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BSI Standards Publication

Ambient air quality — Measurement of bioaerosols

Part 1: Determination of moulds using filter sampling systems and culture-based analyses

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National foreword

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This publication is not to be regarded as a British Standard.

It is being issued in the Draft for Development series of publications and is of a provisional nature. It should be applied on this provisional basis, so that information and experience of its practical application can be obtained.

Comments arising from the use of this Draft for Development are requested so that UK experience can be reported to the international organization responsible for its conversion to an international standard. A review of this publication will be initiated not later than 3 years after its publication by the international organization so that a decision can be taken on its status. Notification of the start of the review period will be made in an announcement in the appropriate issue of *Update Standards.*

According to the replies received by the end of the review period, the responsible BSI Committee will decide whether to support the conversion into an international Standard, to extend the life of the Technical Specification or to withdraw it. Comments should be sent to the Secretary of the responsible BSI Technical Committee at British Standards House, 389 Chiswick High Road, London W4 4AL.

The UK participation in its preparation was entrusted to Technical Committee EH/2/1, Stationary source emission.

A list of organizations represented on this committee can be obtained on request to its secretary.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

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Compliance with a British Standard cannot confer immunity from legal obligations.

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Luftbeschaffenheit - Messen von Bioaerosolen - Teil 1: Bestimmung von Schimmelpilzen mittels Probenahme auf Filtern und kulturellem Nachweis

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The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

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Foreword

This document (CEN/TS 16115-1:2011) has been prepared by Technical Committee CEN/TC 264 “Air quality”, the secretariat of which is held by DIN.

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

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Introduction

Airborne particles of biological origin are called bioaerosols. Depending on the emission source bioaerosols vary in composition; one component of ambient bioaerosols with possible ecological and health relevance can be moulds. Natural and anthropogenic sources for mould spores are widely distributed in the environment. Anthropogenic sources can for example be agriculture and construction activities or waste treatment.

Mould is a common name for filamentous fungi from different taxonomic groups (Zygomycetes, Ascomycetes, 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 of 2 µm to 10 µm, some up to 30 µm and only few up to 100 µm. Spores of some mould genera are small and become airborne very easily (e.g., *Aspergillus, Penicillium*) while others are bigger and/or embedded in a slime matrix (e.g., *Stachybotrys, Fusarium*) and less mobile.

The procedure described in this document is based on VDI 4252 Part 2 [1], VDI 4253 Part 2 [2] and is related to the ISO standards on indoor air ISO 16000-16 [3] and ISO 16000-17 [4].

1 Scope

This Technical Specification describes the measurement of moulds in ambient air in order to identify, quantify and characterize bioaerosol pollution in ambient air resulting from emissions from different sources.

The method described specifies the sampling of moulds as part of the suspended particulate matter (SPM, here particles with aerodynamic diameter up to ca. 30 µm) using a filter sampling system with gelatine/poly-carbonate filter combination followed by the culture-based analyses on DG18 agar. The sampling duration can be varied between 10 min to 24 h. The health effect of bioaerosols is not limited to any particle fraction, therefore, this document describes the sampling of moulds as part of the suspended particulate matter as a convention method.

NOTE The sampling method described in this document in principle is likely to be appropriate for the sampling of actinomycetes and other spore-forming bacteria (resistant to desiccation). For these species a special analytical procedure using different culture media should be applied, but this is not within the scope of this document.

The standard method set out in this Technical Specification is accepted by convention as reference method. The measured quantity, here the number of colony forming units per cubic meter (CFU/m^3), is determined by the inlet design of the sampling head, the associated operational parameters and the analytical procedure.

Standardized methods for sampling, detection and enumeration of moulds including standards for sampling strategies are important for comparative assessment of moulds in ambient air. Before doing any measurements a plan for the measurement strategy is necessary (see CEN/TS 16115-2 [5]).

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

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.

EN ISO 8199:2007*, Water quality — General guidance on the enumeration of micro-organisms by culture (ISO 8199:2005)*

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 density 1 g/cm³ 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

[ISO 7708:1995, 2.2 [6]]

3.2
ambient air
outdoor air in the lower troposphere excluding workplace air

[EN 14907:2005, 3.1.1 [7]]

3.3
analytical blank value
value determined by a blank sample covering the analytical procedure to ensure that no significant contamination occurs during the complete analytical procedure including autoclaving, agar preparation, suspension and extraction of the filters, dilution, incubation, counting, etc.

3.4
bioaerosol
airborne particles of biological origin

[EN 13098:2000, 3.3 [8]].

NOTE Bioaerosols in the sense of this document are all aggregations of particles in the atmosphere to which fungi (spores, conidia, fragments of hyphae), bacteria, viruses and/or pollen as well as their cell membrane components and metabolites (e.g. endotoxins, mycotoxins) are attached or that consist of the above mentioned components.

3.5
biological sampling efficiency
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, 3.4 [8]]

NOTE The biological sampling efficiency considers the sampling stress occurring during sampling and analysis in addition to the physical sampling efficiency. It refers to the proportion (in percent) of collected organisms which have not lost the ability to be cultured subsequently. It is strain- and species specific.

3.6
colony count
number of all visible colonies of microorganisms on a culture medium after incubation under the selected conditions

3.7
Colony Forming Unit
CFU
unit by which the culturable number of microorganisms is expressed

[EN 13098:2000, 3.5 [8]].

NOTE 1 One Colony Forming Unit can originate from one single microorganism, an aggregate of many microorganisms or from one or many microorganisms attached to one particle.

NOTE 2 The number of outgrowing colonies depends on cultivation conditions.

3.8
culture-based analyses
cultivation
growing of microorganisms on culture media

[ISO 16000-16:2008, 3.6 [3]]

NOTE The prerequisites for the detection are the abilities to grow and propagate.

3.9
face velocity
air flow rate divided by the face area

NOTE 1 The face velocity is expressed in metres per second.

[Adapted from EN 779:2002, 3.11 [9]]

NOTE 2 In this document, the face velocity is defined as the volume flow rate divided by the effective filter area.

3.10
field blank value
value determined by a blank sample covering the complete measurement procedure including preparation, sampling, transport and analyses to ensure that no significant contamination has occurred during all steps of measurement and to check that the operator can achieve a quantification level adapted to the task

NOTE A field blank sample is a sample taken in an identical manner as the real sample, but without sucking air through the sampling device. 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.

3.11
filtration
sampling of particles suspended in gas or liquid by flow through a porous medium

[EN 13098:2000, 3.11 [8]]

NOTE In this document, filtration is understood as the separation of moulds from a defined volume of air by means of filters.

3.12
indirect method
suspension of deposited microorganisms with subsequent plating of aliquots on a suitable culture medium, incubation and counting of colonies growing under the conditions selected

[Adapted from ISO 16000-17:2008, 3.3 [4]]

3.13
microbial air pollution
concentrations of airborne microorganisms that exceed natural concentrations or differ in type from the naturally occurring mircoorganisms

3.14
microorganism
microbial entity, either cellular or non cellular, that is capable of multiplication or transfer of genetic material, or entities that have lost these properties

[EN 13098:2000, 3.16 [8]]

3.15
mould
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, 3.9 [3]]

3.16
physical sampling efficiency
capacity of the sampling device to collect particles with specific sizes suspended in ambient air

[Adapted from EN 13098:2000, 3.17 [8]]

3.17
PM2,5
fraction of suspended particulate matter which passes through a size-selective inlet with a 50 % cut-off efficiency at 2,5 µm aerodynamic diameter

[EN 14907:2005, 3.1.5 [7]]

NOTE By convention, the size-selective standard inlet design prescribed in EN 14907:2005, 5.1.2, used at the flow rate given in EN 14907:2005, 5.1.5, possesses the required characteristics in order to sample the $PM_{2,5}$ fraction in ambient air.

3.18
PM10
fraction of suspended particulate matter which passes through a size-selective inlet with a 50 % cut-off efficiency at 10 µm aerodynamic diameter

NOTE Definition in analogy to PM2,5; adapted from EN 14907:2005, 3.1.5 [7]]

3.19
Suspended Particulate Matter
SPM
notion of all particles surrounded by air in a given, undisturbed volume of air

[EN 14907:2005, 3.1.6 [7]]

NOTE The bioaerosol sampling head shows a mean cut-off value of 30 µm aerodynamic diameter without a rigid upper separation limit due to its construction design and the specified flow rate, both determining the face velocity at the filter.

3.20
total sampling efficiency
product of the physical sampling efficiency and the biological preservation efficiency

[EN 13098:2000, 3.19 [8]]

4 Symbols and abbreviations

Not applicable.

5 Basic principle of the method

5.1 Sampling

In this document the measurement object are the airborne moulds as part of the suspended particulate matter (SPM, here particles with aerodynamic diameter up to approximately 30 µm).

During filtration, a defined air quantity is sucked through a filter – on or in which separation of the suspended particles occurs (see Annex A). Airborne moulds are collected on gelatine filters resulting in a high total sampling efficiency. Polycarbonate filters are used below the gelatine filters as supporting and protective filters to enhance stability (see Annex B).

Ambient air sampling devices validated for different fractions of particles are commercially available and are widely used for ambient air particle sampling according to national guidelines and European Standards:

— VDI 2463 Part 7 [11] and VDI 2463 Part 8 [12] for suspended particulate matter;

— EN 12341:1998 for PM10 [10];

— EN 14907:2005 for PM2,5 [7].

