INTERNATIONAL STANDARD

ISO 16000-16

First edition
2008-12-15

Indoor air —

Part 16: Detection and enumeration of moulds — Sampling by filtration

Air intérieur —

Partie 16: Détection et dénombrement des moisissures — Échantillonnage par filtration

Reference number
ISO 16000-16:2008(E)

PDF disclaimer

This PDF file may contain embedded typefaces. In accordance with Adobe's licensing policy, this file may be printed or viewed but shall not be edited unless the typefaces which are embedded are licensed to and installed on the computer performing the editing. In downloading this file, parties accept therein the responsibility of not infringing Adobe's licensing policy. The ISO Central Secretariat accepts no liability in this area.

Adobe is a trademark of Adobe Systems Incorporated.

Details of the software products used to create this PDF file can be found in the General Info relative to the file; the PDF-creation parameters were optimized for printing. Every care has been taken to ensure that the file is suitable for use by ISO member bodies. In the unlikely event that a problem relating to it is found, please inform the Central Secretariat at the address given below.

COPYRIGHT PROTECTED DOCUMENT

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 16000-16 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 6, *Indoor air*.

ISO 16000 consists of the following parts, under the general title *Indoor air*:

— *Part 1: General aspects of sampling strategy*

— *Part 2: Sampling strategy for formaldehyde*

— *Part 3: Determination of formaldehyde and other carbonyl compounds — Active sampling method*

— *Part 4: Determination of formaldehyde — Diffusive sampling method*

— *Part 5: Sampling strategy for volatile organic compounds (VOCs)*

— *Part 6: Determination of volatile organic compounds in indoor and test chamber air by active sampling on Tenax TA® sorbent, thermal desorption and gas chromatography using MS/FID*

— *Part 7: Sampling strategy for determination of airborne asbestos fibre concentrations*

— *Part 8: Determination of local mean ages of air in buildings for characterizing ventilation conditions*

— *Part 9: Determination of the emission of volatile organic compounds from building products and furnishing — Emission test chamber method*

— *Part 10: Determination of the emission of volatile organic compounds from building products and furnishing — Emission test cell method*

— *Part 11: Determination of the emission of volatile organic compounds from building products and furnishing — Sampling, storage of samples and preparation of test specimens*

— *Part 12: Sampling strategy for polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polycyclic aromatic hydrocarbons (PAHs)*

— *Part 13: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs) — Collection on sorbent-backed filters*

— *Part 14: Determination of total (gas and particle-phase) polychlorinated dioxin-like biphenyls (PCBs) and polychlorinated dibenzo-*p*-dioxins/dibenzofurans (PCDDs/PCDFs) — Extraction, clean-up and analysis by high-resolution gas chromatography and mass spectrometry*

— *Part 15: Sampling strategy for nitrogen dioxide (NO_2)*

— *Part 16: Detection and enumeration of moulds — Sampling by filtration*

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

— *Part 23: Performance test for evaluating the reduction of formaldehyde concentrations by sorptive building materials*

— *Part 24: Performance test for evaluating the reduction of volatile organic compounds and carbonyl compounds without formaldehyde concentrations by sorptive building materials*

The following parts are under preparation:

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

— *Part 19: Sampling strategy for moulds*

— *Part 25: Determination of the emission of semi-volatile organic compounds by building products — Micro-chamber method*

— *Part 28: Sensory evaluation of emissions from building materials and products*

The following parts are planned:

— Part 20: Detection and enumeration of moulds — Sampling from house dust

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

— Part 22: Detection and enumeration of moulds — Molecular methods

— Part 27: Standard method for the quantitative analysis of asbestos fibres in settled dust

Furthermore,

— ISO 12219-1 (under preparation), *Indoor air — Road vehicles — Part 1: Whole vehicle test chamber — Specification and method for the determination of volatile organic compounds in car interiors*,

— ISO 16017-1, *Indoor, ambient and workplace air — Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography — Part 1: Pumped sampling*, and

— ISO 16017-2, *Indoor, ambient and workplace air — Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography — Part 2: Diffusive sampling*

focus on volatile organic compound (VOC) measurements.

Introduction

Mould is a common name for filamentous fungi from different taxonomic groups (Zygomycetes, Ascomycetes [Ascomycota], Deuteromycetes). They form a mycelium (hyphae) and spores — namely conidiospores (conidia), sporangiospores or ascospores — by which they become visible macroscopically. Most spores are in the size range 2 µm to10 µm, some up to 30 µm and a very few up to 100 µm. Spores of some mould genera are small and become airborne very easily (e.g. *Aspergillus, Penicillium*) while others are bigger and/or embedded in a slime matrix (*Stachybotrys, Fusarium*) and less mobile.

Mould spores are widely distributed in the outdoor environment and, therefore, also occur in varying concentrations indoors. Growth of moulds in indoor environments, however, should be considered a public health problem because epidemiological studies have revealed that dampness and/or mould growth in homes and health impairment of occupants are closely related.

Standardised methods for sampling, detection and enumeration of moulds including standards for sampling strategies are important for comparative assessment of mould problems indoors. Before taking any measurements, a measurement strategy is required.

The procedure specified in this part of ISO 16000 is based on VDI 4252-2 [7], which is widely used for detection and enumeration of fungi in ambient air and was adapted to be suitable also for indoor environments.

Indoor air —

Part 16: Detection and enumeration of moulds — Sampling by filtration

WARNING — The use of this part of ISO 16000 may involve hazardous materials, operations and equipment. This part of ISO 16000 does not purport to address any safety problems associated with its use. It is the responsibility of the user of this part of ISO 16000 to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

1 Scope

This part of ISO 16000 specifies requirements for long-term (0,5 h to several hours) sampling of moulds in indoor air by filtration. Following the instructions given, a sample is obtained for subsequent detection of moulds by cultivation after suspension according to ISO 16000-17, which is part of the complete measurement procedure.

This part of ISO 16000 is not suitable for personal sampling.

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 16000-17, *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
aerodynamic diameter
diameter of a sphere of relative density 1 with the same terminal velocity due to gravitational force in calm air as the particle, under the prevailing conditions of temperature, pressure and relative humidity

NOTE Adapted from ISO 7708:1995, 2.2.

3.2
biological preservation efficiency
capacity of the sampler to maintain the viability of the airborne microorganisms during collection and also to keep the microbial products intact

[EN 13098:2000 [6]]

3.3
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.

