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Indoor air —

Part 43: Standard method for assessing the reduction rate of culturable airborne fungi by air purifiers using a test chamber

Air intérieur —

Partie 43: Méthode normalisée d'évaluation du taux d'abattement de champignons cultivables aéroportés par des purificateurs d'air en chambre d'essai

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Foreword

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This document was prepared by Technical Committee ISO/TC 146, *Air quality*, Subcommittee SC 6, *Indoor air*.

A list of all parts in the ISO 16000 series can be found on the ISO website.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

An indoor microbial environment is important to the health of occupants, particularly with regard to increased time spent indoors.

Air purifiers are used to reduce the concentration of microorganisms in indoor air.

The efficiency of such air purifiers to reduce airborne microorganisms can be investigated in test chambers at constant temperature and relative air humidity.

Indoor air —

Part 43: Standard method for assessing the reduction rate of culturable airborne fungi by air purifiers using a test chamber

WARNING — The test given in this document shall be performed by persons who are familiar with techniques in connection with microorganisms. The test fungus *Penicillium roqueforti* is a common mould widespread in nature. It has been used for cheese making for a long time. However, it produces spores which can cause an allergic response in people who are sensitive to mould spores. Users of this document shall be aware of national and international safety procedures for working with allergic mould spores, to prevent any exposure in the test environment. The examination and preparation of the cultures should be carried out in a Class II Biological Safety Cabinet.

1 Scope

This document specifies a standard method to evaluate the capacity of air purifiers to reduce the concentration of airborne fungi and clean the air in the indoor environment.

The test is applicable to air purifiers which are commonly used in single room space.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 16000-9:2024, *Indoor air — Part 9: Determination of the emission of volatile organic compounds from samples of building products and furnishing — Emission test chamber method*

3 Terms and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1
air purifier
electrically-powered device that is basically built of a fan and a set of components possessing the ability to capture and/or (partially or totally) destroy air pollutants

3.2
colony forming unit
cfu
unit by which the number of culturable fungi is expressed

[SOURCE: ISO 16000-36:2018, 3.2, modified — “Bacteria” was changed to “fungi”.]

3.3
fungal background concentration
concentration of culturable fungi inside the test chamber prior to testing

3.4
natural decay rate
reduction rate of airborne culturable fungi, which is measured by comparing the concentration of fungi immediately after nebulizing a fungal suspension inside the chamber with the concentration counted after a defined time (testing time) without running the *air purifier* (3.1)

Note 1 to entry: Natural decay rate is expressed in percent.

3.5
fungal reduction rate
reduction rate of airborne culturable fungi, which is measured by comparing the concentration of fungi immediately after nebulizing a fungal suspension inside the chamber with the concentration counted after a defined running time (testing time) of the *air purifier* (3.1)

Note 1 to entry: Fungal reduction rate is expressed in percent.

3.6
impaction
sampling of airborne culturable fungi by inertial separation on a solid agar surface (culture medium or adhesive-coated slides)

Note 1 to entry: See ISO 16000-18.

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

4 Principle

The efficiency of air purifiers is tested using nebulized fungal suspensions inside a test chamber at constant temperature and relative humidity. The efficiency is calculated by the reduction rate of airborne culturable fungi in a defined period of time, considering homogeneity and natural decay rate of the fungi.

5 Apparatus and materials

5.1 Apparatus

5.1.1 Test chamber

The static chamber shall be made from suitable material, i.e. one that emits minimal pollutant, is corrosion proof, such as stainless steel, and shall maintain sufficient airtight capacity.

The volume of the chamber should reflect the later application of the air purifier. The minimum volume shall not be below 8 m^3 and is typically between 15 m^3 and 30 m^3.

The inside of test chamber shall be kept clean and free from microbial contamination. It shall have a suitable environmental control system to maintain a constant temperature and humidity. To achieve this, the test chamber should include the following:

— a system capable of removing contamination and maintaining aseptic condition inside the chamber, such as a UV-C lamp;

— a facility to transfer items into and out of the chamber without cross-contamination (this can include a special system such as a glove box on a rail system);

— a facility to control power inside the chamber from outside;

