INTERNATIONAL STANDARD

ISO 16000-17

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Indoor air —

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

Air intérieur —

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

Reference number
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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 16000-17 was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 6, *Indoor air*.

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

— *Part 1: General aspects of sampling strategy*

— *Part 2: Sampling strategy for formaldehyde*

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

The following parts are under preparation:

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

— *Part 19: Sampling strategy for moulds*

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

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

The following parts are planned:

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

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

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

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

Furthermore,

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

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

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

focus on volatile organic compound (VOC) measurements.

Introduction

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

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

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

The procedure specified in this part of ISO 16000 is based on VDI 4253-2 [5] and VDI 4300-10 [6].

Indoor air —

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

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

1 Scope

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

2 Normative references

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

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

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

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

3 Definitions

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

3.1
filamentous fungus
fungus growing in the form of filaments of cells known as hyphae

NOTE 1 Hyphae aggregated in bundles are called mycelia.

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

[ISO 16000-16:2008]

1) To be published.

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

[EN 13098:2000 [4]]

NOTE In this part of ISO 16000, filtration is understood as the separation of microorganisms or moulds from a defined volume of air by means of filters.

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

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

[EN 13098:2000 [4]]

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

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

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

[ISO 16000-16:2008]

3.6
microorganism
any microbiological entity, cellular or non-cellular, capable of replication or of transferring genetic material, or entities that have lost these properties

[EN 13098:2000 [4]]

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

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

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

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

[ISO 16000-16:2008]

4 Principle

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

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

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

5 Apparatus

Usual microbiological laboratory equipment, and in particular the following.

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

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

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

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

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

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

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

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

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

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

6 Culture media and diluents

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

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

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

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

6.1 Dicloran 18 % glycerol agar (DG18 agar)

The components are listed in Table 1.

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

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

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

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

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

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

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

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

6.2 Malt-extract agar

The components are listed in Table 2.

Table 2 — Composition of malt-extract agar

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

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

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

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

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

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

6.3 Potato dextrose agar

The components are listed in Table 3.

Table 3 — Composition of potato dextrose agar

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

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

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

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

6.4 Saline solution

The components are listed in Table 4.

Table 4 — Composition of saline solution

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

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

6.5 Saline solution with polysorbate 80

The components are listed in Table 5.

Table 5 — Composition of saline solution with polysorbate 80

Component	Quantity
Sodium chloride (NaCl)	8,5 g
Polysorbate 80	0,1 g
Water	1 000 ml

Dissolve the NaCl in the water and sterilise in an autoclave at (121 ± 3) °C for (15 ± 1) min. Allow to cool and add the polysorbate 80.

7 Procedure

7.1 General

Samples to be analysed are either agar plates from sampling by impaction according to ISO 16000-18 or filters from sampling by filtration according to ISO 16000-16. Samples from direct plating or material suspensions can be treated accordingly.

7.1.1 Samples from impaction

Agar plates are directly incubated (see 7.3).

7.1.2 Samples from filtration

Filters are resuspended and aliquots spread on agar plates (see 7.2) followed by incubation (see 7.3).

Process the samples in the laboratory preferably without delay, but not later than 48 h after the end of sampling. Store samples in the laboratory in the dark at a temperature not exceeding the incubation temperature (< 25 °C), protected against adverse influences (humidity, desiccation, contamination). Document the storage conditions.

Carry out all procedures under conditions that protect the samples from any contamination. Check aseptic conditions regularly by controls and results shall be documented.

7.2 Processing of filters

7.2.1 General

Airborne moulds deposited on filters are processed using the indirect plating method.

NOTE Aggregates of either spores or aggregates of particles may be dissolved by suspension as well as by dilution, which may result in higher numbers of colonies after incubation. An aggregate of 30 spores may result in 30 colonies under these circumstances. By contrast, a reduction of the number of colonies may occur if the detachment of the mould spores from the filters is incomplete or by damage of the fungal cells during processing in the laboratory.

7.2.2 Resuspension

In the aseptic atmosphere of a safety cabinet (5.6), transfer the filters (gelatin and polycarbonate filter) into a sterile container containing 5 ml of saline solution with polysorbate 80 (see 6.5) using a sterile pair of tweezers.

Shake (5.8) the filters steeped in this solution in their container intensively in a horizontal position in a water bath maintained at 35 °C to 40 °C (5.7) for 15 min. Make sure that the spore-loaded surface of the filter lies flat and faces upwards and can move freely within the suspension during shaking.

Process the sample according to 7.2.3 within 1 h after suspension.

7.2.3 Dilution

Based on the original suspension, set up a serial dilution series.

Immediately prior to dilution, shake the suspension for 1 min on a test tube shaker (5.8). Add 1 ml of the suspension to 9 ml saline solution (6.4) using a sterile disposable pipette or a cotton-stuffed glass pipette. In the same way, carry out two further dilution steps resulting in dilutions 1:10, 1:100, and 1:1 000.

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

7.2.4 Plating

Plate 0,1 ml of the original suspension and the dilutions (7.2.3) in parallel on DG18 agar (6.1) as well as on malt-extract agar (6.2) or potato dextrose agar (6.3). Use at least two parallel plates for each dilution and incubation temperature (7.3).

If, according to the sampling protocol, low concentrations of moulds are expected, 1 ml of the original solution can be plated on four plates for each agar medium (using 250 µl per plate) to enhance the sensitivity of the method.

Determine laboratory blank samples for all dilution steps.

7.3 Incubation

Incubate agar plates upside up in incubators at (25 ± 3) °C for 7 days. DG18 agar plates may require prolonged incubation times — 10 days — especially if differentiation of fungi is anticipated.

