INTERNATIONAL STANDARD

ISO 16000-18

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Indoor air —

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

Air intérieur —

Partie 18: Détection et dénombrement des moisissures — Échantillonnage par impaction

Reference number
ISO 16000-18:2011(E)

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Foreword

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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 16000-18 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 is a common name for filamentous fungi from different taxonomic groups (Ascomycetes, Zygomycetes, and their anamorphic states formerly known as deuteromycetes or fungi imperfecti). They form a mycelium and spores by which they become visible macroscopically. Most spores are in the size range 2 µm to 10 µm, while some go 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 or embedded in a slime matrix (e.g. *Stachybotrys*, *Fusarium*) and are less mobile.

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

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

This part of ISO 16000 specifies a method for active short-term sampling (1 min to 10 min) whereas an active long-term sampling procedure (0,5 h to several hours) is specified in ISO 16000-16.

This part of ISO 16000 is based on parts of VDI 4300 Part 10:2008[11].

ISO 16017[8][9] and ISO 12219[3]–[7] also focus on volatile organic compound (VOC) measurements.

Indoor air —

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

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 short-term (1 min to 10 min) sampling of moulds in indoor air by impaction on solid agar media. Following the instructions given, a sample is obtained for subsequent detection of moulds by cultivation in accordance with ISO 16000-17.

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 16000-17, *Indoor air — Part 17: Detection and enumeration of moulds — Culture-based method*

3 Terms and definitions

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

3.1
aerodynamic diameter
diameter of a sphere of density 1 g/cm^3 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], 2.2.

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[10]]

3.3
colony forming unit
cfu
unit by which the culturable number of microorganisms is expressed

[EN 13098:2000[10]]

NOTE 1 One colony can originate from one 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.

3.4
cut-off value
particle size (aerodynamic diameter) for which the sampling efficiency is 50 %

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

[ISO 16000-16:2008, 3.6]

3.6
filamentous fungus
fungus growing in the form of filaments of cells known as hyphae

NOTE 1 Hyphae aggregated in bundles are called mycelia.

NOTE 2 The term “filamentous fungi” differentiates fungi with hyphal growth from yeasts.

[ISO 16000-16:2008, 3.3]

3.7
impaction
sampling of particles suspended in air by inertial separation on a solid surface

NOTE For the purposes of this part of ISO 16000, the solid surface consists of agar (see also ISO 4225:1994[1], 3.18, 3.49 which define devices using impaction).

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

[EN 13098:2000[10]]

3.9
mould
⟨air quality⟩ filamentous fungi from several taxonomic groups, namely Ascomycetes, Zygomycetes, and their anamorphic states formerly known as deuteromycetes or fungi imperfecti

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

[ISO 16000-16:2008, 3.9]

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

[EN 13098:2000[10]]

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

[EN 13098:2000[10]]

4 Principle

A defined quantity of air is drawn through an impactor containing one or several plates with agar medium (DG18 and malt-extract or potato dextrose agar). The particles in the air stream impact on the agar surface due to their inertia when the direction of the air flow is diverted to bypass the solid surface.

Airborne moulds are thereby collected directly on the agar plates.

The sampling device is constructed for the detection of particles in the size of mould spores (>1 µm to ~30 µm). To achieve this, the cut-off value of the sampling device should preferably be 1 µm or less and shall not be more than 2 µm.

NOTE Two main types of impactors are widely used and available commercially: a) slit samplers; b) sieve samplers. In slit samplers, air is drawn through a narrow slit and particles are impacted on to a rotating agar plate. In sieve samplers, air is drawn through a perforated plate (sieve) with holes of a defined diameter and particles are impacted on to an agar plate fixed below. Sieve samplers can be operated as stacks with different sieves leading to different flow velocities to collect different particle size fractions (i.e. the six-stage Andersen impactor). Validation data are only provided for single stage sieve impactors (mainly with ≥300 holes) using agar plates with a diameter of 9 cm.

After sampling, the mould spores are cultivated and resulting colonies counted in accordance with the procedure specified in ISO 16000-17.

5 Apparatus and materials

5.1 Sampling device

A detailed example of a single stage sieve impactor is given in Annex A. Impactors with several stages are only used for special purposes when a size fractioning of the particles is required.

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

5.1.2 Impactor, slit or sieve type.

5.1.3 Agar plates, diameter 9 cm, containing DG18 agar and malt-extract or potato dextrose agar (see Clause 6).

5.1.4 Vacuum pump ensuring a constant flow rate during continuous operation.

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

5.1.6 Timer for presetting the time and duration of sampling.

5.1.7 Protective housing (optional, mainly for outdoor use) to protect the impactor from harmful environmental conditions.

5.2 Equipment for preparing the agar plates

Usual microbiological laboratory equipment, and in particular the following.

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

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

5.2.3 Autoclave capable of being operated at (121 ± 3) °C and (115 ± 3) °C.

5.3 Equipment for sampling

5.3.1 Plastic bags to protect the agar plates during transport.

5.3.2 Insulated and refrigerated container for transport of agar plates below 25 °C.

5.3.3 Disinfectant, e.g. isopropanol or ethanol (70 % volume fraction).

5.3.4 Compressed air (oil-free, optional) for drying the equipment after disinfection.

6 Culture media and reagents

6.1 General

All reagents and chemicals shall be of recognized quality “for microbiology” or better. Water used shall be distilled or of equivalent quality.

Use of commercially available, dehydrated substrates is encouraged, provided they comply with the descriptions given. They shall be prepared according to the instructions from the manufacturer.

6.2 Dichloran 18 % glycerol agar (DG18)

The components are listed in Table 1.