3.4
filtration
collection of particles suspended in gas or liquid by flow through a porous medium

[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.

3.5
colony forming unit
cfu
unit by which the culturable number of microorganisms is expressed

[EN 13098:2000 [6]]

NOTE 1 One colony forming unit can originate from one single microorganism, from aggregates of many microorganisms as well as from one or many microorganisms attached to a particle.

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

3.6
cultivation
⟨air quality⟩ growing of microorganisms on culture media

3.7
field blank
⟨air quality⟩ sample taken in an identical manner as the real sample, but without sucking air through the sampling apparatus

NOTE The resulting blank represents the number of cfu entering the sample simply by handling the filter during sampling. The results of the field blanks are not used for correction of measurement results but to detect sampling errors (see ISO 16000-17).

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

[EN 13098:2000 [6]]

3.9
mould
⟨air quality⟩ filamentous fungi from several taxonomic groups namely Zygomycetes, Ascomycetes (Ascomycota) and Deuteromycetes (fungi imperfecti)

NOTE Moulds form different types of spores depending on the taxonomic group they belong to, namely conidiospores (conidia), sporangiospores or ascospores.

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

[EN 13098:2000 [6]]

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

[EN 13098:2000 [6]]

4 Principle

During filtration, a defined air quantity is sucked through a filter — on or in which separation of the suspended particles occurs.

Airborne moulds are collected on gelatine filters resulting in a high total sampling efficiency (see Annex A). Polycarbonate filters are used below the gelatine filters to enhance stability (see Annex A). Filters other than those of gelatine may be used provided they have been shown to have a relative recovery of at least 90 % of the mass recovered on the gelatine type.

The sampling device is constructed for the detection of particles of the size of mould spores (> 1 µm to ~30 µm). To achieve this, the flow velocity of the filter shall be in the range 100 mm/s to 250 mm/s.

NOTE 1 If a filter with a diameter of 80 mm is used, this flow velocity is achieved by a flow rate of about 1,5 m^3/h to 3,3 m^3/h (25 l/min to 55 l/min).

NOTE 2 This method has been validated for a flow velocity of 217 mm/s. The physical sampling efficiency for other velocities may be lower.

NOTE 3 Particles > 30 µm are also retained by the filters. If the filter holder is operated in a hanging position (e.g. outdoor measurements with strong winds or rain) it is nonetheless possible that bigger particles may not reach the filter holder due to their inertia.

After sampling, the mould spores are cultivated and counted. This procedure is specified in ISO 16000-17.

5 Apparatus and materials

5.1 Sampling device

The following components are needed.

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

5.1.2 Sampling head, to position the filter holder with the inserted filters in a hanging position, if necessary.

A bent pipe or hose connection can be used to connect the sampling head to the sampling apparatus. The inner diameter of the pipe or hose shall be 8 mm to 10 mm.

5.1.3 Filter holder, sterile (disposable or sterilizable), to insert the filters.

5.1.4 Filters, of gelatine [1)], sterile, of pore size 3 µm, and of polycarbonate, sterile, of pore size 0,8 µm (see Annex A).

5.1.5 Vacuum pump, ensuring a constant flow rate during continuous operation.

The flow rate has to be adjusted to produce a flow velocity at the filter in the range 100 mm/s to 250 mm/s (see Clause 4).

1) Sartorius Stedim Biotech, Göttingen, is an example of a suitable commercial supplier. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this supplier.

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

5.1.7 Timer, for presetting time and duration of sampling.

5.1.8 Protective housing, to protect the sampling device from harmful environmental conditions (optional, mainly for outdoor use).

The distance between the upper edge of the protective housing and the lower edge of the sampling head should be at least 400 mm.

5.2 Materials

5.2.1 Sterile container, for filter containment during transport; e.g. Petri dishes.

5.2.2 Insulated container, for sample transport.

5.2.3 Protective gloves, preferably sterile disposable gloves or disinfected.

5.2.4 Disinfectant, e.g. *iso*-propanol or ethanol (70 % volume fraction).

5.2.5 Sterile tweezers, to handle the filters.

6 Measurement procedure

6.1 Preparation for sampling

Assemble the sampling equipment according to Figure 1.

A detailed example of a suitable sampling device is given in Annex B.

Connect the gas volume meter between the pump and the sampling head in order to determine the sampling air volume without any interference caused by the leakage flow rate of the pump. The volume of the sampled air is displayed in operating cubic metres to an accuracy of 0,01 m^3. The temperature and pressure within the gas volume meter and in the air are continuously monitored.

The sampling air volume in operating cubic metres referenced to air conditions, V_B, is calculated electronically using Equation (1):

$$V_B = V_G \frac{T_A \; p_G}{T_G \; p_A} \tag{1}$$

where

p_A is the air pressure;

p_G is the air pressure within the gas volume meter;

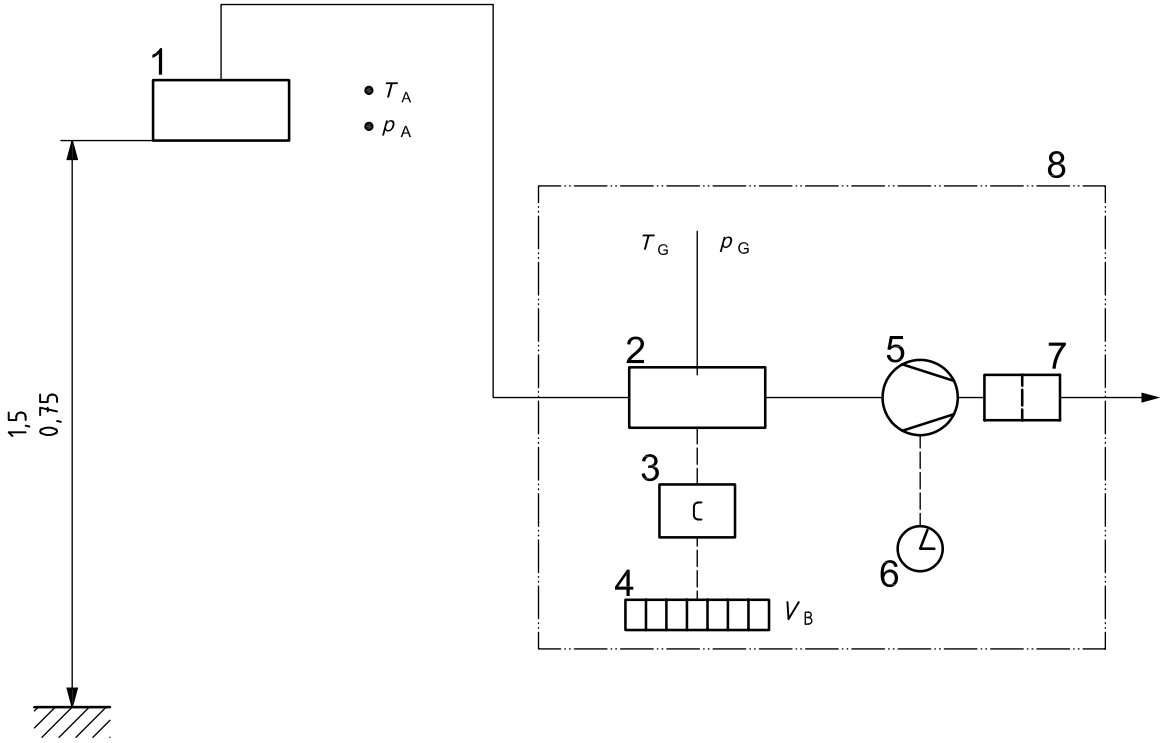
T_A is the air temperature;

