mould damage, the affected material is examined by contact plate and swab samples, if superficial mould growth is suspected. For materials exhibiting no visible mould growth, microscopic examinations are normally ineffective because of the low concentration of mould spores. In interpreting the results of the contact plate or swab sample analyses, it shall be borne in mind that the material surfaces are normally contaminated with mould spores originating from settled dust. The composition of the moulds identified allows a distinction between normal contamination and mould infestation and/or colonization. If required, other material surfaces not affected by moisture in the same room are examined for reference. If the moisture source is the likely cause of the interior mould growth, a bulk sample of the material should be examined by the suspension method. If the mould damage is attributable to microbiologically contaminated water, the sample shall also be analysed for bacteria (faecal indicators in the case of waste water). If the damp material is difficult to access, e.g. behind suspended ceilings or in beam-and-column constructions, screening investigations using the impaction (see ISO 16000-18) or filtration method (see ISO 16000-16) and a determination of the total spore count can be advisable to confirm the likelihood of mould damage before making an intrusive inspection into the building structure. A determination of the mould concentration in house dust can also be useful to support the suspicion. If the suspicion is confirmed, it is necessary to open the structure, and collect and examine a material sample.

6.2.3.3 Establishing the extent of the damage

If material dampness is confirmed to be the underlying cause of the mould damage, the next step is to establish the damage extent. If there is reason to assume that all damp materials are similarly affected due to the specific moisture source, a moisture measurement can be sufficient to establish the extent of the damage. If the moisture damage is due to groundwater intrusion, e.g. in hillside buildings, or if moisture penetration into the material interior is expected due to the material characteristics, core samples shall be drilled and analysed layer by layer. The number of core samples needed depends on the damage extent. Samples should also be collected from areas adjacent to the moisture damage site.

6.2.3.4 Other investigation objectives

If the presence of mould growth has been confirmed, it can become necessary to conduct a mould contamination assessment, investigations to establish the prerequisites for remediation or investigations prompted by health problems (see 6.2.2.4 to 6.2.2.6).

6.2.4 Investigations prompted by non-structural/structural building anomalies

6.2.4.1 General aspects

If non-structural building anomalies or structural building defects point to a mould infestation of concealed building components, it shall be verified whether this suspicion can be confirmed. If there is supporting evidence from prior non-structural investigations (e.g. building moisture measurements, surface temperature measurements, air tightness measurements) that building components do not satisfy the minimum thermal insulation requirements (see, for example, References [1] and [4]) or the air tightness criteria for exterior building components (see, for example, also Reference [5]), the deficient components should, if necessary, also be carefully examined for mould growth.

Objectives of investigations of suspected mould damage prompted by non-structural/structural anomalies include

— verification of the likelihood of the suspected mould damage (see 6.2.4.2);

— locating the source (see 6.2.4.3);

— confirmation of contamination (see 6.2.3.2);

— establishing the damage extent (see 6.2.3.3);

— contamination assessment (see 6.2.3.4);

— establishing the prerequisites for remediation monitoring (see 6.2.3.4);

— investigations prompted on orders of a physician due to health problems (see 6.2.3.4).

For the adoption of additional procedures in the case of confirmation of a mould source, see 6.2.3.2 to 6.2.3.4.

6.2.4.2 Verification of the likelihood of the suspected mould damage

Suspicion of mould damage frequently exists with beam-and-column or flat roof buildings, for instance. As intrusive inspections into the building structure can be very costly, the likelihood of an indoor mould source should first be confirmed by impaction or filtration sampling and determination of the total spore count (see ISO 16000-16 and ISO 16000-18). Additionally, house dust samples can be analysed for moulds as further supporting evidence. In order to check the building envelope for leaks (e.g. pavilion buildings, roof structures), it can be helpful to simulate the pressure variations during normal usage while air sampling is under way. This can be done by creating a negative pressure in the room using a blower door (see EN 13829[7]).