For special purposes (e.g. thermotolerant *Aspergillus* spp.), malt-extract agar plates or potato dextrose agar plates may additionally be incubated at (36 ± 2) °C. For selective cultivation of *Aspergillus fumigatus*, incubation temperatures of (45 ± 2) °C are used.

CAUTION — At incubation temperatures above 25 °C, take special care to avoid desiccation of the agar plates.

Incubate agar plates in such way that sufficient oxygen will allow optimal mould growth, e.g. avoid incubation in tight polyethylene bags. Incubate agar plates in a vibration-free incubator, to minimize the risk of secondary colony inoculation due to spreading of spores. Also avoid extensive airflows in view of desiccation of the agar plates.

7.4 Examination and counting

Examine agar plates for the first time after 2 days to 3 days and subsequently in regular intervals for up to at least 7 days.

Count thermotolerant moulds (36 °C or 45 °C) after 1 day to 3 days, as they grow very rapidly.

Handling of the agar plates may lead to distribution of spores on the plate resulting in secondary colonies in the course of incubation. Take care not to count secondary colonies.

The optimal range for genus/species identification and quantification using a standard culture plate with a diameter of ~90 mm is between 20 and 40 colonies. For quantitative results, at least 10 colonies of the respective genus/species should be on the agar plate and a maximum number of 100 colonies in total. Some moulds may spread very fast inhibiting the growth of other colonies (e.g. *Rhizopus*, *Chrysonilia*, *Mucor*, *Botrytis*) — even on plates containing dicloran — and the capacity of the plate can run down even at lower colony counts.

Record the maximum number of colonies counted within the 7 days of incubation for each agar medium and sampling volume (impaction) or dilution (filtration), respectively.

7.5 Identification of mould species

Identification of the moulds growing on the agar plates is essential for most questions concerning mould problems in indoor environments. Identification is based on macroscopic and microscopic morphological characteristics. These characteristics are usually better identified on malt-extract agar or potato dextrose agar compared to DG18 agar.

NOTE Malt-extract agar and potato dextrose agar have been shown to give comparable results for identification of mould genera and species.

The extent of identification depends on the purpose of the investigation.

To detect sources of mould growth indoors, it is important to recognise differences in species/genera composition between indoors and outdoors as well as moisture indicators e.g. *Aspergillus versicolor* or *Chaetomium* spp.

Identification to species level within certain genera (e.g. *Phialophora*, *Trichoderma*, *Acremonium*, *Chaetomium*, *Penicillium* and *Fusarium*) is not recommended for this purpose as it is very difficult and scarcely achievable in routine analysis.

Detailed species identification may, however, be important for investigations related to health problems. In this case, species identification should be anticipated even for the genera mentioned in the previous paragraph.

For identification of most species, pure cultures have to be prepared from the colonies growing on the isolation medium and transferred to special media for identification.

Either the identification of mould species shall be carried out by laboratories with mycological expertise and experience in the identification of microfungi or the identifications shall be checked by such laboratories, in order to guarantee the reliability of the examination results. Mycological expertise and experience should be tested by proficiency tests for the identification of indoor moulds.

Recommended literature for identification of indoor moulds is given in References [7] to [15]. Identification to genera and species level is mostly based on phenotypical characteristics, e.g. sporulating structures, colony colour and growth behaviour. Morphological characteristics are supplemented by biochemical and molecular characteristics for species identification in some genera.

7.6 Calculation and expression of results

7.6.1 General

Calculation of the concentration of moulds in indoor air is primarily based on the colony counts obtained from DG18 agar plates. Only mould species that do not or only sporadically grow on DG18 agar (e.g. *Chaetomium*, *Stachybotrys*) are counted on malt-extract agar or potato dextrose agar. The results of DG18 agar and malt-extract agar or potato dextrose agar are not compiled, but the calculation is based on the colony count of the agar where best growth of the respective species/genera occurs.

The concentrations are calculated for each species or genera identified. Non-identified mould species are summarized as “other species”. It should, however, be a goal to identify the dominant mould species at least on the genus level (see 7.5). Sterile mycelia are counted separately. The total concentration of moulds is calculated as the sum of the concentrations of the species/genera including non-identified and sterile colonies.

The results are rounded to two significant figures.

NOTE Yeasts are not quantitatively detected by this method. Yeast colonies appearing on the agar plates can, nevertheless, be included in the test report as a separate item.

7.6.2 Impaction

Usually at least four agar plates (two different sampling volumes, analysed in parallel) are available per sample and medium.

The concentration, as the number of colony forming units per cubic meter of indoor air, C_I, is calculated from Equation (1):

$$C_\mathrm{I} = \frac{n_\mathrm{cfu}}{V_\mathrm{I}} \tag{1}$$

where

n_cfu is the total number of colony forming units on the agar plates;

V_I is the total sampling volume, in cubic metres.

EXAMPLE With one agar medium: sampling was performed on two different air volumes — 100 l and 200 l — that were analysed in parallel.