T_G is the temperature within the gas volume meter;

V_G is the sampling air volume measured by the gas volume meter.

Prepare the required number of sampling devices in accordance with the measurement task and the measurement strategy. The air flow rate of the sampling apparatus should not exceed 10 % of the air change rate of the room in question. If the air change rate is unknown, the air volume sampled per hour shall not exceed 10 % of the volume of the room.

Dimensions in metres



Key

1	filter holder with optional sampling head for suspension	5	vacuum pump	p_A	ambient air pressure
2	gas volume meter (e.g. orifice plate, thermal mass flow rate meter)	6	timer	p_G	sample gas pressure
3	electronic circuit for conversion into operating cubic metres	7	filter for abraded material [a]	T_A	ambient air temperature
4	display for sampling air volume in operating cubic metres	8	optional protective housing against adverse environmental conditions	T_G	sample gas temperature
				V_B	sample gas volume

[a] If a rotary vane vacuum pump is used; air flow shall be released horizontally.

Figure 1 — Schematic setup of the sampling device

NOTE 1 Low volume sampling can be used for small rooms, for example children's rooms. High volume sampling may be considered for very large indoor areas if the noise produced by the sampler is of no consequence, provided that the constraints on sampling rate or air volume specified above are observed.

The fluctuation of the flow velocity during sampling should be not more than ± 2 %. During sampling, the flow rate of the sampled air shall not be reduced by more than 10 % as a result of the increasing filter loading.

NOTE 2 There are self-regulating sampling systems to overcome these faults.

It is recommended to check the equipment for completeness and functionality with a check list.

Verify the calibration validity of the sampling device. Conduct new calibration, if necessary, prior to the measurements (see Clause 8).

Use sterile filters and sterile filter holders for the measurements. Maintain filter sterility up to the moment of sampling. Protect filters from dust, heat, and strong vibrations during transport.

6.2 Sampling

Sampling is usually conducted at a height of 0,75 m to 1,5 m above ground. For special purposes, other heights might be applicable. Take care that no settled house dust is sucked into the sampling device when sampling at low heights.

NOTE The orientation of the filter holder in indoor environments with only small air currents is of minor importance. A hanging sampling head is only necessary in case of strong air movements (e.g. comparative measurements outdoors).

Mount the sterile filter holders containing the sterile filters on the sampling apparatus without any contamination (preferably by using sterile disposable gloves). Inspect the filters in the filter holders visually for integrity and exact, airtight fitting of the seat.

Start the sampling device in accordance with the manufacturer's operating instructions.

Record the measurement task and measurement location in the sampling protocol (an example is given in Annex C), as well as data concerning the exact positioning of the measurement apparatus, type of sampling equipment, date and time of each individual sampling, sampling period, flow rate and, for ambient air measurements, wind direction and wind velocity. Also record other parameters (e.g. temperature, relative humidity) as well as specific circumstances, anomalies or interferences that may be relevant to the analyses of the mould problem (e.g. perception of odour, type and location of possible additional emission sources).

After sampling, remove the filters and filter holders from the sampling apparatus using sterile or disinfected gloves, and check the filters for integrity. Pack the filters in sealed containers (see 5.2) in order to avoid any secondary contamination.

Multiple measurements using different sampling periods are recommended. This is especially important when the level of the anticipated concentration of moulds is not known.

Take a minimum of one field blank for each measurement object preferably in the middle of the measurement series. For this purpose, place a sterile filter holder with filter in the sampling head with the pump switched off, then remove, pack and analytically process the filter in the same manner as the loaded filters. Avoid a prolonged exposure of the filter to the surrounding air.

6.3 Sampling period

The sampling period is determined by the measurement task and the expected mould concentration. Usual sampling times are 30 min to several hours.

6.4 Transport and storage

Protect loaded filters from disturbing influences (sunshine, humidity or desiccation, heat and dust, etc.), and transport them to the laboratory immediately after sampling with the sampling side facing upwards in sealed containers (see 5.2). The temperature during transport shall not exceed the incubation temperature, (25 $\pm$ 3) °C. If necessary, cool samples during transport, but take care not to freeze them. Record the conditions during transport (temperature, humidity, duration). Process samples, if possible, immediately, and not later than 48 h after the end of the sampling period. Store samples in the laboratory at a temperature not exceeding the incubation temperature.

7 Sampling efficiency and method limitations

The limitations of the method are determined by the physical and biological sampling efficiency.

Physical sampling efficiency of both gelatine and polycarbonate filters is in excess of 95 % for moulds with an aerodynamic diameter range of $>$ 1 µm using a flow velocity at the filter of v = 217 mm/s (Annex A).

The effect of desiccation — influencing the biological preservation efficiency — is not uniform, but depends on temperature, relative humidity, sampling time at the moment of measuring, as well as the type of mould. Fortunately, most mould spores are relatively insensitive towards desiccation, so that the filter method can usually be successfully applied in this field. Validation trials have shown reduced recovery of *Cladosporium* spp. during very hot and sunny weather (see Clause D.2).