Table 1 — Composition of dichloran 18 % mass fraction glycerol agar (DG18 agar)

Component	Quantity
Peptone[a]	5,0 g
Glucose	10,0 g
Potassium dihydrogenphosphate (KH_2PO_4)	1,0 g
Magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$)	0,5 g
Dichloran (2,6-dichloro-4-nitroaniline) 0,2 % mass fraction in ethanol (100 %)	1,0 ml
Chloramphenicol	0,1 g
Glycerol	220 g[b]
Agar	15,0 g
Water	1 000 ml

[a] Different peptones are used by different manufacturers (e.g. casein peptone, mycological peptone). This does not usually influence the quantitative results of the measurements, but can have an influence on the appearance of the colonies. Positive controls for comparison of recovery and of morphological appearance of the colonies are, therefore, important.

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

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

Plates of DG18 agar in bags may be kept for up to 1 week at (15 ± 3) °C in the dark.

NOTE 1 Dependent on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful, providing they have been shown not to influence the test results.

NOTE 2 DG18 agar is suitable for the detection of a wide spectrum of xerophilic fungi (i.e. preferring dryness). Glycerol reduces the water activity, a_a to 0,95. Chloramphenicol inhibits bacteria, especially gram-negative bacteria. Dichloran inhibits the spreading of fast-growing mould colonies and thus prevents overgrowing of slow-growing colonies.

6.3 Malt-extract agar

The components are listed in Table 2.

Table 2 — Composition of malt-extract agar

Component	Quantity
Malt-extract	30,0 g
Peptone from soy	3,0 g
Agar	15,0 g
Water	1 000 ml

NOTE 1 The addition of chloramphenicol (0,05 g/l) can be necessary if samples contain high concentrations of bacteria.

NOTE 2 Dependent on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful providing they have been shown not to influence the test results.

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

Plates of malt-extract agar in bags will keep for up to 1 month at (5 ± 3) °C in the dark.

IMPORTANT — Many commercial malt-extract agars with different compositions are available. Check that the ingredients correspond to the composition given in Table 2.

6.4 Potato dextrose agar

The components are listed in Table 3.

Table 3 — Composition of potato dextrose agar

Component	Quantity
Potato extract	4,0 g
Glucose	20,0 g
Agar	15,0 g
Water	1 000 ml

NOTE 1 The addition of chloramphenicol (0,05 g/l) can be necessary if samples contain high concentrations of bacteria.

NOTE 2 Dependent on the concomitant flora, other antibiotics, e.g. streptomycin or ampicillin, can be useful, providing they have been shown not to influence the test results.

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

Plates of potato dextrose agar in bags will keep for up to 1 month at (5 ± 3) °C in the dark.

7 Measurement procedure

7.1 Preparation for sampling

Prepare the required number of impactors and agar plates in accordance with the measurement task and the measurement strategy. For routine measurements with a single stage impactor, different air volumes in parallel (e.g. 2 × 50 l and 2 × 100 l) are recommended at each sampling point as well as one field blank for each monitoring exercise. A minimum of four to five DG18 agar plates and four to five malt-extract or potato dextrose agar plates are, therefore, needed for each sampling point.

Check the equipment for completeness and functionality with a check list.

Verify the calibration validity of the sampling device; otherwise conduct new calibration prior to the beginning of the measurements (see Clause 9).

Use a sterile device containing the slit or sieve for each measurement point. Alternatively, disinfect the slit or sieve with ethanol or isopropanol (70 % volume fraction) and air dry it afterwards (e.g. with compressed air). Subsequently, draw air through the impactor without agar plates for several minutes at the new sampling point prior to sampling with the agar plate in place.

Assemble the sampling train according to Figure 1.

7.2 Sampling

Perform function control at regular intervals. Function control implies primarily the volumetric flow control (see Clause 9).

Sampling is usually conducted 0,75 m to 1,5 m above ground. For special investigations, other heights can be applicable. Take care when sampling at low heights that no settled house dust is sucked into the sampling device. The orientation of the inlet of the impactor in indoor environments without air currents is of minor importance. A hanging sampling head is only necessary in case of strong air movements (e.g. comparative measurements outdoors).

Place the agar plates in the impactors with the lid removed. Take care to avoid contamination of the agar plates or the sampling device.

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

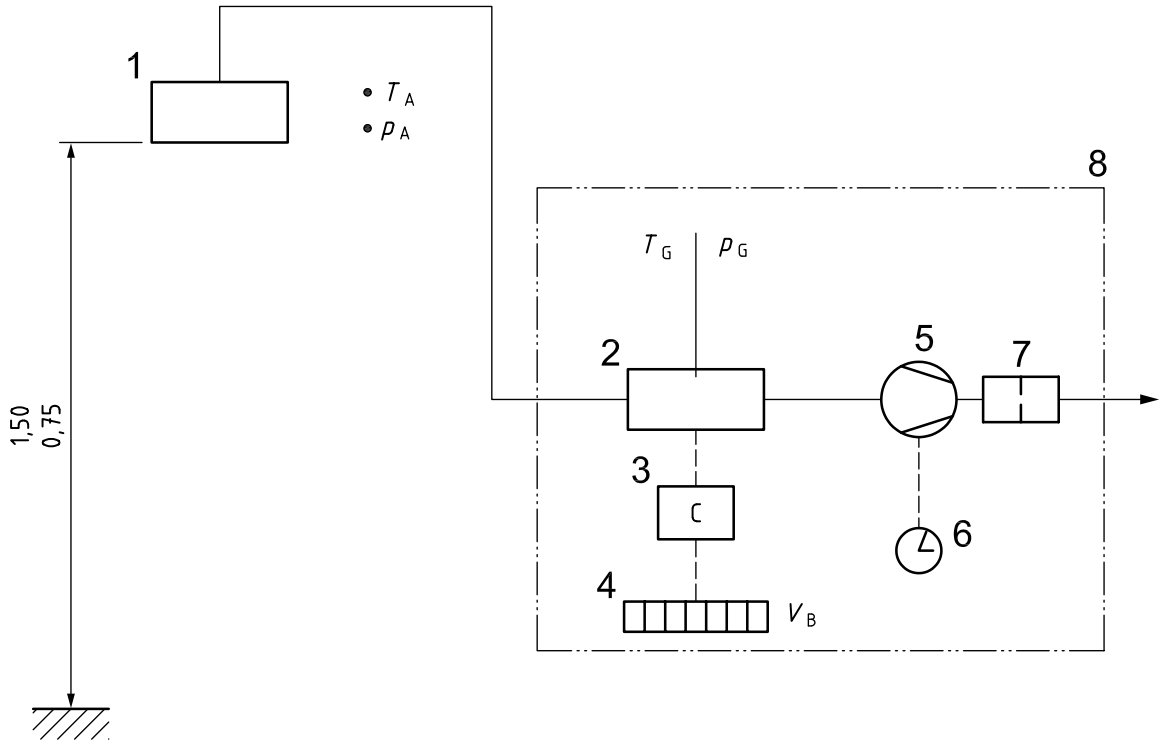
After sampling, remove the agar plates from the sampling apparatus, close the lid and pack them in plastic bags in order to avoid any secondary contamination.