For sampling of bioaerosols an adapted bioaerosol sampling head, e.g. with larger filter diameters, has been developed resulting in changes of the face velocity at the filter compared with the standards given above.

NOTE 1 The sampling head was modified in respect of the reference method described in the national guideline VDI 2463 Part 8 [12]. The applicability of this adapted sampling head for bioaerosols has been confirmed by comparison measurements in 2001 (see Annex A), whereas the reference sampling head according to VDI 2463 Part 8 was validated in 1987/88 during an international WRAC validation campaign (WRAC = Wide range aerosol classifier) [13; 14].

The reason for the modification of the standardised sampling head for suspended particulate matter was the reduction of the face velocity in order to decrease the sampling stress. Additionally, the modifications enable the use of disposable or sterilizable filter holders ensuring aseptic conditions during the handling of filter and sampling head and avoiding carry-over effects and contaminations.

In a comparison measurement campaign it was shown that the adapted bioaerosol sampling head described in A.1 using a filter with a diameter of 8 cm (effective filter diameter of 7 cm) resulting in a face velocity of approximately 20 cm/s (19,5 cm/s to 23,8 cm/s depending on the flow rate of 2,7 m^3/h to 3,3 m^3/h) gave comparable results to the non-modified standardised sampling head for sampling SPM (here up to 30 µm) with regard to the *physical sampling efficiency*. Additionally a validation trial using this bioaerosol sampling head for the detection of moulds in ambient air under real conditions including sampling and analyses was performed (see A.2) showing comparable *biological sampling efficiency*. The performance characteristics and minimum requirements of the bioaerosol sampling head are given in Clause 8.

In general, any sampling head can be used, that assures a comparable physical sampling efficiency of the SPM fraction SPM (here up to 30 µm) and a comparable biological sampling efficiency with regard to the bioaerosol sampling head described in this document. Additionally, aseptic handling of the filter shall be assured und contaminations shall be avoided.

NOTE 2 If only the inhalable fraction is of interest, PM10 sampling can be performed. In this case fractions of bioaerosols which are not inhalable but can cause irritation by contact e.g. to mucous membranes are missed. When using the filter combination gelatine/polycarbonate filters with $PM_{2,5}$ resp. PM_{10} sampling heads, the physical requirements for particle sampling (cut-off efficiency, flow rate, etc., see EN 14907 [7] resp. EN 12341 [10]) apply.

After sampling the mould spores are cultured and counted according Clause 7.

5.2 Analyses

With the methods described here, mesophilic and thermotolerant moulds are quantified by culture-based analyses of the viable and culturable propagules on selective agar. The quantitative determination of the mould concentration is performed by counting the visually recognisable colonies. The density of the colonies grown on the culture medium shall always allow proper enumeration of the colonies. The density of the colonies results from the number of dilution steps after suspension of the cells from the filter. Therefore, in principal several dilution steps need to be plated out.

6 Sampling

6.1 Sampling equipment

The following components are needed:

6.1.1 Stand, to position the bioaerosol sampling head at the sampling height needed.

6.1.2 Bioaerosol sampling head[1)], to position the filter holder with the inserted filters in a hanging position, if necessary.

[1)] Sampling heads for bioaerosols as well as complete sampling devices are commercially available from several manufacturers, e.g. Leckel, Berlin/Germany; Derenda, Stahnsdorf/Germany; Digitel, Hegnau/Switzerland. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named.

A bent pipe or hose connection can be used to connect the bioaerosol sampling head to the sampling device. If using the bioaerosol sampling head described here, the inner diameter of the pipe or hose shall be 8 mm to 10 mm. The length of the connecting hose should not exceed 1,5 m. If using other sampling heads, these dimensions shall be adapted accordingly in order to ensure the physical requirements (e.g, face velocity, flow rate, see Table 3).

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

6.1.4 Filter, gelatine filter [2)], sterile, pore size 3 µm, and polycarbonate filter [3)], sterile, pore size 0,8 µm (see Annex B).

The physical sampling efficiency of both gelatine and polycarbonate filters shall be > 95 % for moulds resp. particles with an aerodynamic diameter range of > 1 µm, using a flow velocity at the filter of v = 21,7 cm/s ± 10 %. The combination of the two filters ensures a sampling efficiency of 99 % (Annex B).

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

The flow rate has to be adapted in order to achieve a face velocity at the filter of 22 cm/s ± 10 %.

NOTE 1 If a filter with a diameter of 8 cm (effective diameter of 7 cm) is used, this face velocity is achieved by a flow rate of about 3 m^3/h ± 10 %. During the sampling duration the filter resistance may increase therefore is recommended to use a pump with a capacity of approximately 6 m^3/h.

NOTE 2 This method has been validated for a face velocity at the filter of 21,7 cm/s (see Annex A).

6.1.6 Gas volume meter, to determine the gas volume sucked at the bioaerosol sampling head, in operating cubic meters.

Display accuracy of the flow rate: 0,01 m^3/h.

NOTE The use of volumetric measuring systems should take into account the manufacturer's specifications with regard to the prevailing conditions during sampling, e.g. difference pressure between ambient and operating conditions, temperature, humidity.

6.1.7 Timer, for presetting time and duration of sampling.

6.1.8 Protective housing, to protect the sampling device from harmful environmental conditions (optional).

The distance between the upper edge of the protective housing and the lower edge of the bioaerosol sampling head should be at least 40 cm.

6.1.9 Commonly used devices for measuring ambient air conditions and operating conditions, e.g. temperature, humidity, pressure.

For long term measurements a data logger may be necessary.

6.2 Materials

6.2.1 Container, sterile, for filter containment during transport, e.g. Petri dishes.

6.2.2 Container, insulated, for sample transport.

6.2.3 Disposable protective gloves, to avoid contamination and ensure occupational safety.

6.2.4 Disinfectant, e.g. iso-propanol or ethanol (70 %, volume content).

[2)] Gelatine filters are commercially manufactured by Sartorius Stedim Biotech GmbH, Goettingen, Germany, however, they are available from many suppliers. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named.

[3)] Polycarbonate filters are available from many manufacturers.

6.2.5 Tweezers, sterile, to handle the filters.

6.2.6 Thermometer, pressure gauge, hygrometer, to measure ambient air conditions during sampling and transportation.

6.3 Sampling procedure

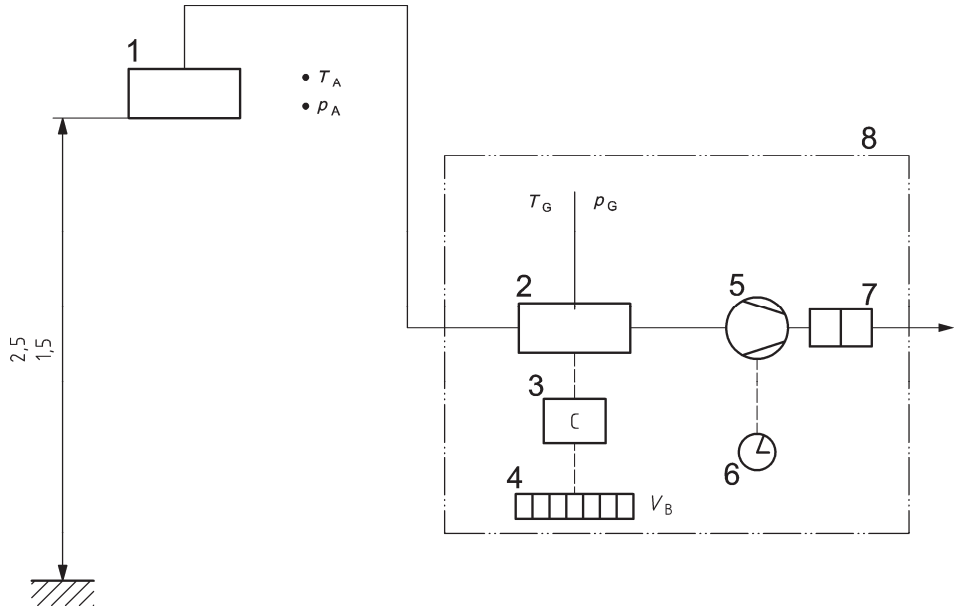
6.3.1 Preparation for sampling

The required number of measurement devices with accessories or other equipment shall be prepared in accordance with the measurement task and the measurement strategy resulting thereof. It is recommended to check the equipment for completeness and functionality using a check list.

The calibration validity of the sampling device shall be verified; otherwise new calibration shall be conducted prior to the beginning of the measurements (see 6.5). The correct function of the sampling equipment shall be documented in the sampling report.

Exclusively sterile filters and sterile filter holders shall be used for the measurements. If factory sterile disposable filters with filter holders are not available, then the sterilized filters shall be inserted in the filter holder at the laboratories safety cabinet and packed in sterility. For this purpose, first the polycarbonate filter and afterwards the gelatine filter shall be placed in the filter holder. Thereby, special attention shall be paid that the filters are tightly inserted into the filter holder. Filter sterility shall be guaranteed up to the moment of sampling. During transport, the filters shall be protected from dust, heat and strong vibrations.

Assemble the sampling device according to Figure 1. A detailed example of a suitable sampling device is given in Annex A.