8 Calibration of flow rate, function control and maintenance of the sampling system

8.1 Calibration of flow rate

Perform calibration of the sampling apparatus by means of a certified reference volume meter having a measurement accuracy of not more than $\pm$ 2 % expressed in operational cubic metres, referenced to air conditions. Connect the reference volume meter to the air inlet of the sampling apparatus. Make sure that the air inlet orifice of the reference apparatus is unobstructed. After a successful adjustment of the flow rate, check the display accuracy of the sampling apparatus against the reference volume meter. The air volume sucked through the sampling apparatus for a duration of 60 min shall be indicated with an accuracy of $\pm$ 1 % compared to the reference volume meter. The time after which a steady flow velocity is reached should not exceed 5 s.

The usual verification of the flow rate (function control) depends on the stability of the apparatus. Carry out a complete calibration prior to starting a new measurement programme or following significant changes, e.g. when new or repaired equipment is used or after pump servicing. If the flow rate determined using the transfer standard deviates more than 2 % from the value required for correct operation of the inlet, adjust the flow controller according to the manufacturer's instructions.

8.2 Function control and maintenance

Carry out maintenance of mechanical parts of the sampling system (inlet and connecting pipe work) including leak check according to the manufacturer’s instructions.

Check the overall method by determining field blanks (see also 6.2).

9 Quality assurance

It is important to ascertain the exact operating mode of the sampling device (lack of leaks/determination of the sampling volume). Additionally, give special attention to the pump and the sampling head equipped with filters.

The laboratory shall implement quality assurance measures to be documented and made available any time (see ISO/IEC 17025 [5]).

10 Sampling protocol

Label the samples for unique identification.

Fill in a sampling protocol for each sample before (or just after) sampling.

The protocol shall at least indicate:

a) date and time of sampling;

b) name and address of the client;

c) type of sampling device used;

d) sampling volume, location, and duration of sampling;

e) activity in the room during sampling;

f) name of the person taking the sample.

The purpose of the analysis and, if applicable, a list of parameters to analyse are also needed as they may subsequently help the laboratory with the choice of methods. Other details can be necessary (e.g. temperature, humidity, exact sampling point, any observations on phenomena which could affect the concentration of airborne moulds).

An example of a sampling protocol is given in Annex C.

NOTE Additional parameters like air pressure, wind direction, wind velocity, and climatic conditions may be of importance for measurements in ambient air.

11 Performance characteristics

Characteristic filter curves and sampling efficiencies for mould spores were determined for the combination of gelatine and polycarbonate filter (see Figure A.1 and Annex A). Sampling efficiency was in excess of 95 % for moulds with an aerodynamic diameter of > 1 µm.

The suitability of the method was primarily tested by comparative measurements in ambient air (see Clause D.1). Some additional experiments were performed in indoor environments in comparison with impaction (see Clause D.2).

Annex A
(informative)

Recovery of spores on gelatine filters in combination with polycarbonate filters

Airborne bioaerosols are enriched on the filter by filtered separation. The mechanisms of filtration (diffusion, impaction, interception, and sedimentation) determine the attainable physical sampling efficiency (see Reference [8]).

Comparative experiments for cultivation of moulds subsequent to separation of mould spores over different filter materials have been conducted. Spores were resuspended from the filters and cultivated on DG18 medium. Gelatine filters were characterised by a high recovery (see also Table A.1), which resulted in a low standard deviation of the measurement results and a better detection limit when compared to polycarbonate (see Reference [9]).

A disadvantage of the gelatine filters is their reduced stability against humidity due to dissolution: the result is insufficient mechanical stability.

The use of humidity-resistant polycarbonate filters for sampling resulted in lower recovery in comparison with gelatine filters. The advantage of a polycarbonate filter compared to other filters is its universal applicability, as well is in its insensitivity towards humidity and temperature.

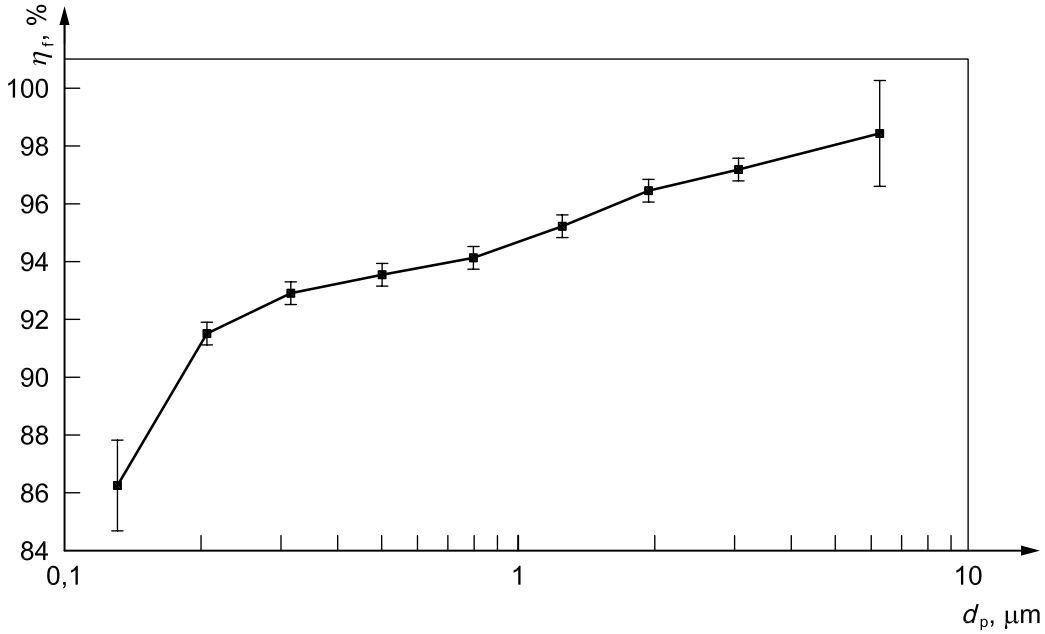
It is, therefore, recommended to use a combination of a gelatine filter with a downstream polycarbonate filter when ambient humidity is high. The polycarbonate filter thus functions as a supporting and protective filter for the gelatine filter, which has a very high sensitivity to humidity.

The use of the filter combination described has proved its reliability and has enabled application for more than 24 h under conditions of high humidity and even rainfall. This filter combination thus represents a separation system suitable for sampling of mould spores even in moist indoor environments or ambient air (see Reference [10]).