Complete a sampling protocol (see Clause 11 and Annex B).

Multiple measurements using different sampling volumes are recommended. This is especially important when the level of the anticipated concentration of moulds is not known. Use a new agar plate for each sampling.

Take a minimum of one field blank for each agar medium at each monitoring exercise. Take the blank preferably in the middle of the measurement series in an identical manner as the real sample, but without drawing air through the sampling apparatus. For this purpose place an agar plate with the lid open in the impactor with the pump switched-off, then remove, close, pack, and analytically process the plate. Avoid a prolonged exposure of the opened agar plate to ambient air. The resulting blank represents the number of cfu entering the sample simply by handling the agar plates during sampling.

Dimensions in metres



Key

1 impactor and stand
2 gas volume meter (e.g. orifice plate, thermal mass flow rate meter)
3 electronic circuit for conversion into operating cubic metres
4 display for sampling air volume in operating cubic metres
5 vacuum pump
6 timer
7 filter for abraded material (if a rotary vane vacuum pump is used)
8 protective housing (if necessary under adverse environmental conditions)
T_A air temperature
p_A atmospheric pressure
T_G temperature of the sample gas
p_G pressure of the sample gas
V_B sample gas volume

Figure 1 — Schematic diagram of the sampling train

7.3 Sampling period and sampling volume

The sampling period and sampling volume are determined by the measurement task and the expected mould concentration. Usual sampling times are 1 min to 10 min. Sampling volumes below 50 l are not recommended because of the error in determining the sampled air volume due to the dead volume of the sampling device (see Annex C).

7.4 Transport and storage

Protect agar plates from disturbing impacts (sunshine, humidity or desiccation, heat and dust, etc.) and transport them to the laboratory immediately after sampling with the sampling surface up in closed containers (see 5.3.2). Transport temperature shall not exceed the incubation temperature of (25 ± 3) °C. Cool samples during transport, if necessary. Take care not to freeze them and avoid very low temperatures because of condensation problems. Document conditions during transport (temperature, humidity, duration). Process samples preferably within 24 h, but not later than 48 h after the end of the sampling period.

8 Sampling efficiency and method limitations

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

The physical sampling efficiency is given by the cut-off value of the sampling device. The cut-off value should preferably be 1 µm or less and shall not be more than 2 µm to allow for efficient sampling of small spores.

The effect of desiccation, influencing the biological sampling efficiency, is not constant, but depends on temperature, relative humidity, sampling time, and the type of mould at the moment of measurement. Risk of desiccation during impaction is low due to short sampling times and impaction directly on agar plates.

The optimal range of colony numbers for counting is 20 to 40 per agar plate (for the range of 10 to 100 colonies per plate, see ISO 16000-17). It is, therefore, essential that the sampling volume be adjusted to the expected concentration of fungi in the air to avoid:

— overloading of the plates leading to an underestimation of the concentration (see Annex C);

— numbers of colonies per plate which are too low to give statistically valid results.

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

9.1 Calibration of flow rate

Calibration of the sampling device shall be performed by means of a certified reference volume meter having a measurement accuracy less than or equal to ±2 % expressed in operational cubic metres, referenced to ambient air conditions. The reference volume meter shall be connected to the air inlet of the sampling apparatus. The air inlet orifice of the reference apparatus shall be unobstructed. After a successful adjustment of the flow rate, the display accuracy of the sampling apparatus shall be checked against the reference volume meter. The air volume sucked through the sampling apparatus for a duration of 30 min shall be indicated with an accuracy of ±1 % compared with the reference volume meter.

The usual verification of the flow rate (function control) depends on the stability of the apparatus. A complete calibration shall be carried out prior to starting a new measurement campaign 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 by more than 2 % from the value required for correct operation of the inlet, the flow controller shall be adjusted according to the manufacturer's instructions. Make sure that the air flow does not fluctuate by more than ±2 % during sampling and that the time to reach the desired sampling velocity in the beginning of the sampling process is kept as short as possible to minimize the influence on the sample volume.

For some sampling devices, verification and adjustment of the nominal flow is not possible by the user, but is conducted by the manufacturer at regular intervals. In this case, a constant flow between calibration intervals shall be guaranteed by the manufacturer and the apparatus shall have an internal control system preventing deviations from the nominal flow.