Key

1 Bioaerosol sampling head
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 volume V_B in operating cubic metres
5 Vacuum pump
6 Timer
7 Filter for abraded material
8 Protective housing

Figure 1 — Schematic set-up of the sampling device

The gas volume meter shall be connected between the pump and the bioaerosol sampling head in order to determine the sampling volume without falsification caused by the leakage flow rate of the pump. The volume of the sampled air is displayed in operating cubic metres and a display accuracy of 0,01 m³. For this purpose the state variables temperature *T* and pressure *p* within the gas volume meter and in the air are being continuously registered, and the sampling volume as measured by the gas volume meters is being converted electronically using Equation (1):

$$V_B = V_G \frac{T_A p_G}{T_G p_A} \quad (1)$$

where

V_B is the sampling volume in operating cubic metres referenced to ambient air conditions, in m^3;

V_G is the sampling volume measured by a gas volume meter, in m^3;

T_A is the ambient air temperature, in K;

T_G is the temperature within the gas volume meter, in K;

p_A is the ambient air pressure, in Pa;

p_G is the air pressure within the gas volume meter, in Pa.

6.3.2 Sampling

The bioaerosol sampling head is normally installed at a height of 1,5 m to 2,5 m with the filter in a hanging position according to Figure 1.

During the complete sampling procedure aseptic techniques shall be ensured as far as possible. The sterile filter holders and sterile filters shall be mounted on the sampling device without any contamination (preferably by using disposable gloves). Prior to placing the filter holders the filters shall be visually inspected for integrity and exact, air-tight fitting of the seat. This check shall be repeated after removal of the filter holder from the sampling device.

The sampling device shall be started in accordance with the manufacturer's operating instructions.

The measurement task and all sampling details shall be recorded in the sampling report (see 6.3.4). In addition all specific characteristics, anomalies and interferences shall be recorded that may be relevant to the measurement result (e.g. odour perception, type and location of possible additional emission sources, performance variations of the emission source(s) subject to the investigation, dust turbulence caused by passing vehicles). A sketch shall depict the surroundings of the measurement location. A sampling report can be found in Annex C as an example.

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 There are self regulating sampling systems which maintain a constant flow rate and thereby avoid these deviations.

Influences that could modify the measurement result shall be avoided. For example, no person is allowed to stand within the range of the flow towards the sampling device.

After sampling, the filters and filter holders shall be removed from the sampling device by using disposable gloves, and the filters shall be checked again for integrity. They shall be sealed in order to avoid any secondary contamination (see 6.3.5).

A minimum of one field blank shall be taken per measurement day. The field blank should be taken preferably in the middle of all measurements performed during one day. A field blank is a sample taken in an identical

manner as the real sample, but without sucking air through the sampling device. For this purpose a sterile filter holder with filter is placed in the bioaerosol sampling head with the pump switched-off, then removed, packed and analytically processed; a prolonged exposure of the filter to the ambient air shall be avoided. The resulting field blank represents the number of CFU entering the sample simply by handling the filter during sampling.

6.3.3 Sampling duration

The sampling duration is determined by the measurement task, and is generally between 10 min and 24 h.

This sampling duration was tested during the national validation campaign (see A.2). In this validation campaign the lower limit of the sampling duration was given by the physical characteristics of the sampling device (volume flow stability). The filter combination has proved successfully under different ambient air conditions, also during rain, and it was shown that the humidity sensitive gelatine filter retained its applicability due to the utilization of the polycarbonate filter as protective filter for 24 h. Longer sampling durations shall be validated.

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.

6.3.4 Sampling report

The samples shall be labelled for unique identification.

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

The report shall at least indicate:

— date and time of sampling;

— name and address of the operator;

— site or facility type, activities and interferences during the sampling;

— measurement task and sampling location (geographic coordinates);

— type of sampling device used;

— sampling volume, flow rate and duration of sampling;

— meteorological parameters as air temperature, air pressure, relative humidity. wind direction, wind velocity, and climatic conditions;

— name of the person taking the sample.

The purpose of the analysis and, if applicable, a list of parameters to analyze are also needed as they may subsequently help the laboratory with the choice of methods. Other details can be necessary (e.g. any observations on phenomena which could affect the concentration of airborne moulds, see 6.3.2).

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

6.3.5 Transport and storage

The samples shall be processed in the laboratory preferably without delay, but not later than 48 h after the end of sampling. The loaded filters shall be protected from disturbing influences (sunshine, humidity or desiccation, heat and dust, etc.) and shall be transported with the sampling side facing upwards in sealed containers (see 6.2). The temperature during transport shall not exceed the incubation temperature (< 25 °C). The conditions during transport (temperature, humidity, duration) shall be recorded.

The samples shall be stored in the laboratory in the dark at a temperature not exceeding the incubation temperature (< 25 °C). The samples shall be protected against adverse influences (humidity, desiccation, contamination). The storage conditions shall be documented.

If necessary, the samples shall be cooled during transport and storage, but care shall be taken not to freeze them.

6.4 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 face velocity at the filter of v = 21,7 cm/s (Annex A and Annex B). The combination of the two filters ensures a sampling efficiency of 99 %

Biological sampling efficiency is mainly influenced by desiccation. The effect of desiccation is not constant, but depends on sampling duration, temperature, relative humidity and other parameters like season as well as on 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 [15].

6.5 Laboratory calibration, function check in the field and maintenance

6.5.1 General

The sampling equipment should be checked and serviced regularly according to the manufacturer's instructions. Particular emphasis should be placed on the calibration and the check of the flow rate. The calibration of the sampling devices is done in the laboratory (see 6.5.2), function check is performed in the field before starting the measurement (see 6.5.3).

6.5.2 Calibration of flow rate in the laboratory and maintenance

On a regular basis (e.g. twice a year) the complete sampling device shall be checked with regard to quality assurance criteria. Hereby the flow rate of the sampling device is controlled against a reference volume measuring device (e.g. mass flow meter, gas volume meter etc., certified by national calibration institute) which is only used for this purpose in the laboratory. Also a general maintenance of the sampling device is to be done during these checks according to the manufacturer's recommendations, this can concern clogged filters of the pump, spare parts and consumables like gaskets, etc.

The volume flow rate of the sampling device against the reference volume measuring device shall be inside a range of ± 5 %. If allowed according to the manufacturer's manual a flow adjustment may be performed. If this is not allowed or not achievable the defective sampling device shall not be used any longer until it has been repaired.

6.5.3 Function check in the field

The usual verification of the flow rate in the field shall be performed before starting the measurement.

A function check prior to the sampling process shall be conducted to assure that the sampling device after transport to the sampling site is working properly. The main tasks are checks of the flow rate and for leaks. In order to check the flow rate a test sampling head with test filters is connected to the sampling device. At the inlet of the sampling head the testing volume measuring device (e.g. mass flow meter, gas volume meter etc.) is connected using an adapter. Then the pump is switched on and the flow rate is observed. The flow rate (calculated or directly readable) at the volume measuring device of the sampling device and the flow rate (calculated or directly readable) at the testing volume measuring device shall be comparable. The tolerance shall be in maximum 5 % and the flow rate shall be within the range of 2,7 m³/h to 3,3 m³/h as given in Table 3.

The data shall be recorded. If no valid data are achieved, the sampling device shall not be used for the measurement and shall be labelled, e.g. "defective".

7 Culture-based analyses

7.1 Quantification equipment

7.1.1 Microbiological laboratory equipment

Usual microbiological laboratory equipment, and in particular:

a) autoclave, capable of operating at (115 ± 3) °C and (121 ± 3) °C;

b) incubator thermostatically controlled at (25 ± 3) °C;

c) incubator thermostatically controlled at (36 ± 2) °C;

d) incubator thermostatically controlled at (45 ± 2) °C;

e) refrigerator thermostatically controlled at (5 ± 3) °C;

f) pH meter with an accuracy of ± 0,1;

g) microbiological safety cabinet Class 2 (laminar flow cabinet);

h) water bath (35 °C to 40 °C) with shaker;

i) test tube shaker (e.g. Vortex® shaker [4)]);

j) test tubes, preferably disposable;

k) orbital shaker (speed: 150 r/min);

l) petri dishes, vented, sterile, diameter approximately 9 cm;

m) sterilized container (e.g. Erlenmeyer flask with minimum bottom diameter of 8 cm).

7.1.2 Culture media and diluents

7.1.2.1 General

All reagents and chemicals shall be of analytical grade. Other grades of chemicals may be used providing they can be shown to lead to the same results. 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. Alternatively DG18 agar plates may also be bought ready for use at specialised manufacturers or may be prepared by the laboratory according to Table 1. It shall be assured, that the composition of commercially available DG18 agar plates is identical to the requirements of Table 1.

[4)] Vortex® shaker is an example of a suitable product available commercially. This is a product that is identified by a trade market name. It has many manufacturers. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named.

7.1.2.2 Dichloran 18 % glycerol agar (DG18 agar)

Table 1 — Formulation of the DG18 agar (pH = 5,6 ± 0,2) [16]

Peptone [5)]	5,0 g
Glucose	10,0 g
Potassium dihydrogen phosphate (KH_2PO_4)	1,0 g
Magnesium sulphate ($MgSO_4 \cdot 7\ H_2O$)	0,5 g
Dichlorane (2,6-dichlor-4-nitroanilin) 0,2 % in ethanol (content by volume)	1,0 ml
Chloramphenicol	0,1 g
Glycerol	220 g [a)]
Agar	15,0 g
Distilled water or water of comparable quality	1 000 ml

[a)] 18 mass percent of approximately 1 220 g final mass = approximately 220 g.