Sampling efficiency of both gelatine and polycarbonate filters is in excess of 95 % for moulds with an aerodynamic diameter range of > 1 µm (see Figures A.1 and A.2).

Table A.1 — Recovery of mould spores on different filters (Reference [10]) in an indoor environment

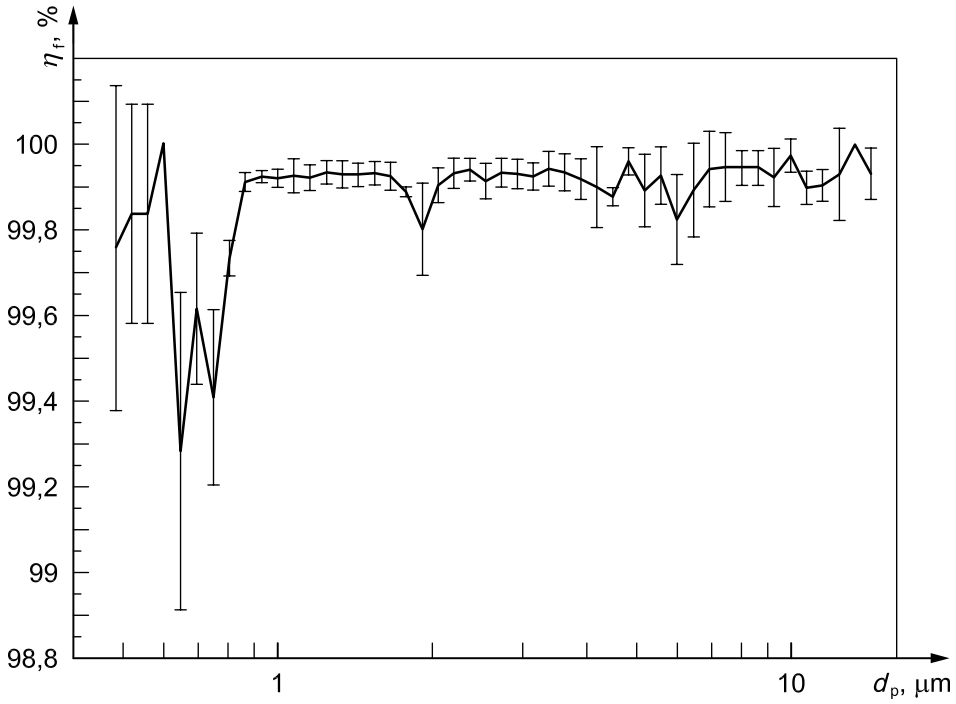
Sample	Measurement number	Sampling on polycarbonate filters Concentration of moulds cfu/m^3	Sampling on gelatine filters Concentration of moulds cfu/m^3
A	1	50	120
	2	30	170
	3	30	120
	4	50	300
	5	—	120
	6	—	240
B	1	50	290
	2	30	260
	3	40	190
	4	40	220



Key

η_f filter efficiency d_p particle diameter

Figure A.1 — Characteristic filter curves of a gelatine filter (pore size: 3 µm; diameter: 80 mm) at a flow rate of 3 m³/h — the values obtained are valid for a flow velocity at the filter v = 217 mm/s



Key

η_f filter efficiency d_p particle diameter

Figure A.2 — Characteristic filter curves of a polycarbonate filter (protective filter, pore size 0,8 µm, diameter 80 mm) at a flow rate of 3 m³/h — the obtained values are valid for flow velocity at the filter v = 217 mm/s

Annex B
(informative)

Technical description of a suitable filtration device

A detailed description of a suitable sampling head and a suitable filter holder which can be used for indoor and outdoor measurements is given in Figures B.1 and B.2. A hanging sampling head is only necessary for outdoor sampling (see Note to 6.2).

The physical parameters of the sampling system are summarised in Table B.1.

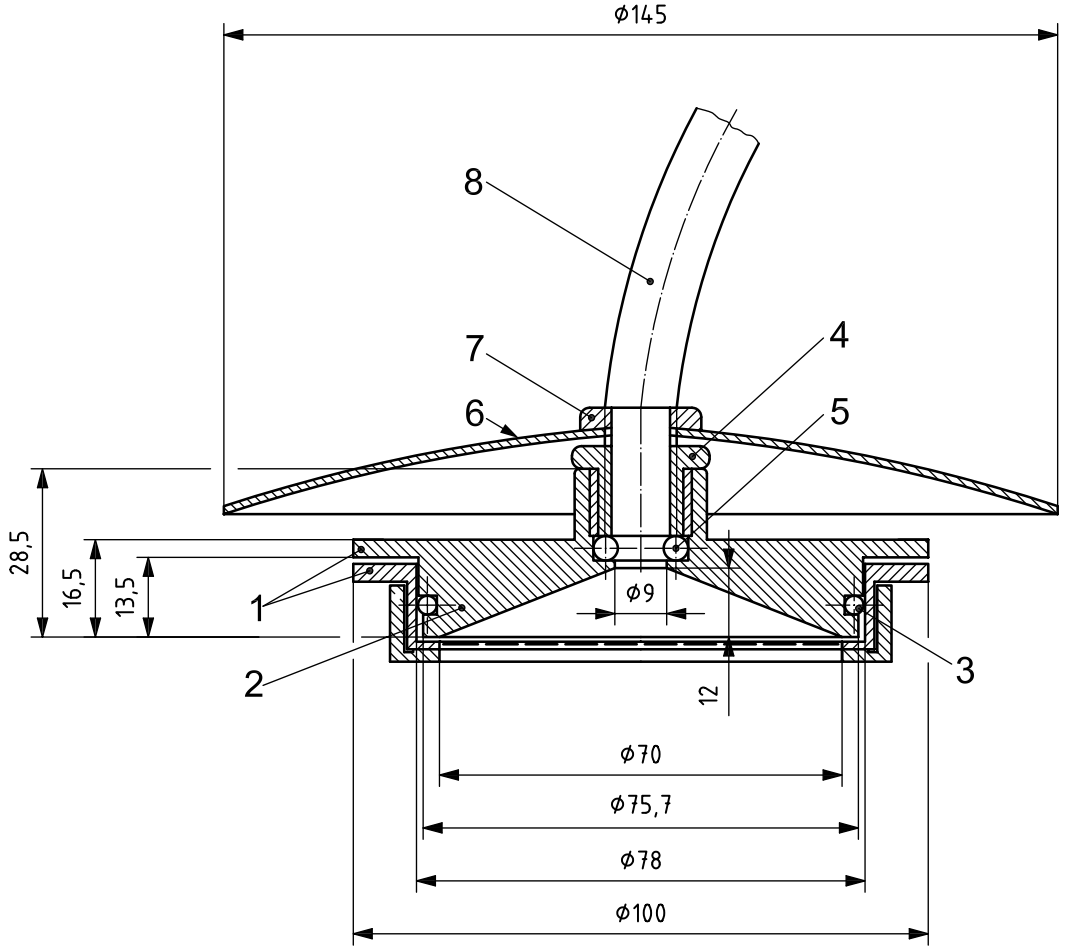
This equipment was used for the sample exchange for method validation with ambient air (Annex D).