9.2 Function control and maintenance of the sampling system

Maintenance of mechanical parts of the sampling system (inlet and connecting pipework), including a leak check, shall be carried out in accordance with the manufacturer's instructions.

10 Quality assurance

Ensure that the sampling device operates correctly, e.g. by verifying that there are no leaks (see 9.2) and by regular calibration of flow rate to ensure consistent determination of the sample volume (see 9.1). In addition, special attention shall be given to the pump and the handling of the agar plates.

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

11 Sampling protocol

The samples shall be uniquely identified and labelled accordingly.

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

The protocol shall include at least the following information:

a) a reference to this part of ISO 16000 (ISO 16000-18:2011);

b) the name and address of the client;

c) the measurement task;

d) the type of sampling device used;

e) the sampling volume, date, hour, location, and duration of sampling;

f) the result of the field blank;

g) the name of the person taking the sample.

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

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

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

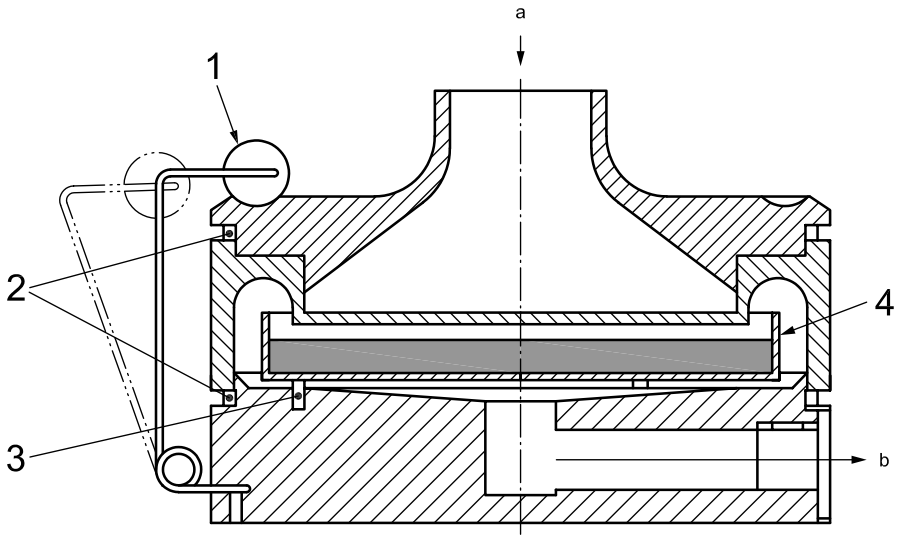
12 Performance characteristics

The suitability of the method was tested by comparative measurements with DG18 agar (see Annex C).

Annex A
(informative)

Technical description of a suitable one stage sieve impactor

A schematic set-up of a suitable sampling device is given in Figure A.1.



Key

1 clamp
2 sealing rings
3 centring device for Petri dish
4 Petri dish with agar medium

a Air inlet.
b Air outlet.

Figure A.1 — Scheme of a one stage impactor

Annex B
(informative)

Sampling protocol

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.

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

Agar type/ Plate No.	Measurement start time	Sampling volume m^3	Measurement period min	Height of measurement m	Temperature °C	Relative humidity %	Remarks, e.g. odour perceptions	Interference during sampling, e.g. activity of persons in the room, HVAC[a]

[a] Heating, ventilation and air conditioning.

Remarks:

Name of laboratory technician: Date and signature:

Annex C
(informative)

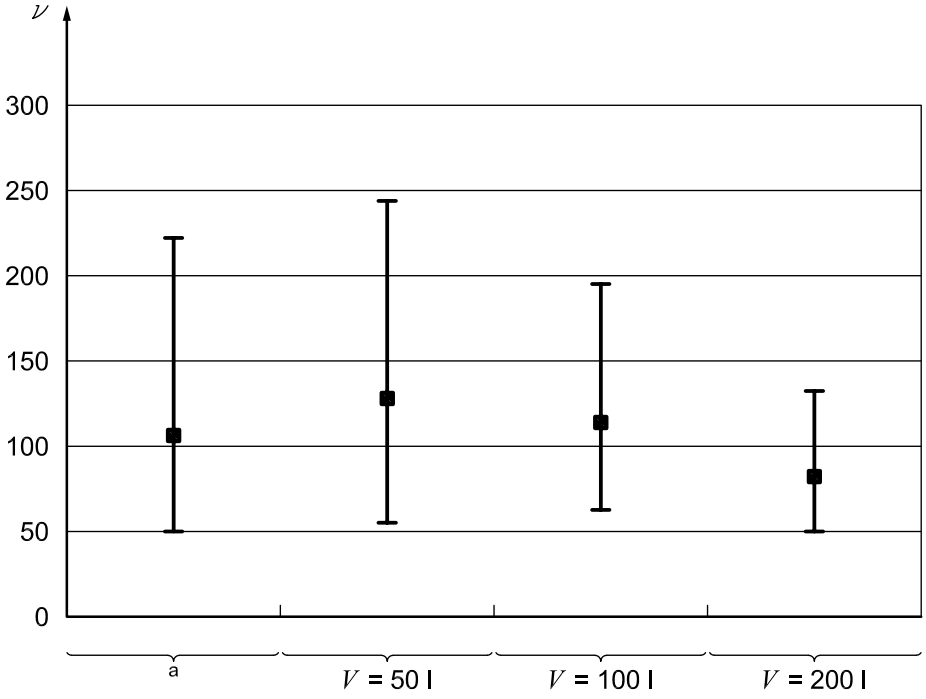
Sample exchange for method validation

The suitability of the sampling method described in this part of ISO 16000 was tested under real conditions in three trials co-ordinated by the Berufsverband Deutscher Baubiologen (Association of German Green Builders) (see Reference [12]).