6.2.4.3 Locating the source

If the suspected mould damage is confirmed, the next step is to locate the mould source. Moisture and temperature measurements as well as hygrothermal studies can provide indications on the location of the mould source. Sites highly susceptible to mould growth (e.g. behind cupboards sitting alongside exterior walls) shall be visually inspected for mould. For this purpose, it can be useful to drill a hole into the defective building structure and carry out a visual examination using a borescope.

6.2.5 Investigations prompted on orders of a physician due to health problems

6.2.5.1 General aspects

If there is strong medical evidence that health problems are linked with one or several specific mould species, the mould species shall be detected and identified as comprehensively as possible. In this connection, it should be noted that mould exposure might not be due only to indoor sources but also to high outdoor mould spore levels. In the case of non-specific health problems, it should be taken into account that the diagnosed symptoms can also have other causes. Therefore, an on-site inspection of the building and its surroundings is an important element for the environment-medical anamnesis. The procedure should be closely coordinated with a physician.

Objectives of investigations of suspected mould damage prompted by health problems include, *inter alia*,

— verification of the likelihood of a specific mould exposure (see 6.2.5.2);

— locating the source (see 6.2.5.3);

— confirmation of contamination (see 6.2.3.2);

— establishing the damage extent (see 6.2.3.3);

— contamination assessment (see 6.2.3.4);

— establishing the prerequisites for remediation monitoring (see 6.2.3.4).

If the presence of a mould source is confirmed, see 6.2.3.2 to 6.2.3.4 for the procedure to adopt.

6.2.5.2 Verification of the likelihood of a specific mould exposure

If there is visible mould growth, contact plate and swab samples as well as microscopical investigations are conducted. If the mould has penetrated into the material interior, an additional material sample should be examined by the suspension method. A comprehensive identification of the mould species is required in all cases. If the field inspection does not reveal any indications of a mould source, it is necessary to collect air samples using the impaction (see ISO 16000-18) or filtration (see ISO 16000-16) method and the total spore count determined in order to verify the presence of significantly elevated mould spore levels. To confirm the suspicion, it can also be useful to analyse house dust samples for moulds. Also for these investigations, a comprehensive identification of the moulds present is required.

6.2.5.3 Locating the source

If the indoor air and dust analyses point to an indoor mould source, it is necessary that the source be located. Moisture and temperature measurements as well as hygrothermal studies can provide indications on the location of the mould source. Sites highly susceptible to mould growth (e.g. behind cupboards sitting alongside exterior walls) shall be visually inspected for mould. For this purpose, it can be useful to drill a hole into the defective building structure and carry out a visual examination using a borescope probe. If the results from the indoor air measurements point to an outdoor source, the outdoor source should be located. Possible outdoor sources are waste containers, compost heaps, accumulations of leaves, composting and waste sorting facilities.

6.2.6 Investigations prompted by odour problems

6.2.6.1 General aspects

Often odour problems in indoor environments are associated with mould damage. As the odour problem can also originate from a great variety of other indoor sources or substances (e.g. solvents, fragrants, emissions from building materials, furniture and household chemicals) or problematic occupant lifestyle habits (e.g. insufficient ventilation), investigations should be conducted to verify whether the suspicion of a link between

the odour problem and an indoor mould source is founded. If yes, it is necessary that the source be located and assessed. Objectives of investigations of suspected mould damage prompted by odour problems include, *inter alia*,

— verification of the suspected link between an odour problem and an indoor mould source (see 6.2.6.2);

— locating the source (see 6.2.6.3);

— confirmation of contamination (see 6.2.3.2);

— establishing the damage extent (see 6.2.3.3);

— contamination assessment (see 6.2.3.4).

If the presence of a mould source is confirmed, refer to 6.2.3.2 to 6.2.3.4 for the procedure to adopt.