Other sampling systems may be used, if they meet the requirements (see 5.1) or lead to comparable results. If other dimensions for the sampler are used, the filter and other equipment have to be adapted to meet especially the requirement for the flow velocity at the filter.

Table B.1 — Physical parameters

Parameter	Value
Flow rate	3 $m^3/h \pm 0,3$ m^3/h
Admissible variation of the flow rate during sampling (see 8.1)	$\leqslant$ 10 %
Display accuracy of the flow rate	0,01 m^3/h
Filter diameter	80 mm
Active filter diameter	70 mm
Flow velocity at filter	217 mm/s $\pm$ 21,7 mm/s
Sampling period (see 6.3)	10 min to several hours

Dimensions in millimetres



Key

1 disposable filter holder
2 adapter
3 seal (silicone)
4 threaded female coupling
5 seal
6 protective cover
7 counter nut
8 exhaust pipe

Figure B.1 — Sampling head with filter holder

The sampling head can accommodate disposable filter holders or sterilisable multiple use filter holders. These are placed on the adapter. The filter holder is thus jammed against the seal. The suction pipe or a suitable hose olive with screw thread is sealed against the sampling head with a sealing ring. The female coupling is sealed against the thread of the pipe adapter with polytetrafluoroethylene tape, for example.

Dimensions in millimetres

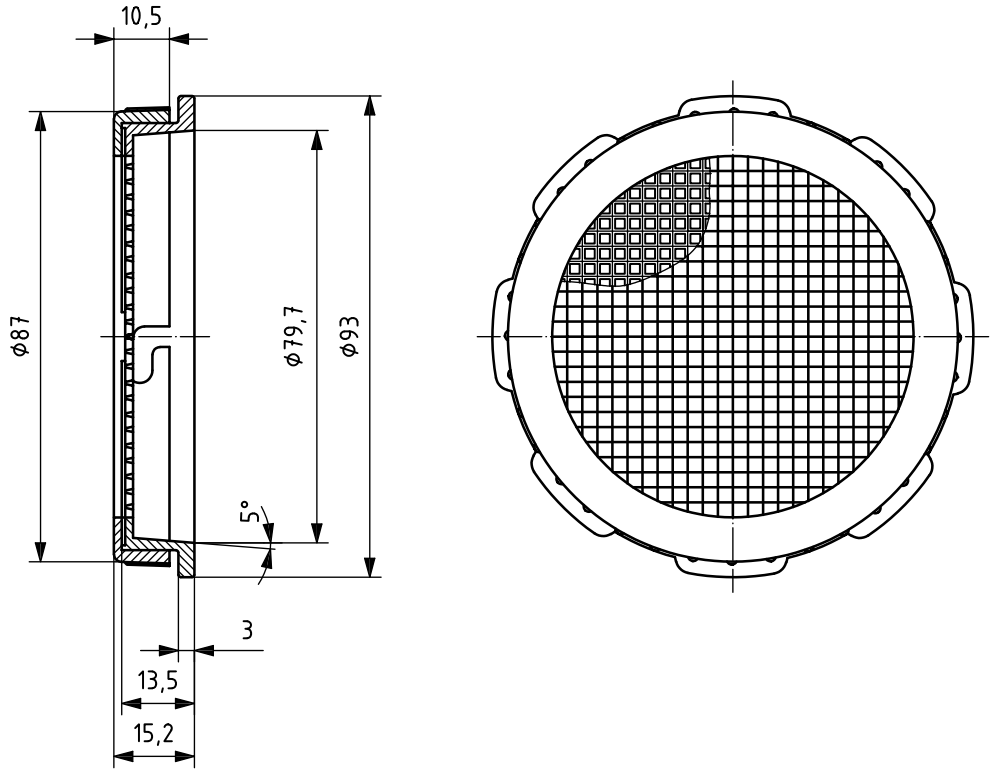


Figure B.2 — Disposable filter holder

Annex C
(informative)

Sampling protocol

Client:	**Assignment No.:**
Object:	**Apparatus type and serial No.:**
Measurement location:	**Date:** **Time:**

Filter No.	Measurement start time	Sampling volume m^3	Measurement period min	Height of measurement m	Temperature °C	Relative humidity %	Remarks

Remarks:

Name of laboratory technician: **Date and signature:**

Description of activities and interference during sampling

Client:	**Assignment No.:**
Object:	**Apparatus type and serial No.:**
Measurement location:	**Date:**

Filter No.	Activities and interference during sampling (potential mould emission sources, odour perceptions, resuspended particles)

Remarks:

Name of laboratory technician: **Date and signature:**

Annex D
(informative)

Trials for method validation

D.1 Ambient air

This method is widely used for ambient air measurements. Therefore, validation data primarily exist for ambient air measurements.

The suitability of the method was tested in the field with the filter combination of gelatine/polycarbonate and the standard deviation of the overall method (sampling, analyses, non-homogeneity of the measurement object) was determined for the purpose of validation (Reference [10]). The studies were conducted under the following four conditions.

— Ambient air measurement on a backyard terrace in an urban environment (residential street, dead end), by one laboratory.

— Ambient air measurement on a backyard terrace in an urban environment (residential street, dead end), by four laboratories.

— Ambient air measurements near a composting plant, by six laboratories.

— Ambient air measurements near a recycling plant, by five laboratories.

The 7 to 12 samples simultaneously obtained were distributed randomly among the six participating laboratories in order to avoid the influence of systematic error.

Measurements were performed during the colder transitional period from autumn to winter at an average temperature of approximately 10 °C. Air humidity was predominantly very high. Measurements were partially performed during intense precipitation. Sampling period was 24 h to 26 h. The filter combination proved successful under these ambient air conditions (in particular, the humidity-sensitive gelatine filter retained its utility due to the use of the protective polycarbonate filter).