The following colony counts were obtained:

Sample volume	Number of colonies
100 l (0,1 m^3)	15
	23
200 l (0,2 m^3)	29
	35

Then:

n_cfu = 15 + 23 + 29 + 35 = 102

V_I = 0,1 + 0,1 + 0,2 + 0,2 = 0,6

and

$$C_\mathrm{I} = \frac{102}{0,6} = 170 = 1,7 \times 10^2$$

NOTE For high numbers of colonies, impactor manufacturers advise the use of a positive-hole correction factor. The positive-hole correction factor is a statistical tool which calculates a probable count from the total raw count, taking into consideration that multiple particles can impact on the same hole. For this reason, the sum of the calculated counts may be less than the positive-hole corrected total. When raw counts reach 80 % of the positive hole value, corrected counts should be considered an estimation. The corrected colony count, $n_\mathrm{cfu,c}$, for a multiple-hole impactor with n_j jets can be calculated as follows:

$n_\mathrm{cfu,c} = n_\mathrm{cfu}[1,075/(1,052 - f)]^{0,438}$ for $f < 0,95$

where

n_cfu is the number of colony forming units or filled impaction sites;

f is $n_\mathrm{cfu}/n_\mathrm{j}$

A statistical correction using the positive-hole correction factor is usually not needed when following this part of ISO 16000 because: i) the correction factor is not relevant in the optimal range for identification of fungi and calculation of results (20 to 40 colonies, maximum 100 colonies); ii) the total number of colonies is calculated by adding the number of colonies for the genera/species identified which usually is $\ll$ 100 colonies; and iii) in most cases the capacity of the plate runs down at low colony counts due to the colony size of the fungi.

7.6.3 Filtration

The quantification of mould colonies is often hampered by disturbing growth of certain mould species. For this reason, usually only agar plates from one dilution step can be utilized if 1:10 dilution steps are used. For calculation of the result, agar plates are chosen which show the least disturbance between colonies and still contain enough colonies for valid quantification (see 7.4). If more than one plate can be used for quantification, calculate the weighted mean according to ISO 8199.

The concentration in the original suspension, in colony forming units per milliliter, C_S, is calculated from Equation (2):

$$C_S = \frac{n_{cfu}}{V_S} \tag{2}$$

where

n_{cfu} is the total number of colonies on the plates of the dilution step used;

V_S is the calculated total volume, in milliliters, of original sample included in the plates of the dilution step used, given by Equation (3):

$$V_S = n_p V_t f_d \tag{3}$$

in which

n_p is the number of plates counted for the dilution step used;

V_t is the test volume, in milliliters, spread on the agar plates;

f_d is the dilution factor for the test volume (f_d = 1 for the original suspension; f_d = 0,1 for a 1:10 dilution, etc.)

EXAMPLE 1 With one agar medium: two agar plates were used per dilution step and 0,1 ml was spread on the plates. The following colony counts were obtained:

Dilution	Number of colonies
10^{-1}	26
	33

Then:

n_{cfu} = 26 + 33 = 59

V_S = 2 × 0,1 × 0,1 = 0,02

and

$$C_S = \frac{59}{0{,}02} = 2\,950$$

The concentration in the air sample, in colony forming units per cubic metre, C_F, can be calculated using Equation (4):

$$C_F = C_S \frac{V_F}{V_I} \tag{4}$$

where

C_S is the concentration, in colony forming units per millilitre, in the original suspension;

V_F is the volume of saline for resuspension of the filter;

V_I is the volume, in cubic metres, of the air sample.

EXAMPLE 2 The volume of the air sample was 0,8 m^3. The filter was resuspended in 5 ml of saline.

Then:

$$C_F = 2\,950 \times \frac{5}{0,8} = 18\,438$$

Result: 1,8 × 10^4 cfu/m^3.

7.6.4 Field blanks

The concentration of fungi in the field blanks is recorded for quality control. Usually no colonies are found on the field blanks. Colony numbers — on an agar plate after impaction or after spreading the undiluted filter suspension — exceeding two indicate sampling errors and the results of the measurement should be interpreted with caution. No correction is made to the measurement results of the samples on the basis of the results of the field blanks.

8 Test report

The test report shall contain at least the following information:

a) a reference to this part of ISO 16000;

b) reference to the sampling standard: ISO 16000-16 for filtration or ISO 16000-18 for impaction;

c) all details necessary for complete identification of the sample;

d) time of analysis;

e) time between end of sampling and start of analysis;

f) duration of incubation;

g) volume and number of samples analysed, if applicable;

h) the results expressed in accordance with 7.6 including reference to the respective culture medium and incubation temperature;

i) statements on error of measurement and/or accuracy of measurement, if possible;

j) any other information relevant to the method.

Annex A
(informative)

Specific characteristics of mould spores

In nature, moulds are ubiquitous and fulfil an important role in the decomposition and mineralisation of organic substances in the nutrient cycle in nature. Some species of fungi are pathogenic to both men and animals.

Moulds are especially adapted to dispersal by air. They develop spores that not only serve for aerial dispersal, but also function as resting stages for long-term survival. In several taxa, different morphological forms of spores (conidia, ascospores, chlamydospores) are found, with each form being specialised in one of the biological functions mentioned above. Spore diameters vary between species, ranging from 2 µm to 10 µm in most airborne species. In a few cases 30 µm or more are reached (geometric diameter). Fragments of hyphae are up to 10 µm wide, but may be substantially longer than 30 µm. The statistical probability that an individual hyphal cell represents a colony forming unit, is negligible.

Units for propagation of moulds in air may be individual spores or conidia, aggregates of spores, or fragments of hyphae that may occur free, adhere to dust particles or are suspended in droplets.

The mould spores have thick cell walls of chitin which make them very resistant to desiccation. Melanin is stored in the membranes of many mould fungi, which protects the spores against damage by ultraviolet light. On account of these characteristics, mould fungi may survive prolonged dryness on the one hand, while on the other they may be dispersed over great distances by air (intercontinental spore dispersal).

Conidia (spores) of several mould genera, e.g. *Penicillium* and *Aspergillus*, are hydrophobic and cannot easily be suspended in water. This is of importance for the laboratory work. Suspensions of conidia in water always have to be made by adding detergents.