6.2.6.2 Verification of the suspected link between an odour problem and an indoor mould source

A field inspection is performed to confirm the likelihood of the suspected link between the odour problem and an indoor mould source, i.e. whether there are indications of damp materials, leaks or humidity caused by poor hygrothermal performance of the building. If the field inspection reveals that chemical and other air pollutants are the more likely problem source, this is confirmed using the procedures presented in different parts of ISO 16000. If the findings from these investigations are negative or the presence of a mould source is more likely from the onset (e.g. musty and earthy odours often point to a mould infestation), the likelihood of an indoor mould source shall be verified by impaction (see ISO 16000-18) or filtration (see ISO 16000-16) sampling and the determination of the total spore count. To confirm the suspicion, it can also be useful to analyse a house dust sample for moulds.

6.2.6.3 Locating the source

If the suspicion is confirmed, the next step is to locate the mould source. Moisture and temperature measurements, as well as hygrothermal studies, can provide indications on the location of the mould source. Sites highly susceptible to mould growth (e.g. behind cupboards sitting alongside exterior walls) shall be visually inspected for mould. For this purpose, it may be useful to drill a hole into the defective building structure and carry out a visual examination using a borescope probe.

6.2.7 Monitoring during the remediation process and remediation verification measurements

6.2.7.1 General aspects

Remediation monitoring begins with the planning stages of a remediation project, continues with the follow-through of the remediation process and concludes with a post-remediation assessment at the end of the project. Monitoring is recommended for large-scale remediation projects, however not for the removal of minor mould damage.[3][16][28]

For remediation planning and the definition of the remediation scope, samples of the mould-infested building components or mould-suspect materials should be collected. The most prevalent methodologies used for this purpose are the suspension method and direct microscopic examination.[3]

Verification measurements during or after the remediation of mould-infested indoor environments (quality control check) are performed for the following reasons:

— verification that the mould growth has been completely removed (see 6.2.7.2);

— verification of remediation effectiveness, i.e. whether the cause of the mould growth has been eliminated and whether the remediation measures have not resulted in a higher than normal mould spore level in the building (see 6.2.7.3).

6.2.7.2 Verification of mould elimination

When large areas are affected by mould growth, the actual extent of the damage frequently does not become apparent until after all infested building materials and components have been removed. If a visual inspection allows no assessment as to whether the infested material has been completely removed, the still-suspect materials shall be examined, taking into account the proportionality principle. The most prevalent methods used for this purpose are the suspension technique and direct microscopic examination. The number of samples to be analysed depends on the extent of the damage. If disinfectants have been used to eradicate the mould growth, the moulds frequently are no longer culturable, even if still present in high concentrations. For this reason, additional non-culture-based sampling and analysis methods are needed for the verification measurements.

NOTE When damp material is dried by technical building drying measures, drying can be inhomogeneous, especially in cavities. Consequently, drying of difficult-to-access areas can be incomplete and long-term residual moisture can induce renewed mould growth. With the technology currently available, the detection of this kind of mould damage requires very elaborate testing.

6.2.7.3 Verification of remediation effectiveness

Depending on the procedure employed, remediation can result in relevant concentrations of airborne mould spore in the building. Cleaning procedures to reduce spore levels after remediation vary greatly in their effectiveness. A cleanliness check is, therefore, required depending on the damage extent and the likelihood of relevant airborne mould spore concentrations after the remediation measure. Where justified by the damage extent and the remediation scope, a visual inspection can be sufficient in the individual case.

If containment measures are implemented to prevent the spread of mould spores during the remediation activities, parallel online measurements of fine dust may be conducted or air samples collected and analysed to demonstrate that the remediation activities have not resulted in any relevant dispersion of mould spores in the building. With such an approach, it is necessary to ensure that all remediation activities associated with relevant mould releases are followed up by measurements. During the remediation activities, airborne mould spore levels can be very high and, therefore, not amenable to a quantitative determination by impaction sampling. In this case, the filtration method in conjunction with appropriate serial dilutions offers the possibility of determining even high concentrations quantitatively (see ISO 16000-16).