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

Add ingredients, except glycerol, and agar as specified in Table 1 in ca. 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.

The pH of the agar is 5,6 ± 0,2 (at 25 °C), which is ensured by the given buffer composition in Table 1.

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 moulds from growing on this agar.

7.1.2.3 Saline Solution with Polysorbate® 80

For suspension of the filters and for the dilution series a saline solution (0,85 % w/v NaCl) with 0,01 % v/v Polysorbate® 80 [6)] is used. 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 [6)]. Due to the viscosity of the Polysorbate® 80 [6)] it is recommended to use a positive-displacement pipette or to weigh the required amount using a calibrated and appropriate balance.

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5) 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 using microbiological reference strains or natural samples for comparison of recovery and of morphological appearance of the colonies are, therefore, important.

6) Polysorbate® 80 is equivalent to Polyoxyethylenesorbitan monooleate or Polyethylene glycol sorbitan monooleate. Polysorbate® 80 is an example of a suitable product available commercially. This chemical is a product that is identified by a trade market name. It is unique and has a sole manufacturer, however, it is available from many suppliers. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named.

7.2 Processing of filters

7.2.1 General

Process the samples in the laboratory preferably without delay, but not later than 48 h after the end of sampling.

Airborne moulds deposited on filters are processed using the indirect plating method. The filters obtained from sampling by filtration are re-suspended in saline solution (0,85 % NaCl) with 0,01 % Polysorbate® 80 [6)] (see 7.1.2.3). Decimal dilutions of the suspension are prepared and aliquots are spread on DG18 agar. 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 by colony morphology and counted. The extent of identification depends on the objective of the investigation. The identification requires appropriately trained personnel (see also 7.4).

NOTE Aggregates should be separated and distributed equally by suspension and dilution as far as possible. This cannot be ensured completely, therefore the measured concentrations may differ from the expected dilution factor.

Carry out all procedures under conditions that prevent the samples from any contamination, whenever possible use a laminar flow cabinet. Aseptic conditions shall be checked regularly by controls and the results shall be documented. 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.

7.2.2 Suspension

In the aseptic atmosphere of a laminar flow cabinet transfer the filter (gelatine and polycarbonate filter) into a sterilized container (e.g. Erlenmeyer flask minimum bottom diameter of 8 cm) containing approximately 10 ml of saline solution with Polysorbate® 80 [7)] (see 7.1.2.3) as suspension volume using a sterile pair of tweezers. The container shall be covered e.g. using sterilized aluminium foil or caps. The suspension volume shall be documented for the further calculation (see 7.5).

Shake the filters intensively (e.g. orbital shaker, speed approximately 150 r/min) in a horizontal position in this solution at 35 °C to 40 °C 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.

Further processing of the sample (see 7.2.3) shall take place within one hour after suspension.

7.2.3 Dilution

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

Immediately prior to dilution, shake the suspension for approximately 1 min on a test tube shaker (see 7.1.1). Transfer 1 ml of the suspension to 9 ml saline solution with Polysorbate® 80 [7)] (see 7.1.2.3) using a sterile disposable pipette or a sterilized cotton-stuffed glass pipette. In the same way, carry out two further dilution steps, e.g. 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.

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7) Polysorbate® 80 is equivalent to Polyoxyethylenesorbitan monooleate or Polyethylene glycol sorbitan monooleate. Polysorbate® 80 is an example of a suitable product available commercially. This chemical is a product that is identified by a trade market name. It is unique and has a sole manufacturer, however, it is available from many suppliers. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named.

7.2.4 Plating

Spreadplate 0,1 ml of the original suspension and the dilutions (see 7.2.3) in parallel on DG18 agar (see 7.1.2.2). Use at least two parallel plates for each dilution and incubation temperature (see 7.3).

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

Determine an analytical blank sample using the agar plate and the saline solution every day when performing the plating. The field blanks (see 6.3.2) have to be determined for the original suspension as well as for all dilution steps (details see 7.5.1).

7.2.5 Membrane filtration

In cases where low mould concentrations are expected (less than ten colonies per 1 ml of the original suspension) the original suspension may be concentrated by membrane filtration (details see Annex D extracted from EN ISO 8199:2007, 8.2.5 [17]). For this purpose, a cellulose nitrate/cellulose ester filter (pore size of 0,45 µm) is placed onto the sterile frit of the vacuum filtration unit with a tweezers and the sterile funnel is applied. The original suspension is again shaken for approximately 10 s and the aliquot of 10 ml is transferred with a sterile pipette (use tips with cotton plug) onto the filtration unit. Finally, the liquid is sucked into a flask by a vacuum pump.

To avoid that additional microorganisms are sucked onto the filter the membrane filtration should be performed using a laminar flow cabinet.

The filter is removed from the vacuum filtration unit using sterile tweezers and transferred to the agar plate so that the loaded obverse of the filter is upside.

7.3 Incubation

Incubate agar plates lid facing upwards in incubators at (25 ± 3) °C for seven days. For special purposes plates can be incubated at (36 ± 2) °C (e.g. thermotolerant *Aspergillus* spp.) or (45 ± 2) °C (*Aspergillus fumigatus*). DG18 agar plates may require prolonged incubation times – ten days – especially if identification of moulds to species level is intended (for identification see ISO 16000-17 [4].).

Incubate agar plates in such a way that sufficient oxygen will allow optimal mould growth, e.g. incubation in tight polyethylene bags should be avoided. Incubate agar plates free from vibrations caused by the incubator, since otherwise growth of secondary colonies by spreading of spores may occur. Also avoid extensive airflows in view of desiccation of the agar plates.

7.4 Examination and counting

7.4.1 General

DG18 agar is suitable for the detection of a wide spectrum of xerophilic (i.e. preferring dryness) moulds. However, in some cases, DG18 agar also enables the growth of actinomycetes and other bacteria as well as yeasts. As the measurement objects are airborne moulds, it is necessary for the appropriately trained laboratory staff to be able to differentiate between the visual appearance of mould and non-mould colonies based on colony morphology.

Identification to genus or species level is possible, it shall be carried out exclusively by laboratories with mycological expertise and experience in the identification of microfungi or shall be checked by these, in order to guarantee the reliability of the examination results. Mycological expertise and experience should be tested by proficiency tests for the identification of moulds. For further details and reference lists see also ISO 16000-17 [4].

7.4.2 Procedure

Examine agar plates for the first time after two to three days and subsequently in regular intervals (each day if possible) for up to at least seven days. Thermotolerant moulds (36 °C or 45 °C) should be counted after one day up to three 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. Avoid counting secondary colonies.

The optimal range for quantification using a standard culture plate with a diameter of ca. 9 cm is between 20 and 40 colonies. For quantitative results, at least ten colonies shall be on the agar plate and a maximum number of approximately 100 colonies in total should not be exceeded. Some moulds may spread very fast inhibiting the growth of other colonies (e.g. *Rhizopus*, *Chrysonilia*, *Mucor*, *Botrytis*) – even on plates containing Dichloran – and the capacity of the plate can run down even at lower colony counts.

Record the maximum number of colonies counted within the seven days of incubation for each dilution step. Also any growth of non-moulds shall be recorded.

7.5 Calculation and expression of results

7.5.1 General

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). Usually only agar plates from one dilution step can be evaluated if 1:10 dilution steps are prepared.

Sometimes the evaluation can be based on two dilution steps (especially, if other dilution steps are used, e.g. 1:2). In this case the weighted mean shall be calculated according to EN ISO 8199:2007, 8.4.2 [17] (an extract is given in Annex E).

7.5.2 Calculation for single dilution step

The concentration in the original suspension is calculated from the colony counts using the following Equation (2):

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

where

C_S is the concentration in the original suspension, in CFU/ml;

n_{CFU} is the total number of colonies on the plates of the dilution step used, in CFU;

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

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

where

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

V_t is the test volume, in ml, 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 CFU

$V_S = 2 \times 0{,}1 \times 0{,}1 = 0{,}02$ ml

and

$$c_S = \frac{59}{0{,}02} = 2\,950 \text{ CFU/ml}$$

From the concentration in the original suspension the concentration in the air sample can be calculated using the following equation:

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

where

C_F is the concentration in the air sample in CFU/m^3;

C_S is the concentration, in CFU/ml, in the original suspension;

V_F is the volume of saline for re-suspension of the filter, in ml;

V_l is the volume, in m^3, of the air sample.

EXAMPLE 2 The volume of the air sample was 0,8 m³. The suspension volume of the filter was 10 ml.

Then:

$$C_F = 2\,950 \frac{10}{0{,}8} = 36876 \text{ CFU} / \text{m}^3$$

Result: $3{,}7 \times 10^4$ CFU/m^3

7.5.3 Quantification limit

Table 2 summarizes the quantification limits depending on sampling volume. Usually, an aliquot of 0,1 ml of the original suspension is analysed. For detection of lower concentrations 1 ml of the original suspension can be plated out on four Petri dishes (250 µl each), the values of CFU per plate can be added up and taken as one result. Furthermore, by membrane filtration of an aliquot of 10 ml of the original suspension the quantification limit can be improved by a factor of 10 (see Annex D).The quantification limits refer to the enumeration of a minimum of 10 CFU/plate and depend on the aliquot volume used (see above).