Table A.1 — Concentrations for fungal species in ambient air, in waste management, and agricultural environments

Typical species in ambient air			Concentrations [a]			
Species	**Particle size, either spore size or conidial size** µm	**a_{H_2O} values, material** [b]	**Indoors** cfu/m^3	**Workplaces, waste management** cfu/m^3	**Ambient air** cfu/m^3	%
Alternaria alternata	(18 to 83) (7 to 18) (Ref. [17])	0,85 to 0,88 (Ref. [17])	10^1 (Ref. [21])	n.r.	10^2 (Ref. [18]); 10^1 (Ref. [21])	—
Aureobasidium pullulans	(7,5 to 16) (3,5 to 7) (Ref. [17])	—	< 5 (Ref. [19])	n.r.	< 5 (Ref. [21])	—
Botrytis cinerea	(8 to 14) (6 to 9) (Ref. [17])	0,93 to 0,95 (Ref. [17])	< 5 (Ref. [20])	n.r.	< 10 (Ref. [20])	—
Cladosporium spp.	3 to 11	—	10^2 (Ref. [20])	—	10^3 (Ref. [18]), (Ref. [20])	up to 90 (Ref. [18])
C. herbarum	(5,5 to 13) (4 to 6) (Ref. [16])	0,85 to 0,88 (Ref. [16])	10^1 (Ref. [20])	10^2 (Ref. [19])	10^2 (Ref. [20])	up to 60
C. cladosporioides	[3 to 7 (11)] [2 to 4 (5)] (Ref. [16])	0,86 to 0,88 (Ref. [16])	10^1 (Ref. [20])	10^3 (Ref. [19])	10^1 (Ref. [20])	up to 30
Epicoccum nigrum	16 to 25 (Ref. [16])	0,86 to 0,90 (Ref. [16])	10^1 (Ref. [21])	n.r.	10^1 (Ref. [21])	—

Table A.1 (*continued*)

Typical species in waste management and agriculture			Concentrations			
Species	**Particle size respectively spore size or conidial size** µm	a_{H_2O} **values, material** [b]	**Indoors** cfu/m^3	**Workplaces, waste management** cfu/m^3	**Ambient air** cfu/m^3	%
Absidia corymbifera	(3,4 to 4,6) (2,8 to 3,8) (Ref. [10])	—	n.r.	—	n.r.	
Aspergillus spp.	2,5 to 5 (Ref. [16])	0,71 to 0,95	10^1 (Ref. [20])	—	< 10 (Ref. [20])	< 3 (Ref. [18])
A. candidus	2,5 to 4 (Ref. [16])	0,75 to 0,78 (Ref. [16])	< 5	10^4 (Ref. [19])	—	+
A. flavus	3,6 (Ref. [16])	0,78 to 0,80 (Ref. [16])	< 5	10^4 (Ref. [19])	—	—
A. fumigatus	2,5 to 3 (Ref. [16])	0,85 to 0,94 (Ref. [16])	sparsely	10^7 (Ref. [19])	up to 20	—
A. nidulans	3 to 3,5 (Ref. [16])	0,85 (Ref. [17])	< 5	10^5 (Ref. [19])	—	—
A. niger	3,5 to 5 (Ref. [16])	0,92 to 0,95 (Ref. [17])	sparsely	10^4 (Ref. [19])	—	—
A. parasiticus	3,5 to 5,5 (Ref. [16])	0,78 to 0,82 (Ref. [16])	—	10^3 (Ref. [19])	—	—
A. versicolor	2 to 3,5 (Ref. [16])	0,78 (Ref. [16])	< 5 (Ref. [21])	10^6 (Ref. [19])	< 5 (Ref. [21])	—
Eurotium herbariorum	4,5 to 7 (8) (Ref. [16])	—	< 5 (Ref. [21])	n.r.	< 10 (Ref. [21])	—
F. graminearum	[41 to 60 (80)] (4 to 5,5) (Ref. [16])	0,89 (Ref. [16])	primarily not airborne	n.r.	—	—
Mucor hiemalis	(3,5 to 5,2) (2,5 to 3,7) (Ref. [10])	—	—	n.r.	—	—
M. racemosus	[5,5 to 8,5 (10)] (4 to 7) (Ref. [10])	0,94 (Ref. [16])	—	n.r.	—	—
Paecilomyces variotii	(3 to 5) (2 to 4) (Ref. [16])	0,79 to 0,84 (Ref. [16])	—	10^6 (Ref. [19])	—	—
Penicillium spp.	—	0,78 to 0,98	10^2 (Ref. [21])	—	10^1 (Ref. [21])	2,5 to 13 (Ref. [18])
P. brevicompactum	3 to 4,5 (Ref. [16])	0,78 to 0,82 (Ref. [16])	10^1 (Ref. [21])	10^4 (Ref. [19])	< 10 (Ref. [20])	+
P. chrysogenum	(3 to 4) (2,8 to 3,8) (Ref. [16])	0,78 to 0,81 (Ref. [16])	up to 10^1 (Ref. [21])	10^2 (Ref. [19])	up to 10^1 (Ref. [21])	+
P. corylophilum	(2,5 to 3.2) (2,5 to 3,0)	—	—	n.r.	—	+
P. crustosum	3 to 4 (Ref. [16])	—	—	10^5 (Ref. [19])	—	—

Table A.1 (*continued*)