If the cleanliness verification is performed after completion of the remediation project, the type of sampling method used, if sampling is required at all, is determined by the remediation scope and type. It is necessary that the remediation goals and sampling methods be agreed in advance. The general mould spore level in the building is checked by determining the mould spore concentration in the indoor air or settled dust. Sampling shall be performed under conditions of use. Which sampling method is selected depends, *inter alia*, on the remediation procedure applied. If disinfection measures have been carried out in the course of the remediation project, the cleanliness verification will comprise a total spore count of the indoor air versus the ambient air. If no disinfection measures have been carried out and a fast decision on the additional procedures to adopt is needed, the determination of the total spore count is likewise to be appropriate. For a greater measurement certainty, mould concentrations and types may be determined by additional impaction sampling (see ISO 16000-18) and compared with the ambient air. Knowledge of the species involved in the original mould damage increases the validity of the post-remediation verification measurement. The analyses of house dust samples are usually only used to confirm the results of other measurements.

If it is necessary to verify the cleanliness of individual surfaces, contact plate or swab samples (see ISO 16000-21) shall be collected and analysed. Such material surface examinations allow, however, no conclusions as to the general cleanliness of the building. The examination of newly installed materials does not normally allow any conclusions as to the quality of the remediation measures.

If temperature and pressure measurements or hygrothermal studies fail to confirm unequivocally that the underlying cause of the mould damage has been eliminated, it is necessary to decide on a case-by-case basis whether a microbiological verification of the remediation effectiveness is required. The measurement methods applied and the timing of the measurements are determined by the type of mould damage and its underlying cause. If a heat bridge was the cause of mould damage, for instance, it is necessary to check that the surface temperature of the original mould growth site is consistently above the critical level, even at low outdoor

temperatures. Microbiological surface sampling should not be carried out before the first winter following the remediation in order to reliably determine whether renewed active mould growth is present.

Suitable methodologies are tape-lift samples or, if required, also the contact plate/swab method.

If the building was poorly sealed and moisture intrusion was the underlying cause of the mould damage, the post-remediation assessment comprises a check of the affected material for dryness and a check of the building for durable sealing.

7 Quality requirements and uncertainty considerations

Results from microbiological examinations can be associated with a high measurement uncertainty. Mould spores are not uniformly distributed in the air, but their distribution is a function of a great variety of parameters (e.g. air circulation, activities in the room, relative humidity). For this reason, individual mould measurements are associated with a major uncertainty factor.[23][27][29]

Depending on the sampling and analysis method employed, not all mould species present can be detected. Some mould species grow very poorly on culture media, especially if they have to survive under stress conditions (e.g. prolonged drying-out). Depending on the composition of the mould population, cultivation can show a significantly lower spore count than actually present. This can be compensated by determining the total spore count, which does not rely on growth on a culture medium.

Blank values for the sampling procedure are an important element in quality control. To determine the blank value for the filtration sampling method, a sterile filter holder with filter medium is mounted to the sampling head with the pump turned off, and removed again (see ISO 16000-16). The blank samples are processed in the same manner as the normal samples. When using the impaction sampling method, a sterile culture plate is placed into the impactor in the middle of the field measurement series and then further processed in the same way as a normal sample (ISO 16000-18).

The reference parameter for the concentration of moulds in indoor air is the concentration in the ambient air. Mould concentrations in ambient air vary greatly with climatic conditions and season.

An assessment of mould growth in indoor environments using microbiological analysis presupposes an identification of the moulds. Specialist laboratories involved and their employees shall possess the necessary qualifications and many years of experience in mould analysis. The mycological laboratory shall be equipped with appropriate rooms, apparatus and the technical infrastructure for the analytical methods employed. The specific equipment needed by a mycological laboratory includes, *inter alia*, incubators, a sterile work bench, a microscope and a stereomicroscope. The mycological laboratory shall have a laboratory unit for work with microorganisms of risk level 2.