Table 2 — Theoretical quantification limits depending on sampling duration and sampling volume assuming 10 CFU/plate

Sampling duration min	**Sampling volume** in m^3 at a flow rate of e.g. 50 l/min	**Quantification limit** in CFU/m^3 when plating 0,1 ml aliquot	**Quantification limit** in CFU/m^3 when plating 1 ml aliquot	**Quantification limit** in CFU/m^3 when membrane filtering 10 ml aliquot
10	0,5	2 000	200	20
60	3,0	333	33	3,3
360	18,0	56	6	0,6
720	36,0	28	3	0,3
1 440	72,0	14	1,4	0,14

7.5.4 Field blanks and analytical blanks

The colony count of the field blanks is recorded for quality control and shall ensure the aseptic conditions during sampling and analyses. Usually no colonies are found on the field blanks. Colony counts – on an agar plate after spreading the undiluted filter suspension – exceeding two colonies indicate sampling errors and the results of the measurement should be interpreted with caution. No correction shall be made to the measurement results of the samples on basis of the results of the field blanks.

An analytical blank is a quality control measure used to check that aseptic conditions are maintained in the laboratory. There should normally be zero colonies found on an analytical blank sample. Rarely an isolated colony may be found on an analytical blank sample. A sporadic and minor event such as this should be noted and laboratory staff should pay extra attention to aseptic technique. The observation of one or two colonies on analytical blank samples on successive days may indicate the development of a persistent low level contamination problem that should be recorded, brought to the attention of the laboratory manager and investigated. The presence of more than two colonies on an analytical blank sample suggests a more serious failure in the aseptic practices in the laboratory. This situation requires an investigation, remedial action and further tests to demonstrate that the problem has been rectified. Any sampling results generated on an occasion where the analytical blank has failed (i.e. > two colonies) should be clearly marked on the laboratory documentation system as such and appropriate re-sampling is advised.

8 Performance characteristics and minimum requirements

Table 3 — Compilation of the performance characteristics and minimum requirements

Sampling duration	10 min to 24 h
Particle fraction	airborne moulds as part of SPM with aerodynamic diameters up to 30 µm (see also Figure B.2)
Standard deviation of the overall process	up to 100 % (details see Table A.2)
Face velocity	Validated range: 19,5 cm/s to 23,8 cm/s For example, in order to assure these face velocities a flow rate of 2,7 m^3/h to 3,3 m^3/h is needed when using a bioaerosol sampling head with a effective filter diameter of 7 cm.
Filter	Filter combination of Gelatine + Polycarbonate
Filter efficiency of the filter combination	> 99 % for an aerodynamic diameter range of > 1 µm with a value of face velocity at the filter fixed at 22 cm/s ± 10 %".
Slope for comparison measurements in terms of SPM mass concentration (conditions for filter weighting described in EN 12341 [10])	1,0 ± 0,1 (example see Figure A.3) By convention the regression line is calculated without offset for bioaerosol sampling heads. Calculation examples with zero offset regression lines are given in ISO 20988 [18]
Minimum number of comparison measurements	n ≥ 40
Correlation coefficient	*R* > 0,90 (example see Figure A.3)
Culture media	DG18 agar according to Table 1
Suspension and dilution	Saline solution with Polysorbate®80[8)] according to 7.1.2.3
Incubation temperatures	(25 ± 3) °C for moulds In special cases (36 ± 2) °C for thermotolerant *Aspergillus* spp. or (45 ± 2) °C for *Aspergillus fumigatus* is possible
Detection limit	1 colony on the plate
Quantification limit	10 colonies on the plate (details see Table 2)
Field blank value	0 – 2 colonies
Analytical blank value	0 – 2 colonies

8) Polysorbate®80 is an example of a suitable product available commercially. This chemical is a product that is identified by a trade market name. It is unique and has a sole manufacturer, however, it is available from many suppliers. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named.

The physical sampling efficiency of bioaerosol sampling heads shall be tested by comparison measurements against the reference sampling head for SPM described in the guideline VDI 2463 Part 8 [12]. Up to now no international published performance characteristics are specified for equivalence test for SPM. Therefore the minimum requirements for the physical sampling efficiency given in Table 3 for bioaerosol sampling devices are specified by convention.

9 Quality assurance

Ambient air samples are taken and separated on filters for the purpose of mould measurements. For the accuracy of the quantitative concentrations subject to determination it is necessary to ensure the exact operating mode of the sampling device (see 6.5). Besides, special attention shall be given to the pump and the bioaerosol sampling head equipped with filters. The laboratory shall implement quality assurance measures for this testing to be documented and made available any time (refer to e.g., EN ISO/IEC 17025 [22]).

The overall method shall be regularly checked by determining field blanks and analytical blanks (see also 7.5.4).

10 Trouble shooting during sampling and analyses

During sampling and analyses several problems can arise. Table 4 gives some advice on a problem solving approach. Figure 2 gives an overview of the complete measurement procedure including all relevant steps as well as the related field blank and analytical blank.

Table 4 — Possible problems during sampling and analyses and possible solutions

Problem	Possible causes	Solutions
Analytical blanks exceed minimum requirement of 0 – 2 colonies	Contamination during analytical process	Review analytical techniques and identify potential source of contamination
	Materials: culture media, solutions for suspension and dilution, disposable plastic ware, etc.	Prepare fresh media and solutions, use sterilised plastic ware, ensure materials sterility, etc.
	Equipment: Autoclave performance and calibration, incubator, flow cabinet, etc.	Check autoclave records and enhance performance, perform decontamination of laminar flow cabinet, etc.
	Operator: Failure to keep sample free from contamination during processing	Review handling procedures, train the personnel
Field blanks exceed minimum requirement of 0 – 2 colonies	Contamination during sampling	Review sampling techniques and identify potential source of contamination
	Contaminated bioaerosol sampling head	Sterilise bioaerosol sampling head, assure aseptic transportation and handling
	Non-aseptic handling of the filter	Single use of disposable gloves, reduce exposure of the filter, assure aseptic transportation and handling
	Contamination during analytical process (see above)	
(Inconsistent) high mould concentrations	Filter overloaded due to high moulds concentration	Perform further dilution steps, decrease sampling time (see Table 2)
	Sampling duration is too long	Perform further dilution steps, decrease sampling time (see Table 2)
	Inappropriate sampling location	Identify possible additional emission sources, optimise sampling strategy

Table 4 (*continued*)

Problem	Possible causes	Solutions
	Inappropriate handling and calculation	Review the complete sampling and analytical procedure (e.g. assure homogeneous vortexing), double-checking of calculations
Lower CFU values (than expected)	Failure or breakdown of the sampling device	Review functioncheck, check general integrity of the sampling device
	Filter desiccation	Reduce sampling duration, identify environmental parameters that could explain it (see 6.4) Review transportation and storage procedures
	Inappropriate environmental conditions during transportation and/or storage	Review transportation and storage procedures
	Poor recovery from filters	Review analytical procedure, train staff (see 7.2 to 7.4)
	Operator mistake (e.g. wrong dilution factor, error in media preparation)	Review analytical procedure, train staff (see 7.2 to 7.4)
	Inappropriate incubation temperature or duration, incubator failure	Review analytical procedure, train staff (see 7.2 to 7.4)
Unstable flow rate during sampling	Defective pumping unit	Check pumping unit
	Defective electronic operation unit	Perform function check
	Leaks	Check hardware integrity
	Filter failure	Visual inspection of the filter unit and exchange, if necessary
	Filter blocked	Identify possible additional emission sources that could lead to filter overloading Reduce sampling duration Check freezing of filter

All issues shall be documented according to the implemented quality assurance measures. The origin of problem shall be investigated and sorted out. If required, new operational procedure shall be implemented in order to avoid re-occurrence of the problem(s).