Typical species in waste management and agriculture			Concentrations			
Species	**Particle size respectively spore size or conidial size**	a_{H_2O} **values, material** [b]	**Indoors**	**Workplaces, waste management**	**Ambient air**	
	µm		cfu/m^3	cfu/m^3	cfu/m^3	%
P. expansum	(3 to 3,5) (2,5 to 3) (Ref. [16])	0,82 to 0,85 (Ref. [16])	—	10^1 (Ref. [19])	—	—
P. glabrum	3 to 3.5 (Ref. [16])	—	< 10 (Ref. [20])	10^4 (Ref. [19])	< 5 (Ref. [20])	—
P. lanosum	2,5 to 3,0	—	sparsely	n.r.	—	+
P. roqueforti	4 to 6 (8) (Ref. [16])	0,83 (Ref. [16])	—	10^4 (Ref. [19])	—	—
Rhizopus oligosporus	[(4) 9 to 10 (15)] [(4) 7 to 10 (11)] (Ref. [10])	—	—	10^4 (Ref. [19])	—	—
Sporobolomyces spp.	(2 to 12) (3 to 35)	—	—	—	up to 10^5 (Ref. [18])	—
Stachybotrys chartarum	(7 to 12) (4 to 6) (Ref. [10])	0,94 (Ref. [16])	—	—	—	—
Trichoderma harzianum	(2,8 to 3,2) (2,5 to 2,8) (Ref. [16])	—	sparsely	—	—	—
T. citrinoviride	(2,2 to 3,7) (1,5 to 2,1)	—	—	10^2 (Ref. [19])	—	—

Legend

\+ species regularly present, even if detected in lower numbers

n.r. species occurs in very low cfu numbers and is not of hygienic relevance

– no literature source available

NOTE 1 Quantitative data without reference are unpublished empirical data (DG18 agar, impaction) of the Hygiene and Environmental Health Institute, Aachen University Hospital, Germany.

NOTE 2 Only very limited numbers of results are shown as examples. Concentrations may vary depending, for example on location of the building and season.

[a] Concentrations are mostly given as order of magnitude (power of ten), in environments with relatively low concentrations of filamentous fungi (e.g. indoor air, ambient air), frequency classes of < 10 cfu/m^3, < 5 cfu/m^3 and “sparsely” are given.

[b] The values (material) are the lowest a_{H_2O} levels for mould development; temperature, exposure time, and material properties also affect growth. The lowest a_{H_2O} values are not all from building materials but may result from laboratory cultures.

Annex B
(informative)

Exchange of samples for validation of the cultivation method

Three different trials have been conducted to check the validity of the described cultivation method: i) trial 1 using a dust sample, ii) trial 2 using contaminated material, and iii) trial 3 using plates from impaction. All samples for the trials were prepared by one reference laboratory and sent by courrier to the participating laboratories. Participating were seven reference laboratories that were acquainted with the cultivation method in detail. A further 25 to 30 laboratories participated in the trial that were more or less acquainted with the cultivation method.

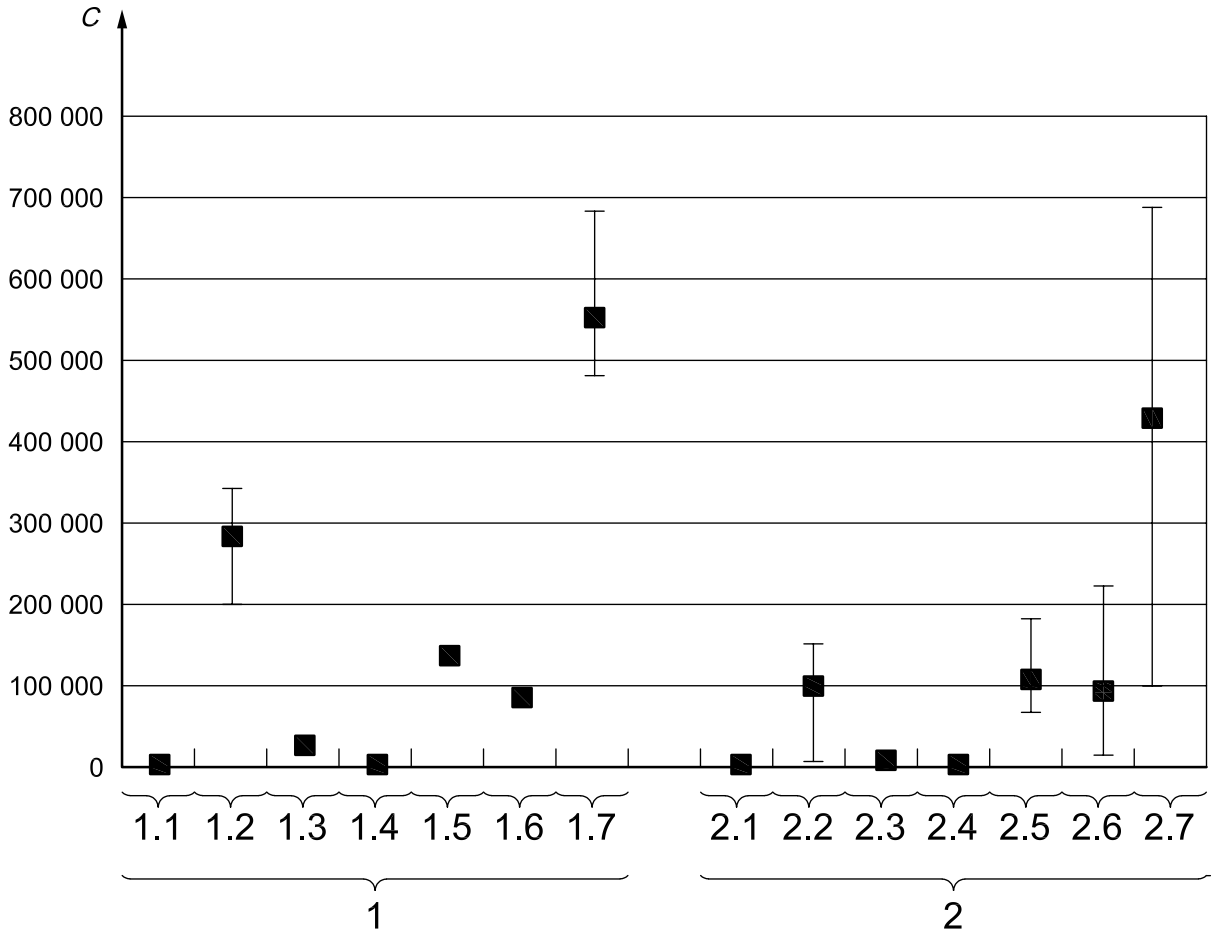
For trial 1, a dust sample was fractionated and portions of the 100 µm fraction were sent to the participating laboratories. Cultivation was performed on DG18 agar and malt-extract agar according to this part of ISO 16000. Results are shown in Figure B.1.