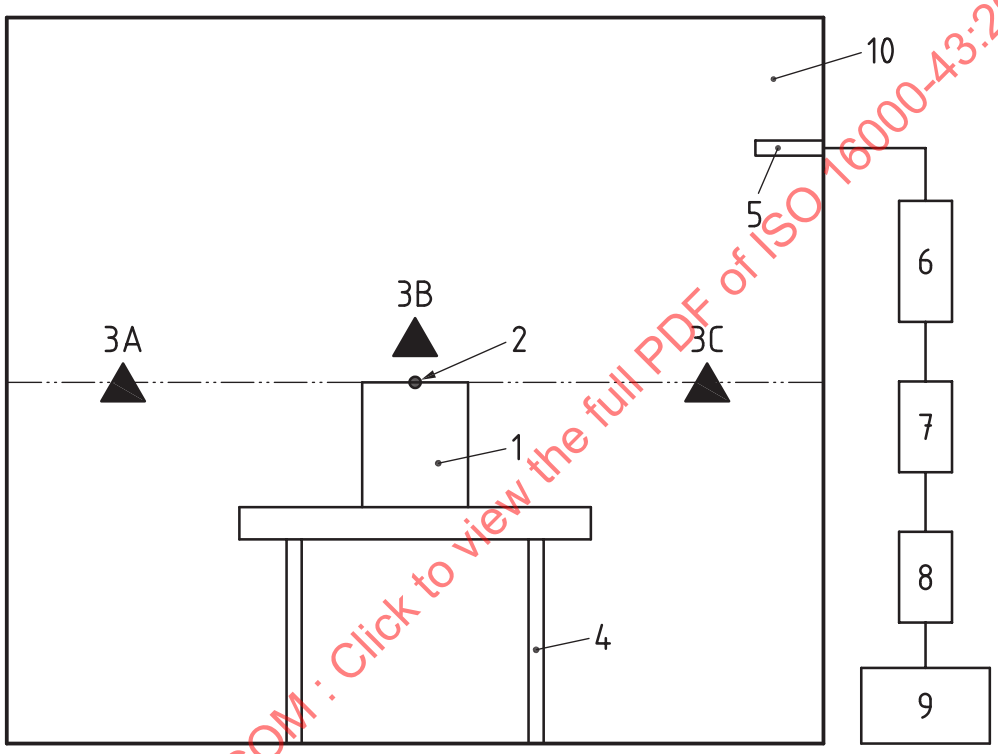
— a facility to generate an aerosol of test fungi inside the chamber and to ensure their homogeneity (this can be achieved by using a spray inlet, through which fungi are nebulized, connected to a spray nozzle in the chamber, with a fan to ensure homogeneous distribution of the fungi inside the chamber);

— an air conditioning system inside the chamber capable of controlling temperature and relative humidity in a stable and precise manner; the air conditioning system shall be switched off during the test;

— a facility to use negative pressure air flow to flush the chamber post-testing;

— an indicator to display main environmental factors of the test including flow rate, temperature and relative humidity.

A test system using a test chamber is shown in Figure 1.



Key

1 air purifier
2 air intake of test unit
3 3A, 3B, 3C position of impactors
4 stand for the air purifier
5 inlet of spray
6 dehumidifier
7 nebulizer
8 filter (to supply clean air)
9 pressure pump
10 test chamber

Figure 1 — Schematic diagram of test system using a test chamber

Example photos of a test chamber are given in Annex A.

In accordance with ISO 16000-9:2024, 8.1:

— the test temperature and acceptable range of variation shall be (23 ± 1) °C;

— the test humidity and acceptable range of variation shall be (50 ± 5) %.

In addition, the test may be performed under other conditions. These conditions shall be documented.

After each test, the interior space of the test chamber is decontaminated using a UV lamp, 70 % ethanol (5.1.12) or adopting other decontamination methods in order to prevent contamination after a test.

5.1.2 Nebulizer.

The nebulizer shall be capable of nebulizing the spore suspension into particles (0,05 µm to 5 µm) to produce, as far as possible, individual fungal particles. It typically comprises a pump to generate a certain air pressure to nebulize the spore suspension, a clean air supplying unit (HEPA filter) and a dehumidifier to remove excess from the generated spore suspension.

5.1.3 Impactor for sampling of fungi.

The impaction method described in this document is only applicable for relatively low concentrations of culturable fungi and small chambers with a volume of at least 8 m^3.

The initial concentration shall be below the upper detection limit of the sampling method. For impaction with a 300 holes sampler and a sampling volume of 50 l or 100 l at 100 l/min flow rate, the upper detection limit is approximately $1,6 \times 10^4$ cfu/m^3 or $3,2 \times 10^4$ cfu/m^3, respectively (299 of 300 possible colonies).

NOTE The detection limit is dependent on the sampling strain, which has different air flows and collection efficiency.