The trials were conducted in two different meeting rooms (one each for trial 1 and 2, respectively) and a church (in trial 3) which were naturally ventilated. Windows were closed 1 h prior to sampling. Parallel samples of 50 l, 100 l, and 200 l were sampled by impaction in all trials. In trial 3, 50 l samples were taken at the beginning and at the end of the sampling programme with comparable results showing that the concentration of fungi was constant over the sampling time.

DG18 agar plates were provided by one central laboratory (Health Agency Baden-Württemberg, Stuttgart) for all participants to avoid media effects. All plates were incubated and analysed by the central laboratory. In trial 1, additional sampling was performed on plates which were incubated and analysed either by the respective participants or sent by the participants to analysis laboratories.

During trial 1 (June 2005), 34 laboratories sampled by impaction with different devices. The concentration of fungi in the room was relatively low. The mean total count using the results from all sampling volumes was about 100 cfu/m^3 (see Figure C.1). Results differed, however, when calculated separately for the different sampling volumes. Significantly higher counts where found when sampling 50 l and 100 l (131 cfu/m^3 and 120 cfu/m^3, respectively) compared to 200 l (85 cfu/m^3). This result indicates that growth inhibition can already occur at relatively low numbers of colonies on the plate (about 20 colonies with a 200 l sampling volume). Desiccation is not a likely explanation for the lower counts when sampling 200 l because of the overall short sampling times and impaction on an agar surface.



Key

95th percentile

median

5th percentile

ν concentration/(cfu/m^3)

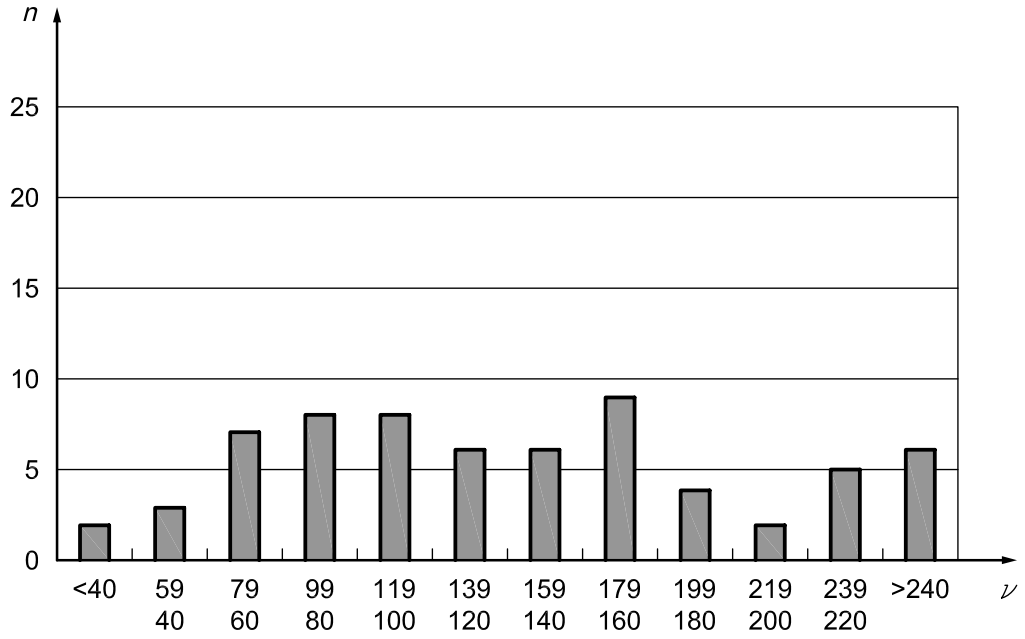
V sampling volume

a Overall.

Figure C.1 — Concentration of total colony counts on DG18 agar after sampling by impaction with different volumes in trial 1

The standard deviation was highest for 50 l sampling and lowest for 200 l sampling. These results indicate that low colony numbers on the plates result in high standard deviation. The same was true when colony counts for different genera were compared. The concentrations of *Cladosporium* spp. and *Penicillium* spp. were of the order of 50 cfu/m^3 with a standard deviation of about 30 %. Other species were present in lower concentrations and the standard deviation of the results increased by 50 % to 200 %. Standard deviation of the total colony count of about 100 cfu/m^3 was 23 %. Colony counts were normally distributed for the 100 l samples and the 200 l samples, but not for the 50 l samples (see Figures C.2 to C.4). These results support the view that colony numbers below 10 should not be used for quantitative results.

The standard deviations of the results obtained from the plates which were analysed by the participating laboratories, rather than the reference laboratory, were much higher. For concentrations of 40 cfu/m^3 to 100 cfu/m^3 standard deviations of 60 % to 80 % were calculated.