Measures for internal and external quality assurance shall be in place. The laboratory commissioned with the mould identification should demonstrate that it is routinely and successfully participating in inter-laboratory trials for the determination of moulds.[9][14][23][27][30]–[32]

Annex A
(informative)

Moisture damage indicators

Elevated mould levels of fungi in indoor environments and the occurrence of certain mould species are highly indicative of excessive moisture. These mould species are called moisture indicators. Examples of such fungal genera and species in moderate climates are given in Table A.1.

Table A.1 — Examples of moisture damage indicators in moderate climates

Acremonium spp.
Aspergillus penicillioides
Aspergillus restrictus
Aspergillus versicolor
Chaetomium spp.
Cladosporium sphaerospermum
Engyodontium (Tritirachium) album
Penicillium chrysogenum
Phialophora spp.
Scopulariopsis brevicaulis
Scopulariopsis fusca
Stachybotrys chartarum
Trichoderma spp.

Annex B
(informative)

Devices for total spore count and detection of culturable fungi

Different devices are available for total spore counts by slit impaction and subsequent microscopy (see Table B.1) as well as for the detection and enumeration of culturable fungi by filtration or impaction (see Table B.2).

Table B.1 — Slit impactors for the determination of the total spore count in indoor air

Sampling system and flow rate l/min	Recommended sampling time min	Sample volume m^3	Cut-off d_{50} µm	Sample preparation/ analysis method	Possible range of results in spores (mycelial fragments)/m^3
Sampler with replaceable slides approx. 30	approx. 5 to 7	0,15 to 0,2	1,8[a,b]	Staining and light microscopy; enumeration of spore types (genera and/or genus groups)	50 to 100 000[d]
Sampler with replaceable slides approx. 15	5 to 10	0,075 to 0,15	(Not known)[a,b]	Staining and light microscopy; enumeration of spore types (genera and/or genus groups)	50 to 100 000[d]
Disposable cassette approx. 15	5 to 10	0,075 to 0,15	1,8 to 2,3[b,c]	Staining and light microscopy; enumeration of spore types (genera and/or genus groups)	50 to 100 000[d]

[a] The collection efficiency depends on the selected medium (adhesion, viscosity).

[b] The collection efficiency depends on the configuration of the outer spore envelope (spore-to-sampling medium contact).

[c] The collection efficiency depends on the configuration of the disposable cassette.

[d] The evaluation range applies to detailed evaluations. Here, the lower value is dependent on the number of detailed evaluations; the upper value achievable in measurement practice varies greatly because spores, skin scales, other particles, etc., trapped on the impaction surface restricts the adhesion of further spores. An overview evaluation of the complete sample trace [which makes sense only for spores with distinct morphological structures (e.g. *Stachybotrys, Chaetomium*)] allows the detection of a single spore on the entire impaction surface, i.e. the entire sample volume. The results of such an evaluation do not, however, enable a quantitative assessment.

Table B.2 — Sampling and analysis methods to determine culturable mould species in indoor air