A blank was always taken prior to sampling. These blank filters were always unloaded. All filters were transported by postal mail. Sample processing and subsequent quantitative detection of moulds was performed on DG18 agar according to ISO 16000-17.

The sample exchange results are listed in Table D.1.

Table D.1 — Results [a] of the sample exchange of gelatine/polycarbonate-filters subsequent to ambient air measurements at different locations

Measurement period November 2002	Sampling in a city/analysed by one laboratory			Sampling in a city/analysed by several laboratories			Sampling near a composting plant/ analysed by several laboratories			Sampling near a recycling plant/ analysed by several laboratories		
	cfu/m^3 [a]											
Filter number	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
1	100	100	20	200	200	50	700	2000	1 000	10 000	400	800
2	90	80	10	200	200	200	600	3000	500	8 000	600	800
3	80	60	20	500	70	300	2 000	800	600	7 000	400	800
4	60	80	20	500	90	200	1 000	1 000	800	7 000	500	900
5	70	30	20	200	400	300	900	3 000	600	10 000	300	1 000
6	40	50	20	500	300	400	500	3 000	400	10 000	900	600
7	100	20	20	500	1 000	400	3 000	1 000	900	8 000	700	700
8	100	30	10	2 000	100	60	2 000	3 000	600	6 000	300	900
9	80	60	20	2 000		200	700	2 000	800	9 000	300	700
10							600	2 000	1 000	8 000	200	
11							3 000					
12							3 000					
Mean	80	60	20	700	300	200	1 000	2 000	700	8 000	400	800
Median	80	60	20	500	200	200	1 000	2 000	700	8 000	400	800
Minimum	40	20	10	200	70	50	500	800	400	6 000	200	600
Maximum	100	100	20	2 000	1 000	400	3 000	3 000	1 000	10 000	900	1 000
Coefficient of variation %	27	49	22	101	97	55	63	37	33	18	49	15

[a] Each value corresponds to the result of an ambient air sample per day of the respective laboratory.

D.2 Indoor air

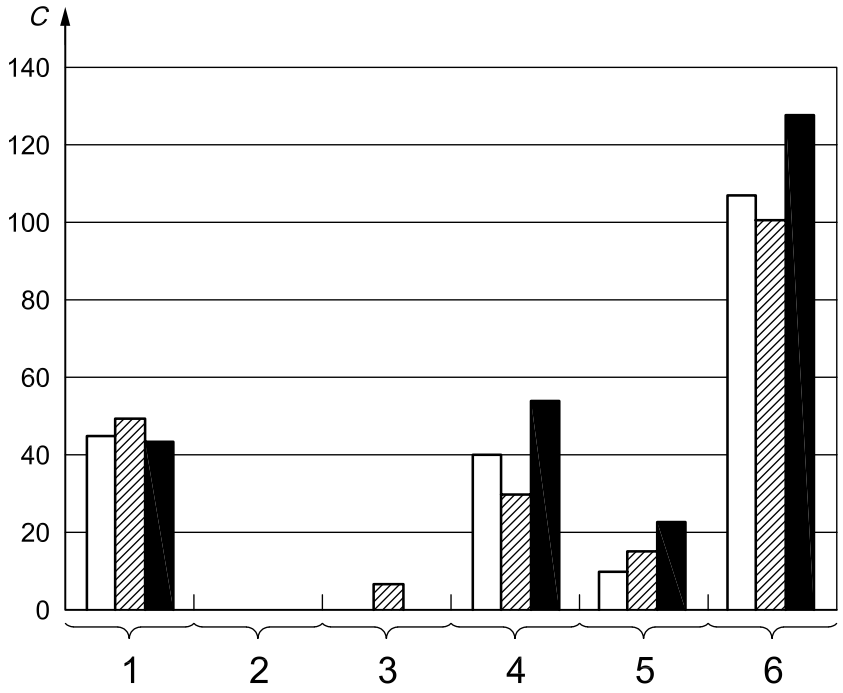
Conditions in indoor air do not differ from ambient air conditions in a manner that would restrict the use of validation data obtained from ambient air measurements (see Clause D.1) (see References [11], [12], and [13]).

Two additional validation trials were conducted indoors. Most participating laboratories used impaction for sampling but some laboratories sampled by filtration.

The trials were conducted in two different meeting rooms (one each for trials 1 and 2) which were naturally ventilated. Windows were closed 1 h prior to sampling. Parallel samples of 50 l, 100 l, and 200 l were analysed for impaction. Higher volumes were sampled by filtration. Sampling time for filtration was 1 h to 3 h.

DG18 agar plates were provided by one reference laboratory for all participants to avoid media effects. One set of plates was transported to the reference laboratory for further analysis. A second set of plates was analysed by the respective laboratories taking part in trial 1. All plates were analysed by the reference laboratory in the second trial.

In trial 1 (June 2005), 36 laboratories took samples during the trial; 34 laboratories sampled by impaction. Filtration was performed with 12 filtration devices from three different manufacturers. Filters were analysed in two different reference laboratories. Results are summarised in Figure D.1.



Key

- ☐ impaction A (n[a] = 34): analysis of the sampling plates in reference laboratory
- ▨ impaction B (n = 26): analysis of the sampling plates in the participating laboratories
- ■ filtration C (n = 12): all results compiled
- C colony forming unit density, cfu/m^3

1 *Cladosporium* spp.
2 *Alternaria* spp.
3 *Aspergillus* spp.
4 *Penicillium* spp.
5 other species
6 total colony count