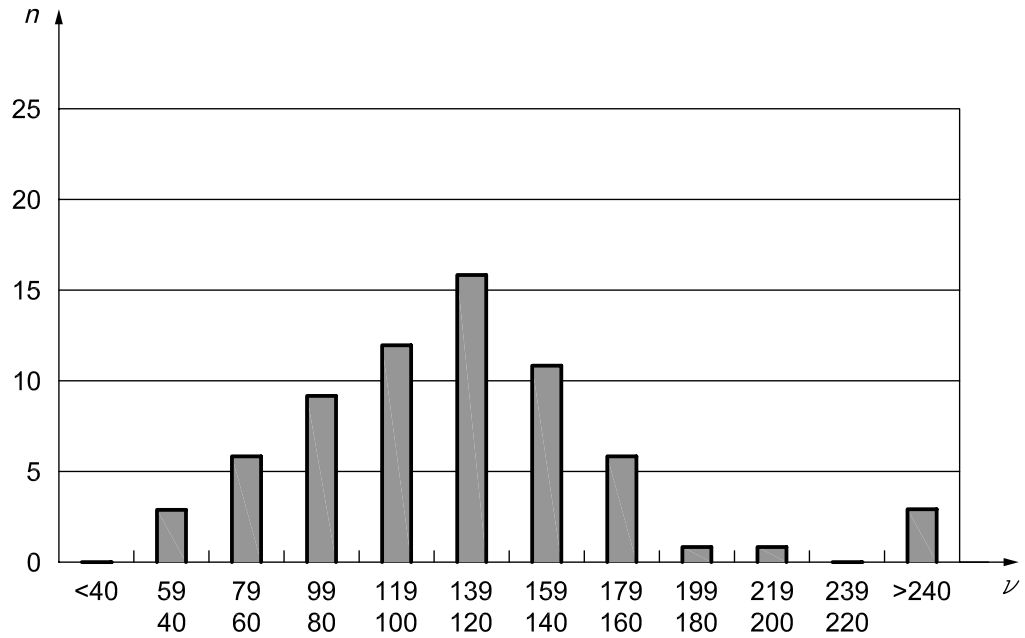


Key

n number of results

ν concentration/(cfu/m^3)

Figure C.2 — Number of results in different concentration ranges when sampling 50 l; results are not normally distributed

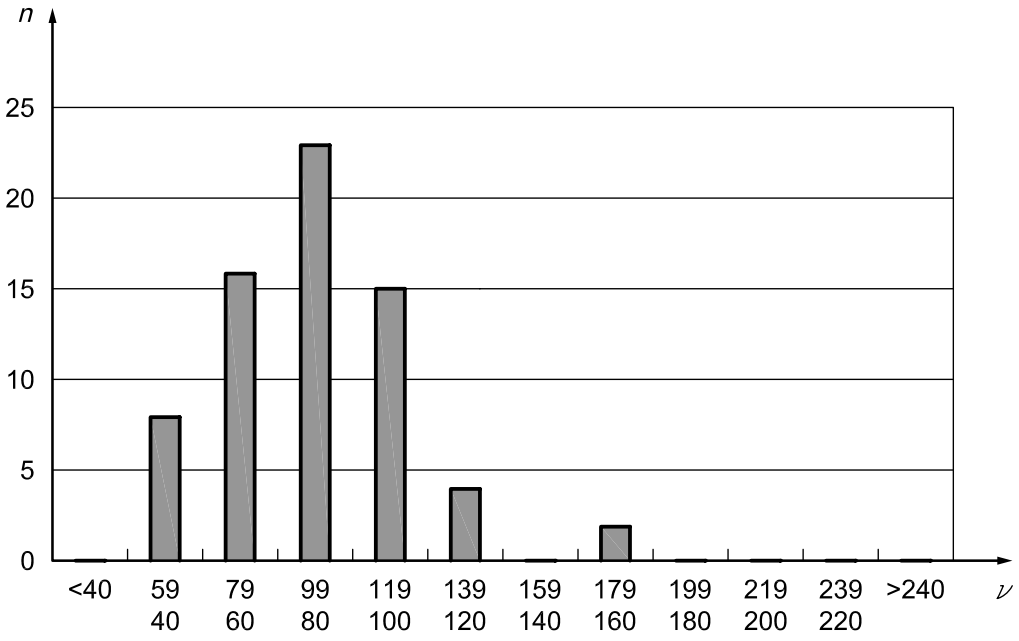


Key

n number of results

ν concentration/(cfu/m^3)

Figure C.3 — Number of results in different concentration ranges when sampling 100 l; results are normally distributed



Key

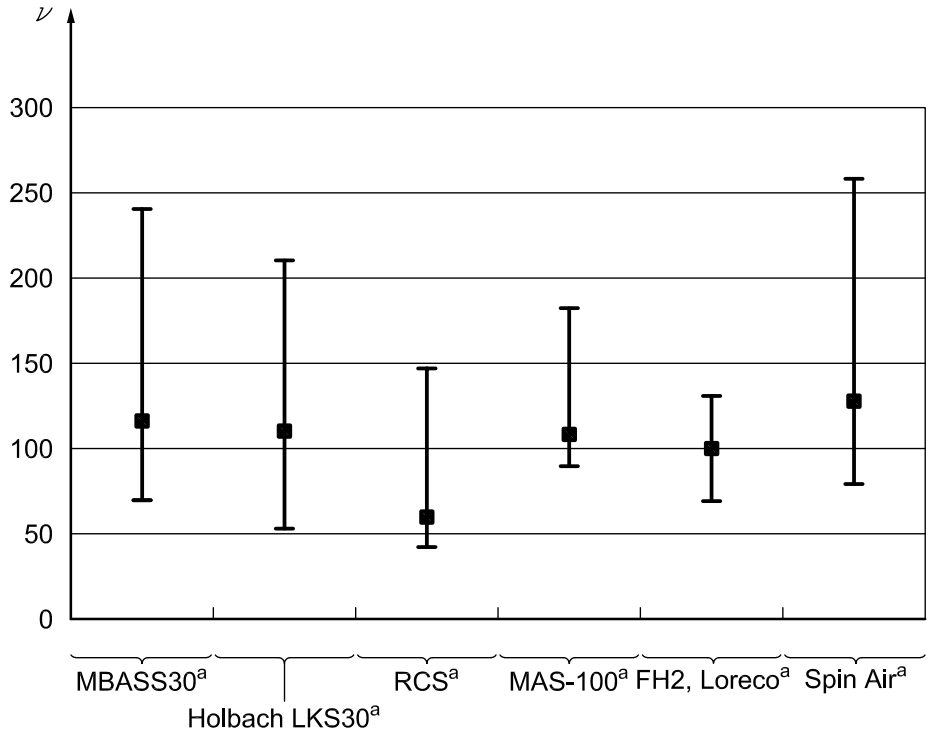
n number of results

ν concentration/(cfu/m^3)

Figure C.4 — Number of results in different concentration ranges when sampling 200 l; results are normally distributed

Different sampling devices were used by the 34 laboratories (with the number of sampling trains used given in parentheses): Holbach LKS30[1)] (15), MBASS30[1)] (11), RCS[1)] (4), MAS-100[1)] (2), Spin Air[1)] (1), FH2 Loreco[1)] (1). Results were comparable for all sampling devices except for the RCS. Median colony counts with the RCS samplers were about half the concentration compared to the other samplers (see Figure C.5).