Results obtained by the seven reference laboratories are in good agreement. Standard deviation of results was much higher, however, for the other participating laboratories. Species variation was very high in the dust sample and did not allow for statistical analysis in detail. Therefore, results are given on the genus level only.

Trial 2 was prepared by cultivating six different fungal species on a material sample (wood) to restrict species diversity in the sample. The following fungi were used: *Aspergillus restrictus*, *Aspergillus sydowii*, *Aspergillus versicolor*, *Penicillium brevicompactum*, *Penicillium expansum*, and *Acremonium strictum*. The wood sample was homogenised and sent to the participating laboratories for analysis. Cultivation was performed on DG18 agar and malt-extract agar according to this part of ISO 16000. Results are shown in Figure B.2.

Results obtained by the seven reference laboratories were again in good agreement. Standard deviation of results was again much higher for the other participating laboratories. Especially, the detection of *Aspergillus restrictus* and *Acremonium strictum* posed problems for many laboratories.

Agar plates from sampling by impaction in indoor air and corresponding ambient air were used in trial 3. All 100 l samples were taken in one reference laboratory during one day using 5 impactors in parallel. Samples in ambient air were taken first (plates 1 to 140) followed by samples in the indoor environment (plates 280 to 420). A source of *Aspergillus versicolor* was present in the indoor environment. Two DG18 and two malt-extract agar plates from indoor as well as from ambient air were sent to each participating laboratory for cultivation and detection of fungi. Each laboratory received one set of plates from the beginning of sampling and one plate from the end of the sampling period. Results are presented in Figure B.3 a) and b).