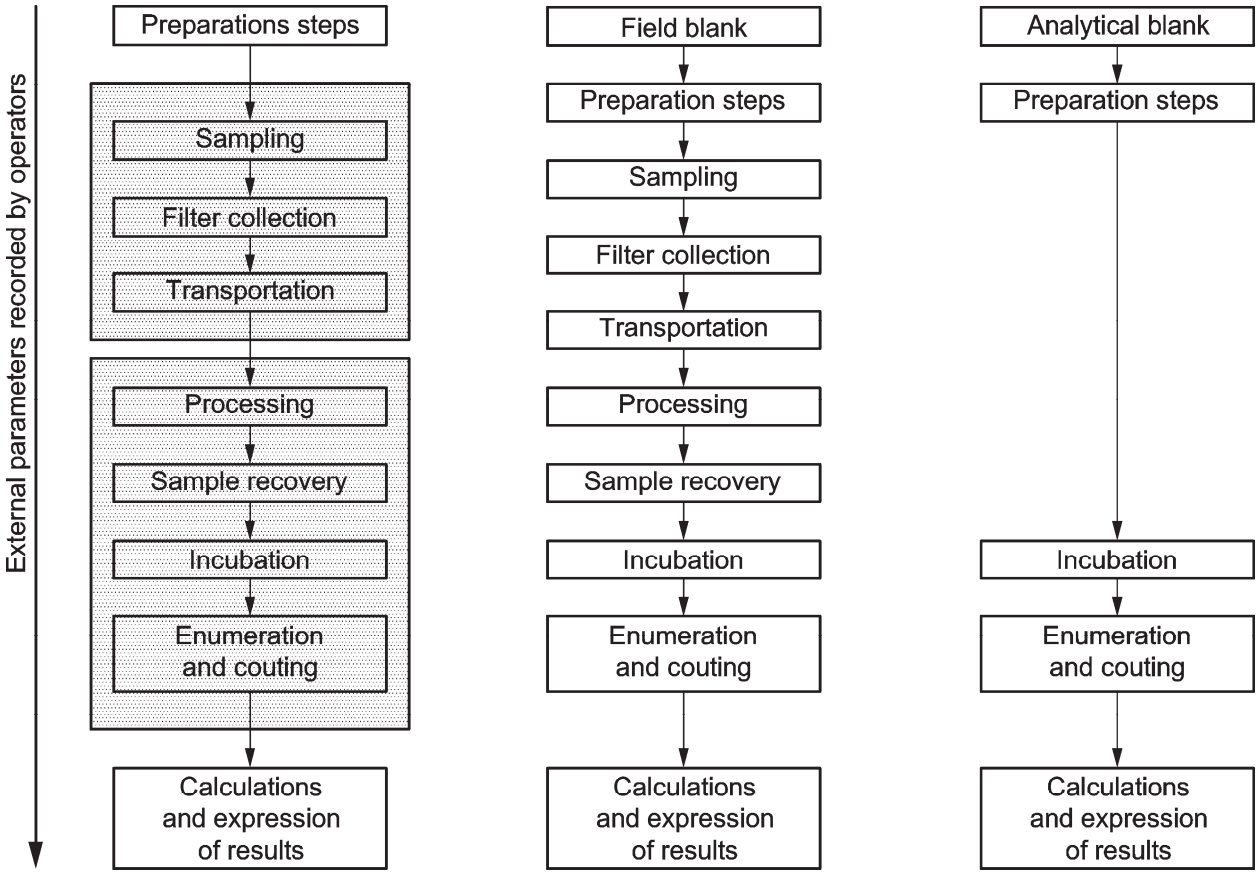


Figure 2 — Schematic description of the sampling and analytical procedure and the corresponding blank values

Annex A
(informative)

Example for a validated sampling device

A.1 Technical description of the validated sampling device

A detailed description of a suitable bioaerosol sampling head and a suitable filter holder which can be used for indoor and outdoor measurements is given in Figure A.1 and Figure A.2.

The physical parameters of the sampling system are summarized in Table A.1.

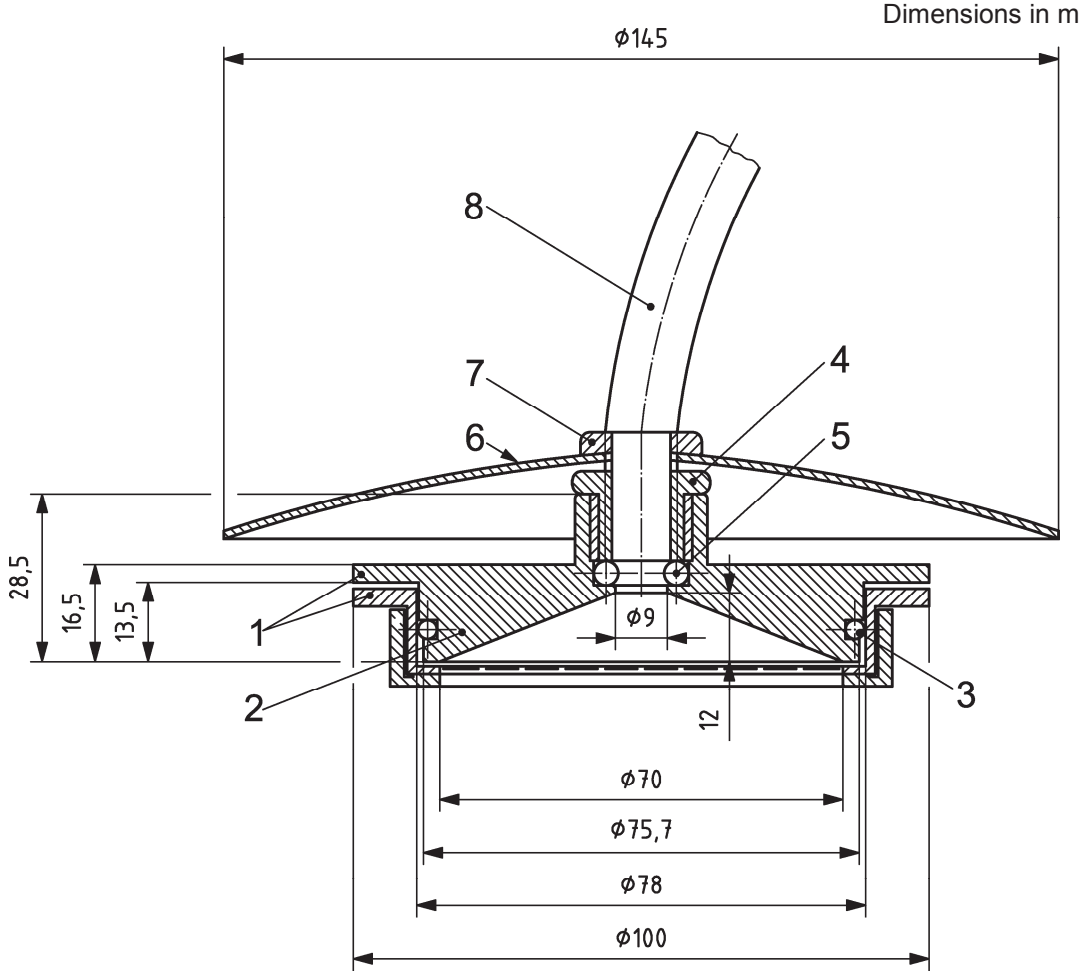
This equipment was used for the sample exchange for method validation with ambient air (see A.2).

Other sampling systems may be used, if they meet the requirements (see Clause 8) or lead to the same results. If other dimensions for the sampling head are used, comparison measurements of the candidate sampling head against the reference sampling head for SPM described in guideline VDI 2463 Part 8 [12] have to be performed. The results of the candidate sampling device have to meet the requirements specified in Table 3.

Table A.1 — Physical parameters

Parameter	Value
Flow rate	3 m³/h ± 10 %
Admissible variation of the flow rate during sampling	≤ 10 %
Display accuracy of the flow rate	0,01 m^3/h
Filter diameter	80 mm
Effective filter diameter	70 mm
Face velocity at filter	21,7 cm/s ± 10 %
Sampling duration	10 min to 24 h

Dimensions in millimetres



Key

1	Disposable filter holder	5	Seal
2	Adapter	6	Protection cover
3	Seal (silicone)	7	Counter nut
4	Threaded female coupling	8	Exhaust pipe

Figure A.1 — Bioaerosol sampling head with filter holder

The bioaerosol sampling head is suitable to insert disposable filter holders or sterilizable 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 bioaerosol sampling head with a sealing ring. The female coupling is sealed against the thread of the pipe adapter with PTFE tape, for example.