1) Equipment available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.



Key

95th percentile

median

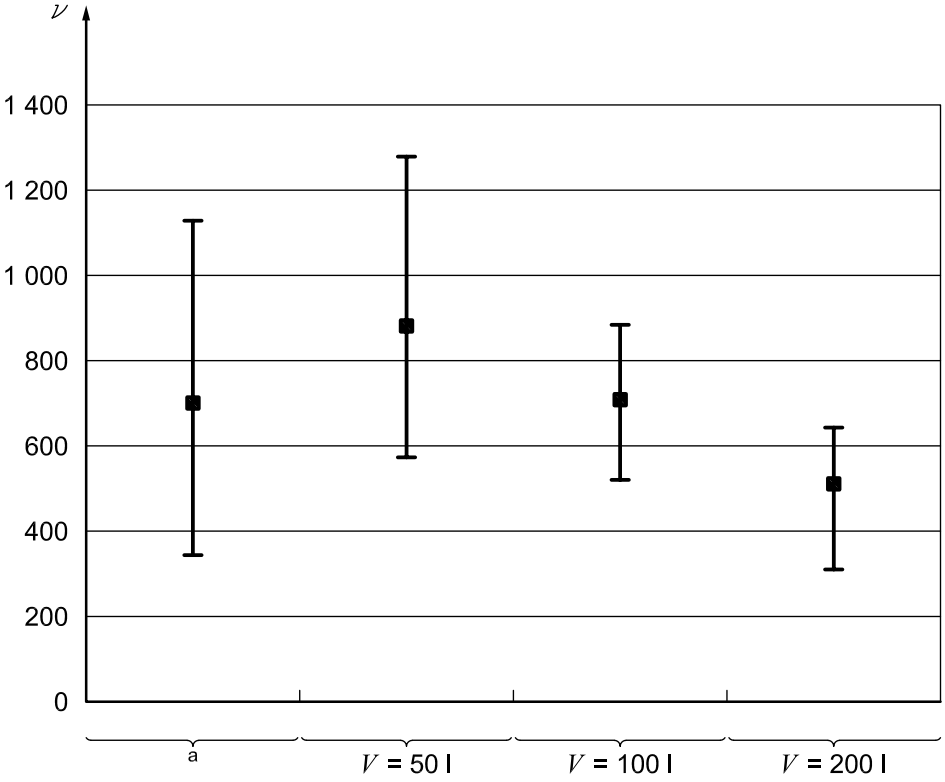
5th percentile

ν concentration/(cfu/m^3)

[a] Equipment available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

Figure C.5 — Concentration of total colony counts on DG18 agar after sampling with different sampling devices in trial 1

During trial 2 (June 2006), 33 laboratories sampled by impaction with different devices. The concentration of fungi in the room was higher than in trial 1. The mean total concentration using the results from all sampling volumes was about 700 cfu/m^3 (see Figure C.6). Again, results differed (statistically significant) when calculated separately for the different sampling volumes. Decreasing mean concentrations were found with increasing sampling volume: 884 cfu/m^3 for 50 l, 704 cfu/m^3 for 100 l; and 508 cfu/m^3 for 200 l samples. The number of colonies on the plates was in the optimal range for counting (about 90 colonies) only for 50 l samples. The number of colonies on the plates was too high for accurate counting for the 100 l and 200 l samples. This explains the lower mean concentrations found with higher sampling volumes. Standard deviations of the results were comparable to the values calculated for trial 1 (20 % in the optimal counting range). Colony counts were normally distributed for all sampling volumes (data not shown).



Key

95th percentile

median

5th percentile

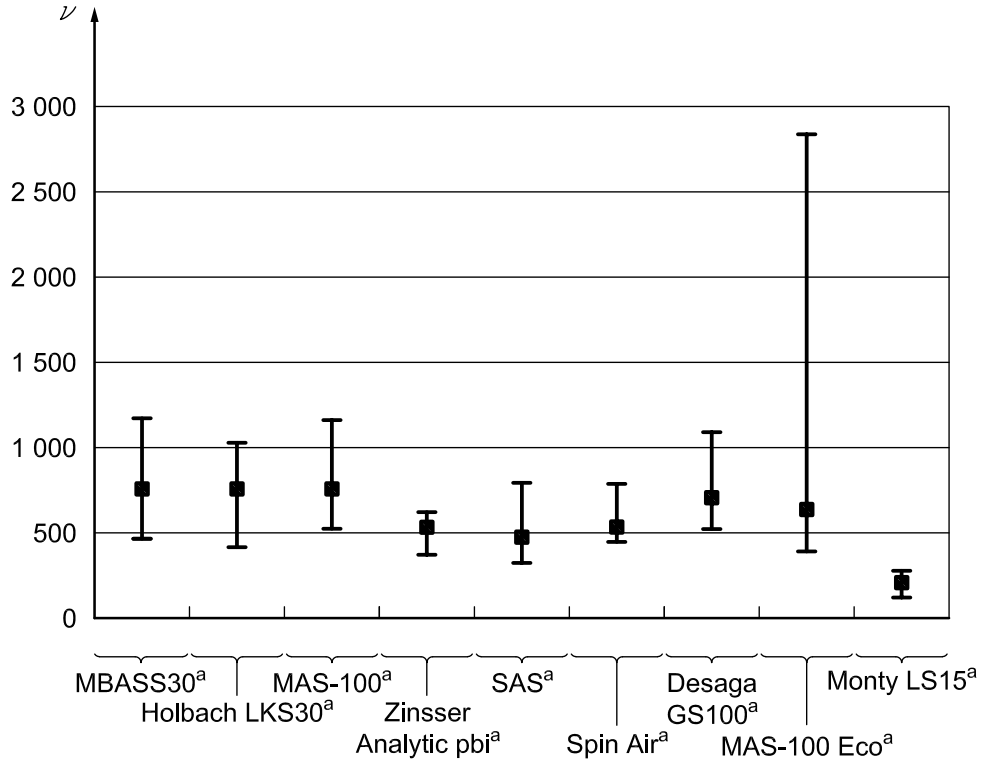
ν concentration/(cfu/m^3)