Collection principle/ sampling system	Recommended sampling time	Sample volume m^3	Collection efficiency or cut-of d_{50}	Sample preparation/ analysis method	Possible range of results cfu/m^3
Filtration: GSP 3,5 with gelatin filter based on BGIA method 9420	1 h	0,2	Collection efficiency for particles > 1 µm: > 95 %	Suspension of gelatine filter in 2,5 ml and plating out of 0,1 ml aliquots[b] of the source suspension and two dilutions (1 × 10,1 × 100)	1 250 to 1 250 000[b]
	3 h	0,6			420 to 420 000[b]
Filtration: GSP 10 with gelatin filter based on BGIA method 9420	1 h	0,6			420 to 420 000[b]
	3 h	1,8			140 to 140 000[b]
Filtration: small filter unit in accordance with ISO 16000-16	1 h	3		Suspension of gelatine filter in 5 ml and plating out of 0,1 ml source suspension and two dilutions	170 to 170 000[b]
	3 h	9			55 to 55 000[b]
Round-hole impactors (flow rate: approx. 100 l/min)	1 min	0,1	Cut-off (0,9 to 1,6) µm	Cultivation on a medium loaded during sampling	100 to 1 000
	2 min	0,2			50 to 500
Round-hole impactors (flow rate: approx. 30 l/min)	approx. 1,5 min	0,05	Cut-off: (0,9 to 2) µm	Cultivation on a medium loaded during sampling	200 to 2 000
	approx. 3 min	0,1			100 to 1 000
	approx. 7 min	0,2			50 to 500
Slit impactors (flow rate approx. 100 l/min	1 min	0,1	Cut-off: 0,8 µm	Cultivation on a medium loaded during sampling	100 to 1 000
	2 min	0,2			50 to 500
Slit impactors (flow rate approx. 30 l/min)	approx. 1,5 min	0,05	Cut-off: (0,8 to 1) µm	Cultivation on a medium loaded during sampling	200 to 2 000
	approx. 3 min	0,1			100 to 1 000
	approx. 7 min	0,2			50 to 500

[a] The measuring range indicated takes into account that an identification to species is only possible on culture plates showing between 10 and 100 colonies/plate after 10 days of incubation. For a semi-quantitative evaluation (4 to 9 colonies/plate), the lower value of the evaluation range decreases by a factor of 2,5. Results outside the ranges indicated allow, at best, a qualitative or orientative statement.

[b] When using filtration methods and concentrations are expected to be low, 0.5 ml may be streaked out on a large plate or several small plates. In this way, the lower limit of the evaluation range can be lowered by a factor of 5.

Annex C
(informative)

Field inspection report to describe sampling procedure and to document potential mould damage

NOTE ISO grants the user of this part of ISO 16000 the right to reproduce or otherwise use the sampling protocol on this page solely for the purpose of implementing this part of ISO 16000.

To document the results of the field inspection for potential mould damage, it is recommended to include the following information in addition to the data prescribed for indoor air measurements (see ISO 16000-1).

The final format and contents of the field inspection report should be determined on a case-by-case basis within the scope of measurement planning.

C.1 Type of sample

❑ Indoor air	❑ Ambient air	❑ Floor dust
❑ Bedding dust	❑ Old dust	❑ Contact plate sample
❑ Tape-lift sample	❑ Wipe sample	❑ Water sample from HVAC
❑ Material sample, specify: _____		❑ Others

C.2 Sampling locations of individual sample _____

C.3 Air sampling method

	Sampler	Flow rate	Volume	Culture medium:	Type of filter:
Impaction	❑ _____	I_I_I_I __/__	I_I_I_I __	_____	
Filtration	❑ _____	I_I_I_I __/__	I_I_I_I __	_____	_____
Total spore count	❑ _____	I_I_I_I __/__	I_I_I_I __		

C.4 Temperature and relative humid in indoor air

Room 1: _____°C _____ %

Room 2: _____°C _____ %

Room 3: _____°C _____ %

Room 4: _____°C _____ %

C.5 Sampling conditions/Activity in room

low ❑ high ❑ Level of activity: _____

C.6 Ambient air

Location: _____

Particularities: _____

Temperature: _____ °C Relative humidity: _____ %

Wind direction: _____ Wind force: _____

C.7 Weather conditions on sampling day

Sunny/Clouded: _____ Precipitation: _____ Frost: _____

Ambient air conditions during the three days preceding the sampling: _____

C.8 House dust

Sampling head: _____ Flow rate of sampler: _____

Type of sampled surface: _____ Size of sampled surface: _____

Sampling period: _____

Last cleaned prior to sampling: _____

Location of sampling sites:

1.: _____ 2.: _____

3.: _____ 4.: _____