Dimensions in millimetres

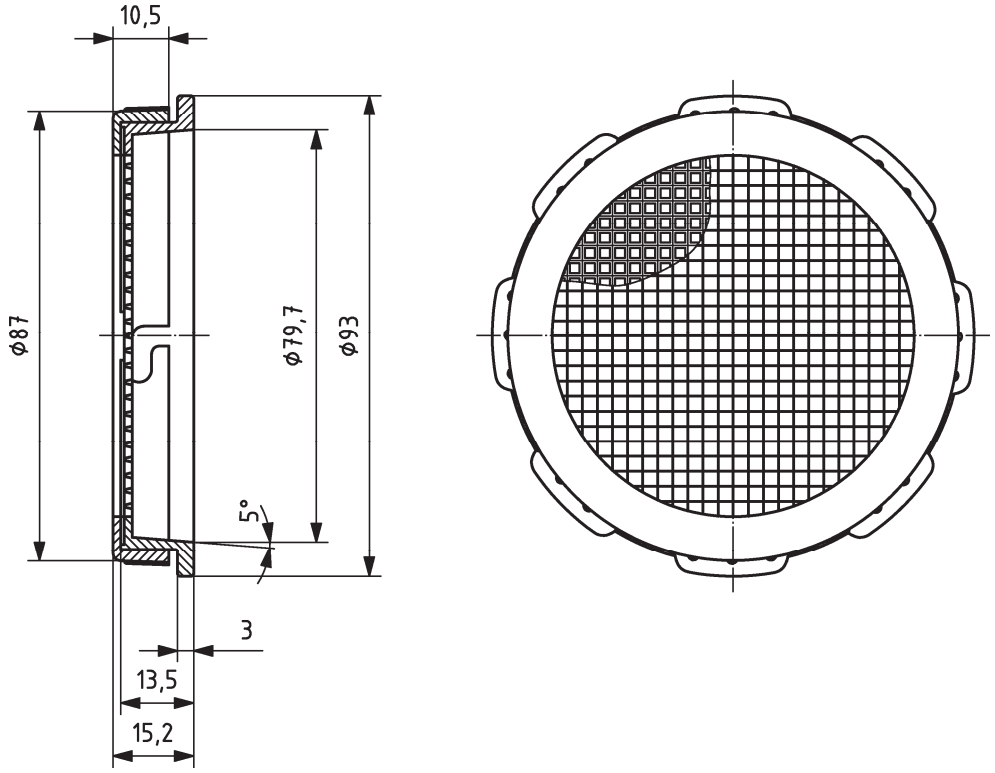
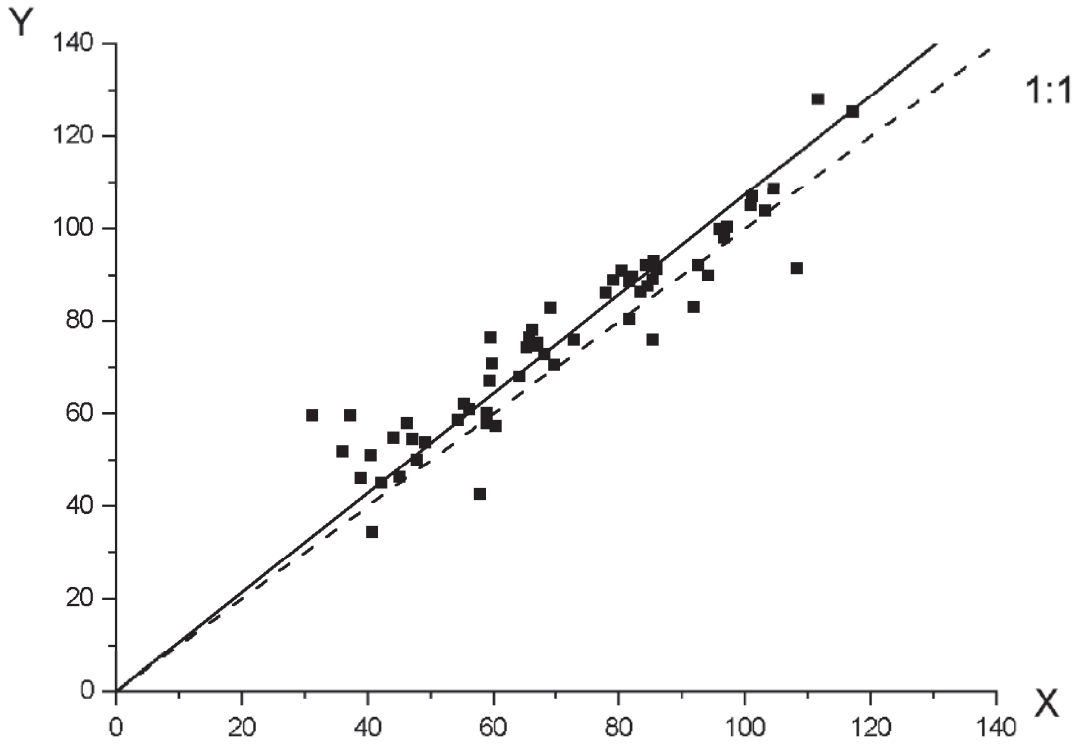


Figure A.2 — Disposable filter holder

In a national comparison measurement campaign it was shown, that this adapted bioaerosol sampling head gave comparable results to the non-modified sampling head, SPM sampling head according to VDI 2464 Part 8 (see Figure A.3). The dashed line corresponds to a 1:1 correlation.



Key

X Particle mass concentration using SPM sampling head (VDI 2463 Part 8 [12])
Y Particle mass concentration using bioaerosol sampling head (VDI 4252 Part 2 [1])

n Number of measurements
R Correlation coefficient
AM Arithmetical average value
S Standard deviation

n = 60
y = 1,07 x in micrograms per cubic metre
R^2 = 0,87
R = 0,933
$AM_{SPM\ head}$ = 71 µg/m^3
$AM_{\ bioaerosol\ head}$ = 76 µg/m^3
$AM_{bioaerosol\ head}$ /$AM_{SPM\ head}$ = 1,1
$S_{SPM\ head}$ = 22 µg/m^3
$S_{bioaerosol\ head}$ = 21 µg/m^3
$S_{bioaerosol\ head}$ / $S_{\ SPM\ head}$ = 0,95

NOTE In order to demonstrate clearly the correlation between the two methods, the regression line has been calculated without offset. Calculation examples for zero offset regression lines are given in ISO 20988 [18].

Figure A.3 — Results of comparison measurements [1] between the bioaerosol sampling head VDI 4252 Part 2 and SPM sampling head VDI 2463 Part 8

A.2 Quality assurance measures and uncertainty trials for method validation

The suitability of the method was tested under real conditions (a filter combination of gelatine/polycarbonate filter for mould sampling (see Annex B)), and the deviations of the overall method (sampling, analyses, non-homogeneity of the measurement object) were determined for the purpose of validation [15]. The studies were conducted under the following five preconditions:

— ambient air measurement on a backyard terrace in urban environment (residents street, dead end), by one laboratory;

— ambient air measurement on a backyard terrace in urban environment (residents 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 participating six laboratories in order to avoid the influence of systematic error.

The 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. The maximum sampling duration was 24 h. The filter combination has proved successfully under these ambient air conditions (especially the humidity sensitive gelatine filter retained its applicability due to the utilization of the polycarbonate filter as protective filter).

A blank was always taken prior to sampling. These blank samples were always unloaded. Sample shipment was by the post. Sample processing and the subsequent quantitative detection of moulds were performed according to VDI 4253 Part 2.

The sample exchange results are compiled in Table A.2 [9)].

Table A.2 — Results [10)] of the sample exchange of gelatine/polycarbonate-filters subsequent to ambient air measurements at different locations

Measurement period November 2002	Sampling in a city / analyzed by one laboratory			Sampling in a city / analyzed by several laboratories			Sampling near a composting plant / analyzed by several laboratories			Sampling near a recycling plant / analyzed by several laboratories		
	CFU/m^3											
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	2 000	1 000	10 000	400	800
2	90	80	10	200	200	200	600	3 000	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					

9) The relative standard deviation in Table A.2 contains here the contributions of measurement uncertainty from sampling and analyses, as well as from the non-homogeneity of the measurement object.

10) Each value corresponds to the result of an ambient air sample per day of the respective laboratory.

Table A.2 (*continued*)

Measurement period November 2002	**Sampling in a city / analyzed by one laboratory**			**Sampling in a city / analyzed by several laboratories**			**Sampling near a composting plant / analyzed by several laboratories**			**Sampling near a recycling plant / analyzed by several laboratories**		
	CFU/m^3											
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**
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
Relative standard deviation in %	27	49	22	101	97	55	63	37	33	18	49	15

Annex B
(informative)

Recovery of spores on gelatine filters in combination with polycarbonate filters

Air-borne bioaerosols are enriched on the filter by filtered separation. The mechanisms of filtration (diffusion, impacting, interception and sedimentation) determine the attainable physical sampling efficiency (see [19]).

Comparative experiments for culture-based analyses of moulds subsequent to separation of mould spores over different filter materials were conducted. Spores were re-suspended from the filters and cultured on DG18 medium. Gelatine filters were characterized by a high recovery (see also Table B.1), which resulted in a limited deviation of the measurement results and a better detection limit (see [20]).

A disadvantage of the gelatine filters is their reduced stability against humidity by means of dissolving: the result is a 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 consist in its universal applications ability, as well is in its insensitivity towards humidity and temperature.

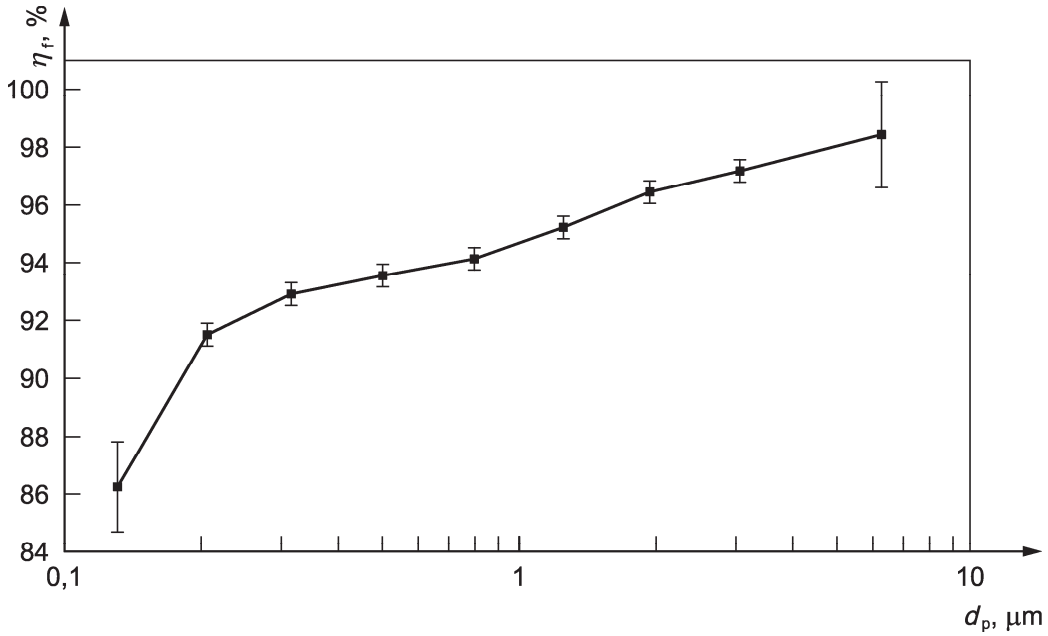
For ambient air measurements it is, therefore, necessary to use a filter combination of gelatine filter with underlying (downstream) polycarbonate filter. The polycarbonate filter thus functions as a supporting and protective filter for the gelatine filter, which has a very high sensitivity for humidity.