V sampling volume

a Overall.

Figure C.6 — Concentration of total colony counts on DG18 agar after sampling by impaction with different volumes in trial 2

Different sampling devices were used by the 33 laboratories (with the number of sampling trains used given in parentheses): Holbach LKS30[1)] (11), MBASS30[1)] (12), MAS-100[1)] (4), Spin Air[1)] (1), Zinsser Analytic pbi[1)] (1), Desaga GS100[1)] (1), SAS[1)] (1), MAS-100 Eco[1)] (1), Monty LS15[1)] (1). Results were comparable for MBASS, LKS30, MAS-100 and Desaga GS100. Slightly lower concentrations were detected by Spin Air, Zinsser Analytic pbi, SAS, and MAS-100 Eco although comparison is difficult because of the low number of results with these sampling devices. Significantly lower concentrations were detected with the Monty LS15 sampler (see Figure C.7). The use of small agar plates when sampling with Zinsser Analytic pbi, SAS, and Monty LS15 may have contributed to the lower concentrations detected with these samplers.



Key

95th percentile

median

5th percentile

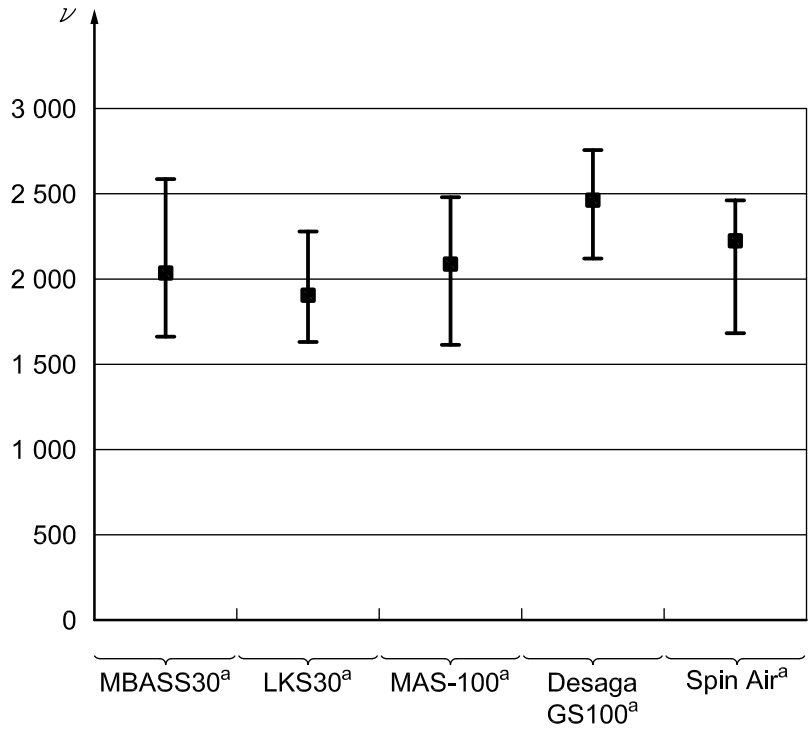
ν concentration/(cfu/m^3)

[a] Equipment available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

Figure C.7 — Concentrations of fungi obtained by sampling with different sampling devices in trial 2

During trial 3 (June 2007), 35 laboratories sampled by impaction with different devices. The concentration of fungi in the room was higher than in trial 1 and trial 2. Mean total concentration was about 2 000 cfu/m^3 (see Figure C.8). Only plates from sampling 50 l could be used for counting (about 100 colonies per plate). Plates from 100 l or 200 l sampling were overgrown with an expected number of colonies of 200 and 400. These results clearly show the limitation of the impaction method at high concentrations of fungi in the air. Standard deviations of the results were about 5 % for total colony count and for the dominant fungal genus *Cladosporium*. Standard deviations were higher (50 % to 100 %) for fungal genera which were only present in low concentrations. Colony counts were normally distributed for the 50 l samples (data not shown).

Different sampling devices were used by the 35 laboratories (with the number of sampling trains used given in parentheses): Holbach LKS30[1)] (13), MBASS30[1)] (15), MAS-100[1)] (5), Spin Air[1)] (1), and Desaga GS100[1)] (1). Results were comparable for MBASS, LKS30, and MAS-100 (see Figure C.8). Slightly higher concentrations were detected by Spin Air and Desaga GS100 although comparison is difficult because of the low number of results with these sampling devices.



Key

95th percentile

median

5th percentile

ν concentration/(cfu/m^3)

[a] Equipment available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named.

Figure C.8 — Concentration of total colony counts on DG18 agar after sampling with different sampling devices in trial 3

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2) Under preparation.

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