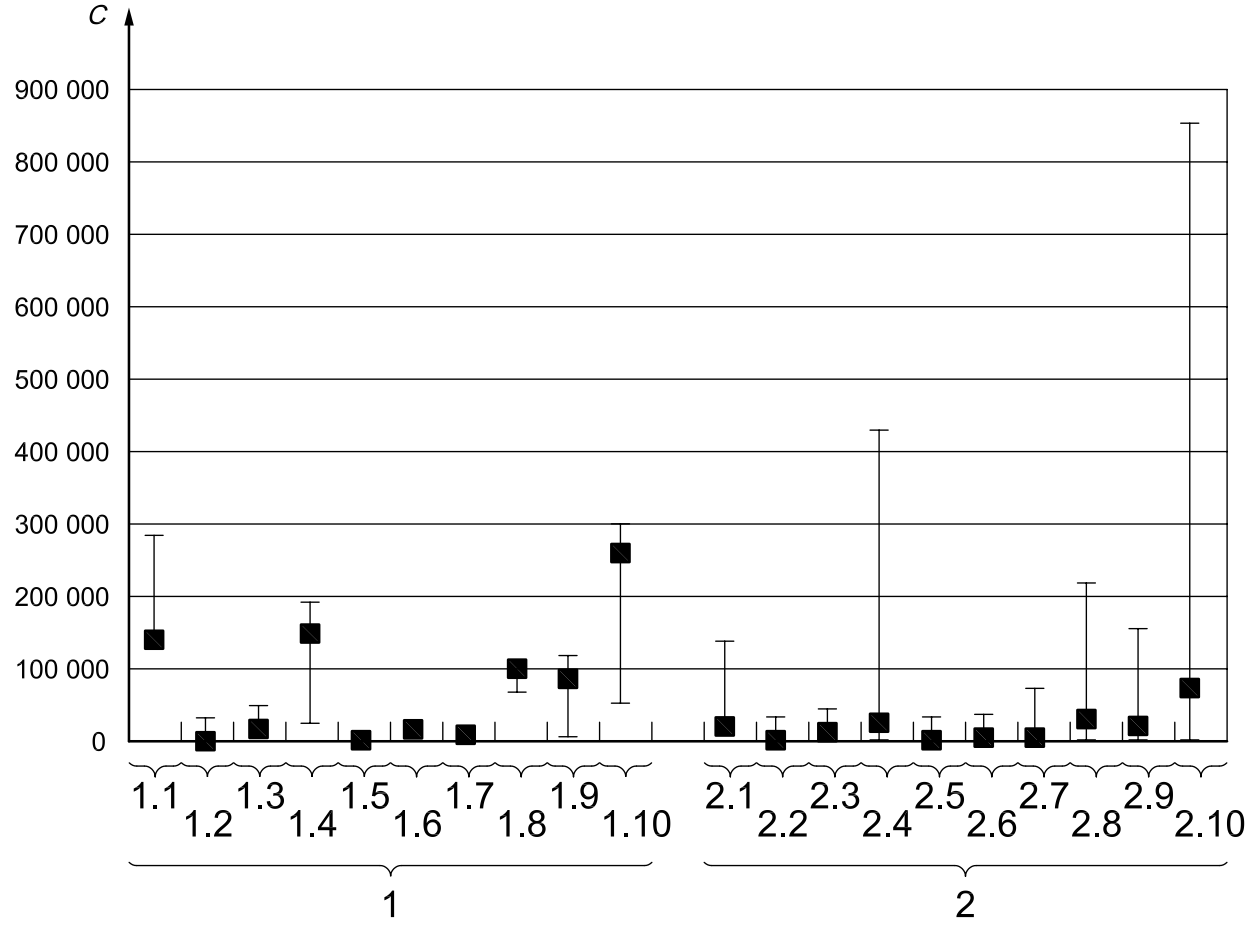


Key

C	colony forming units per cubic metre		
1	reference laboratories	2	other laboratories
1.1	*Alternaria* spp.	2.1	*Alternaria* spp.
1.2	*Cladosporium* spp.	2.2	*Cladosporium* spp.
1.3	*Aspergillus* spp.	2.3	*Aspergillus* spp.
1.4	*Eurotium* spp.	2.4	*Eurotium* spp.
1.5	*Penicillium* spp.	2.5	*Penicillium* spp.
1.6	other species	2.6	other species
1.7	total colony count	2.7	total colony count

NOTE Results are presented separately for the seven reference laboratories and other participating laboratories. The median as well as the 25th and 75th percentile are given for each genus and for the total colony count.

Figure B.1 — Results of colony counts using a dust sample

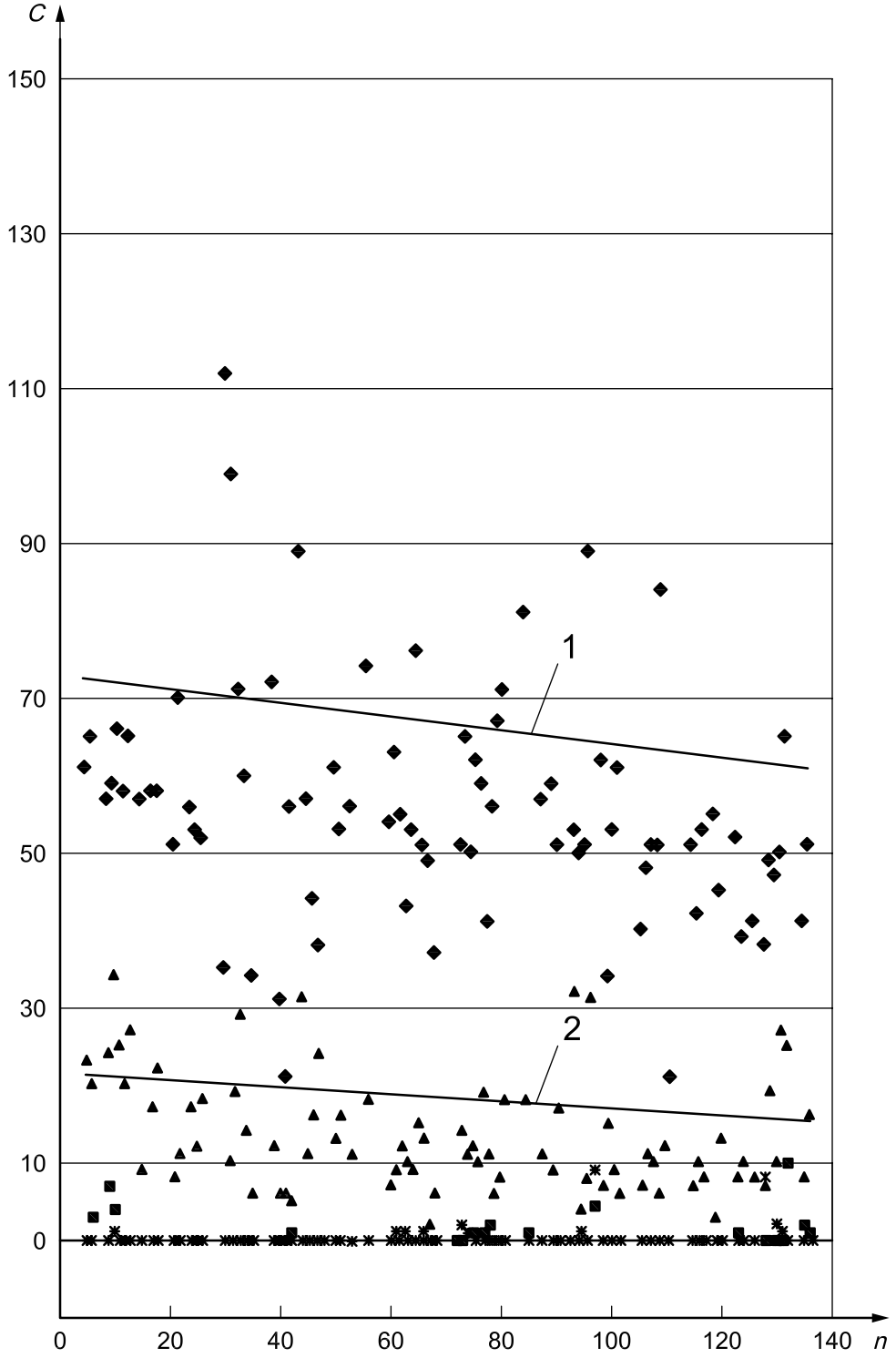


Key

C	colony forming units per cubic metre		
1	reference laboratories	2	other laboratories
1.1	*Aspergillus restrictus*	2.1	*Aspergillus restrictus*
1.2	*Aspergillus sydowii*	2.2	*Aspergillus sydowii*
1.3	*Aspergillus versicolor*	2.3	*Aspergillus versicolor*
1.4	total *Aspergillus* spp.	2.4	total *Aspergillus* spp.
1.5	*Penicillium brevicompactum*	2.5	*Penicillium brevicompactum*
1.6	*Penicillium expansum*	2.6	*Penicillium expansum*
1.7	total *Penicillium* spp.	2.7	total *Penicillium* spp.
1.8	*Acremonium* spp.	2.8	*Acremonium* spp.
1.9	other species	2.9	other species
1.10	total colony count	2.10	total colony count

NOTE Results are presented separately for the seven reference laboratories and other participating laboratories. The median as well as the 5th and 95th percentile are given for each species, genus and for the total colony count.