The use of the filter combination described has proven its reliability and has enabled application for more than 24 h, also under high humidity or 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 [15; 21]).

Sampling efficiency of both gelatine and polycarbonate filters is in excess of 95 % for moulds with an aerodynamic diameter range of > 1 µm (see Figure B.1 and Figure B.2). The efficiency of the filter combination is > 99 %.

Table B.1 — Recovery of mould spores on different filters [15]

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

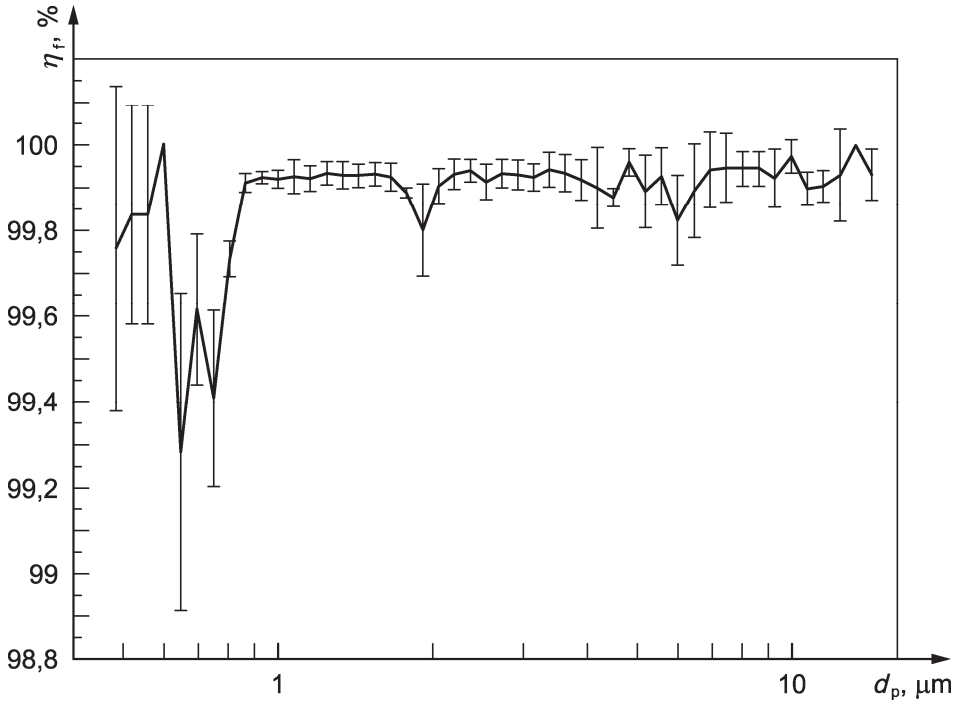


Key

d_P Particle diameter

η_f Filter efficiency

Figure B.1 — Characteristic filter curves of a gelatine filter (pore size 3 µm, diameter 8 cm) at a flow rate of 3 m³/h. The obtained values are valid for face velocity at the filter v = 21,7 cm/s [1].



Key

d_P Particle diameter

η_f Filter efficiency

Figure B.2 — Characteristic filter curves of a polycarbonate filter (protective filter, pore size 0,8 µm, diameter 8 cm) at a flow rate of 3 m³/h. The obtained values are valid for face velocity at the filter v = 21,7 cm/s [1].

Annex C
(informative)

Examples for sampling and analyses reports

C.1 Sampling report

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

Filter No.	Measure-ment start Time	Sampling volume m³	Sampling duration min	Distance to source m	Tempe-rature °C	Relative humidity %	Air pressure hPa	Wind direction	Wind velocity m/s	Climatic conditions

The geographic conditions shall be documented in a sketch or on a topographic map.

Remarks:

Name of laboratory technician: Date and signature:

Description of activities and interference during ambient air measurement

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

Filter No.	Activities and interference during measurement (potential secondary emission sources, odour perceptions, drifts of resuspended particles)

Remarks:

Name of laboratory technician: Date and signature:

C.2 Analytical report

Sample names/date of sample receipt/conditions for storage

Sample names/Lab. No.:	
Air volume sampled	
Date of sampling:	
or date of sample receipt:	Date/Time Date/Time
Condition of the sample after transport	
Storage temperature of filter sample	°C

Processing of samples

Start of processing:	Date/Time

Specifications of culture medium:

Manufacturer and type:	
Commercial agar plates:	☐ (tick box)
Self made agar plates:	☐
Start of incubation:	Date/Time
Incubation temperatures:	°C

Data recording sheet

Sample name/Lab. No.:

Incubation temperature: °C

Start of counting: Date

End of counting: Date

Dilution	Plate No.	Number of colonies on the day of counting					Remarks
		1	2	3			
$A_{1m\ell}$ (10^{-0})	1						
$A_{10m\ell}$ (10^{-0})	1						
A (10^{-0})	1						
	2						
B (10^{-1})	1						
	2						
C (10^{-2})	1						
	2						
D (10^{-3})	1						
	2						
E (10^{-4})	1						
	2						
F (10^{-5})	1						
	2						
G (10^{-6})	1						
	2						
H (10^{-7})	1						
	2						

The counting has been carried out by: .....

Remarks:

Protocol

Data recording sheet No./ sample name/ Lab. No.	**Dilution or plate,** e.g. A 1, 2 or $A_{1m\ell}$	**Colony counts**	**Day of counting (Date)**	**Mean value of colony counts**	**Volume of air sampled** m^3	**Result** CFU/m^3

The calculation was carried out by: ……

Annex D
(informative)

Membrane filtration technique

D.1 General

This annex has been taken from EN ISO 8199:2007, 8.2.5, designed for water analyses. The requirements may be adapted accordingly to the ambient air samples.

D.2 Filtration

Connect the sterile filtration device to a source of vacuum. Place a sterile membrane filter (mean pore size of 0,45 µm), grid-side upwards, on the porous disc of the filter base, with only the outer part of the membrane filter being grasped by flat-ended sterile forceps. Position the sterile funnel securely on the filter base. Pipette or pour one of the following into the funnel (with the vacuum stopcock turned off):

a) a known volume of the sample, or dilution of it, carefully mixed (at least 10 ml);

b) the contents of a flask or bottle containing the test portion and sufficient diluent to bring the total volume to at least 10 ml;

c) at least 10 ml of diluent, to which the test portion, measured with a pipette, is added directly and mixed with the pipette.

Open the stopcock and apply sufficient vacuum (about 70 kPa) to filter the suspension through the membrane. Close the stopcock as soon as the sample has been filtered. It may be advisable to rinse the funnel by filtering one to three 10 ml to 30 ml portions of sterile diluent while the filter is still in place.

D.3 Transfer of membrane

Remove the funnel (make sure the stopcock is closed before doing this) and transfer the membrane on an agar medium in a Petri dish with sterile forceps, ensuring that no air bubbles are trapped between the membrane and the medium.

To filter another sample, either use a separate sterile device or disinfect the funnel, for example by flaming. Alternatively, follow the manufacturer's instructions for disinfection.

Annex E
(informative)

Calculation by weighted mean

This annex has been taken from EN ISO 8199:2007, 8.4.2, designed for water analyses. The requirements may be adapted accordingly to the ambient air samples.

The weighted mean is calculated according to Equation (E.1):

$$C_S = \frac{Z}{V_{tot}} \times V_S \quad \text{(E.1)}$$

where

C_S is the estimate of the number of CFU in the reference volume V_S of the sample;

Z is the sum of colonies counted on plates or on membranes derived from dilutions d_1, d_2, ..., d_i, or derived from separate volumes of the test portion (sample or dilution);

V_S is the reference $_{volume}$ chosen to express the concentration of the micro-organisms in the sample;

V_{tot} is the calculated total volume of original sample included in the plates enumerated. V_{tot} is either the sum of the separate volumes from the test portion (sample or dilution) or calculated by Equation (E.2):

$$V_{tot} = (n_1 V_1 d_1) + (n_2 V_2 d_2) + ... + (n_i V_i d_i) \quad \text{(E.2)}$$

where

V_{tot} is the calculated total volume of original sample included in the plates enumerated;

n_1, n_2, ..., n_i is the number of plates counted for dilution d_1, d_2, ..., d_i;

V_1, V_2, ..., V_i is the test volume used with dilution d_1, d_2, ..., d_i;

d_1, d_2, ..., d_i is the dilution used for the test volume V_1, V_2, ..., V_i ($d = 1$ for an undiluted sample, $d = 0{,}1$ for a ten-fold dilution, etc.).

NOTE The final result thus obtained is a function of the weighted average of the counts from each plate.

Unless otherwise stated, round off the calculated results to two significant figures, when reporting final results. In order to do this, if the third figure is less than 5 do not modify the preceding figure; if the third figure is greater than or equal to 5, increase the preceding figure by one unit.

Express the result as a number preferably between 1,0 and 9,9 multiplied by the appropriate power of 10, or a whole number with two significant figures.

An example of the calculation for the weighted mean for a dilution of 1:2 (enumerated in duplicate) is as follows:

EXAMPLE

Dilution	Number of colonies
I (0,05 ml)	15 23
II (0,1 ml)	29 35

Then:

n_{CFU} = 15 + 23 + 29 + 35 = 102;

V_S = 0,05 + 0,05 + 0,1 + 0,1 = 0,3

and

C_S = 102/0,3 = 340 = 3,4 x 10^2 CFU/ml

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