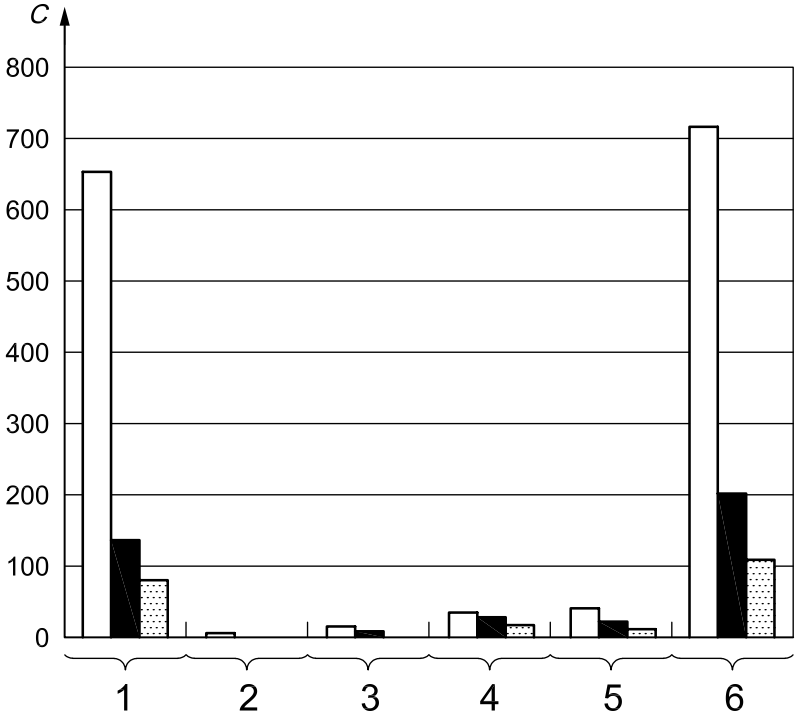
NOTE All filtration results were compiled.

[a] No. sampling devices.

Figure D.1 — Results of mean colony counts after sampling in a meeting room with different sampling devices during trial 1

Results obtained by impaction and filtration were comparable in trial 1.

In trial 2 (June 2006), 37 laboratories took samples during the trial; 33 laboratories sampled by impaction; three laboratories sampled by filtration using six different filtration devices. Filters were analysed in three different reference laboratories. Results are summarised in Figure D.2.



Key

- □ impaction A (n^a = 34): analysis of the plates in reference laboratory
- ■ filtration 1 h to 2 h (n = 4): analysis of the plates in the participating laboratories
- ⊡ filtration 3 h (n = 12)
- C colony forming unit density, cfu/m^3

1 *Cladosporium* spp.
2 *Alternaria* spp.
3 *Aspergillus* spp.
4 *Penicillium* spp.
5 other species
6 total colony count

NOTE All filtration results were compiled.

[a] No. sampling devices.

Figure D.2 — Results of mean colony counts after sampling in a meeting room with different sampling devices during trial 2

Sampling by filtration resulted in lower colony counts compared to sampling by impaction in trial 2. This was especially obvious for *Cladosporium* spp. where only 21 % (filtration 1 h to 2 h) or 12 % (filtration for 3 h) of the colony count obtained by impaction were detected by filtration.

Trial 1 and trial 2 were conducted during the same month in 2005 and 2006. Nevertheless, the meteorological conditions were very different. In 2005, the weather was relatively cold and rainy. In 2006, the week before the trial and the day of the trial were hot and sunny. The different weather conditions may explain the differences in recovery by filtration. Desiccation stress which was much higher in trial 2 strongly affected the recovery of *Cladosporium* spp.

Bibliography

[1] ISO 7708, *Air quality — Particle size fraction definitions for health-related sampling*

[2] ISO 12219-1, *Indoor air — Road vehicles — Part 1: Whole vehicle test chamber — Specification and method for the determination of volatile organic compounds in car interiors*

[3] ISO 16017-1, *Indoor, ambient and workplace air — Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography — Part 1: Pumped sampling*

[4] ISO 16017-2, *Indoor, ambient and workplace air — Sampling and analysis of volatile organic compounds by sorbent tube/thermal desorption/capillary gas chromatography — Part 2: Diffusive sampling*

[5] ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

[6] EN 13098, *Workplace atmospheres — Guidelines for measurement of airborne micro-organisms and endotoxin*

[7] VDI 4252-2, *Measurement of airborne microorganisms and viruses in ambient air — Active sampling of bioaerosols — Separation of airborne mould on gelatine/polycarbonate filters*

[8] LIU, B.Y.H., PUI, D.Y.H., RUBOW, K.L. Characteristics of air sampling filter media. In: Marple, V.A., Liu B.Y.H., editors. *Aerosols in the mining and industrial work environments*, Vol. **3**, *Instrumentation*, pp. 989-1037. Ann Arbor Science, Ann Arbor, MI, 1983

[9] RABE, R., KAULFÜRST-SOBOLL, H. Methodische Verbesserungen bei der Messung luftgetragener Mikroorganismen [Methodological improvements in measuring airborne microorganisms]. *Gefahrst. Reinhalt. Luft* 2002, **62**, pp. 461-466

[10] GABRIO, T., CREUTZNACHER, H., FISCHER, G., HEROLD, T., KÄMPFER, P., PHILIPP, W., RABE, R., TESSERAUX, J., WEIDNER, U., WOPPOWA, L. Ergebnis eines Probenaustausches gemäß Richtlinien VDI 4252 Blatt 2 und VDI 4253 Blatt 2 [Results of a sample exchange in accordance with guidelines VDI 4252-2 and VDI 4253-2]. *Gefahrst. Reinhalt. Luft* 2004, **64**, pp. 295-299

[11] GABRIO, T., DILL, I., TRAUTMANN, C., WEIDNER, U. Schimmelpilze in Luft — Probenahme und Bestimmung, Validierung von Probenahmeverfahren zur Bestimmung von Schimmelpilzen in Luft [Mould fungi in air — Sampling and regulation, validation of sampling procedures for the detection of mould fungi in air]. *Bundesgesundheitsbl. Gesundheitsforsch. Gesundheitsschutz* 2005, **48**, pp. 3-11

[12] GABRIO, T., SEIDL, H.P., SZEWZYK, R., TRAUTMANN, C., WEIDNER, U. Aussagekraft von Luft- und Hausstaubuntersuchungen im Zusammenhang mit Schimmelpilzproblemen im Innenraum [Validity of air and house dust investigations in connection with interior mould problems]. *Gefahrst. Reinhalt. Luft* 2005, **65**, pp. 106-113

[13] SEIDL, H.P., GABRIO, T. Qualitätssicherung bei der Messung von Schimmelpilzen [Quality assurance with the measurement of mold fungi]. In: *Mikrobielle Luftverunreinigungen*: *KRdL Experten Forum*, 2005-10-13 to 14, Freising-Weihenstephan, pp. 157-176. Normenausschuss KRdL, Düsseldorf, 2005. (*Kommission Reinhaltung der Luft-Schriftenreihe*, Vol. 35)

ICS 13.040.20

Price based on 20 pages