Figure B.2 — Results of colony counts using a material sample



Key

1 $C = -0{,}087\ 6n + 72{,}768$ $r^2_{nC} = 0{,}002\ 1$

2 $C = -0{,}045\ 5n + 21{,}327$ $r^2_{nC} = 0{,}002\ 9$

C colony forming units per cubic metre

n agar plate number

◆ total colony count

■ *Aspergillus* spp.

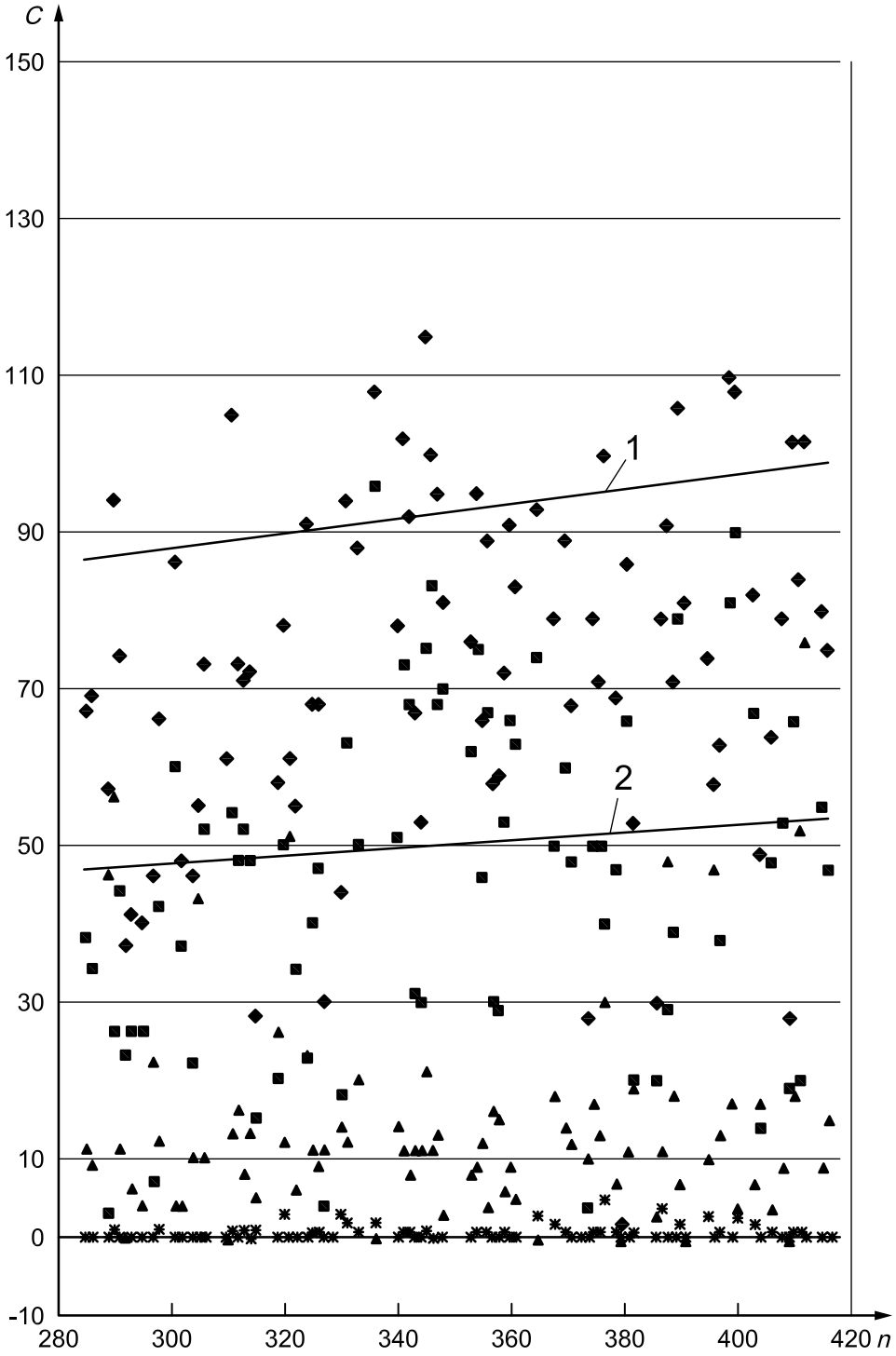
✱ *Eurotium* spp.

▲ *Penicillium* spp.

NOTE Four extreme values (200 to 600) were deleted from the figure. Lines indicate a time trend.

a)

Figure B.3 — Results of colony counts using agar plates from sampling by impaction in indoor air



Key

1 $C = -0{,}097\ 2n + 58{,}558$ $r^2_{nC} = 0{,}000\ 8$

2 $C = -0{,}052\ 2n + 31{,}859$ $r^2_{nC} = 0{,}001$

C colony forming units per cubic metre

n agar plate number

◆ total colony count

■ *Aspergillus* spp.

✱ *Eurotium* spp.

▲ *Penicillium* spp.

NOTE Six extreme values (200 to 1 000) were deleted from the figure. Lines indicate a time trend.

b)

Figure B.3 (*continued*)

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