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

5.1.5 Autoclave, thermostatically controlled at (121 ± 3) °C and a pressure of (103 ± 5) kPa.

5.1.6 Incubator, thermostatically controlled at (25 ± 1) °C.

5.1.7 Cryogenic freezer, thermostatically controlled at (–70 ± 2) °C.

5.1.8 Class II Biological Safety Cabinet.

5.1.9 Balance, capable of weighing to ± 0,01 g.

5.1.10 Inoculating loop, 4 mm in ring diameter, sterile.

5.1.11 Petri dishes, vented, sterile, 90 mm to 100 mm diameter.

5.1.12 Disinfectant, isopropanol or ethanol (70 % volume fraction).

5.1.13 pH-meter, capable of measuring to ± 0,2 unit.

5.1.14 Timer.

5.1.15 Haemocytometer, a device used for counting red blood cells or fungal cells (spores).

5.2 Materials

5.2.1 Test fungi

Select a non-pathogenic species of live, e.g. *Penicillium roqueforti* KACC 47196.

The test fungi shall be obtained from a culture collection (e.g. KACC = Korean Agricultural Culture Collection).

For specific questions, other fungi may be used. All strains used shall be listed in the test report.

5.2.2 Culture media and reagents

5.2.2.1 General

For the preparation of culture media and reagent, use ingredients of uniform quality and chemicals of analytical grade. Prepare culture media with distilled or deionized water equivalent to ISO 3696 quality 3 and free from fungal growth inhibiting substance. Alternatively, use complete media and follow strictly the manufacturer's instructions. If other culture media are used, the media shall be recorded in the test report. If other fungi are used, a media suitable for each fungus shall be used and the relevant information shall be recorded in the test report.

5.2.2.2 Potato dextrose agar (PDA)

The components are listed in Table 1.

Table 1 — Composition of potato dextrose agar

Component	Quantity
Potato starch	4,0 g
Dextrose	20,0 g
Agar	15,0 g
Water	1 000 ml

Dissolve ingredients in distilled or deionized water (see Table 1). Adjust pH with sodium hydroxide or hydrochloric acid. The final pH should correspond to 7,0 to 7,2 at 25 °C. Sterilize by autoclaving at 121 °C for 15 min. Store at (5 ± 3) °C for not longer than one month.

5.2.2.3 Phosphate buffer solution

The components are listed in Table 2.

Table 2 — Composition of phosphate buffer solution

Component	Quantity
Potassium dihydrogen phosphate	34,0 g
Water	1 000 ml

Dissolve ingredients of distilled or deionized water (see Table 2). Adjust pH with sodium hydroxide or hydrochloric acid. The final pH should correspond to 7,0 to 7,2 at 25 °C. Sterilize by autoclaving at 121 °C for 15 min. Store at (5 ± 3) °C for not longer than one month.

6 Preparation of the stock cultures and working cultures of the test fungi

6.1 Preparation and maintenance of stock culture

Inoculate the fungal stock culture on potato dextrose agar (5.2.2.2) medium using a sterile loop (5.1.10) and incubate it at (25 ± 1) °C, at not less than 50 % relative humidity (RH) for 5 days. After incubating, apply sterile physiological saline solution onto the cultured medium plate, stir the fungal mycelia using a sterile glass rod to make a suspension, and filter the fungal suspension through several layers of sterile gauze to obtain a spore suspension. Add the same volume of 20 % (volume fraction) sterile glycerol or 10 % (volume fraction) dimethylsulfur oxide (DMSO) to the fungal suspension to attain 10 % (volume fraction) glycerol or 5 % (volume fraction) DMSO suspension and mix well. Distribute the aliquots into screw capped plastic tubes of 1 ml and store at (–70 ± 10) °C in a cryogenic freezer (5.1.7) for a maximum of two years.

6.2 Preparation and maintenance of working cultures of the test fungi on agar plates

Prepare a working culture of the test fungi from the stock culture (6.1). Equilibrate the frozen stock culture to room temperature (15 °C to 30 °C) and inoculate the fungal suspension to a potato dextrose agar (5.2.2.2) plate. After cultivation, store the plates at (5 ± 3) °C for not longer than one month.

6.3 Preparation of working culture suspensions

From the incubated fungal culture plate (6.2), apply sterile phosphate buffer solution (5.2.2.3) onto the cultured medium plate, stir the fungal mycelia using a sterile glass rod to make a suspension. Take the suspension with a sterile pipette and filter the fungal suspension through several layers of sterile gauze to obtain a spore suspension above 10^5 cfu/ml. Keep the test fungal suspension in a cold storage (5 ± 3 °C) if it is not in immediate use; do not keep it longer than 4 h.

To check the test fungal suspension concentration: put 50 µl to 100 µl of the fungal suspension prepared on physiological saline solution onto a haemocytometer (5.1.15), place a coverslip over it and count the number of fungal spores with five replicates using a light microscope.

7 Procedure

7.1 General

Prevent any fungal contamination and exposure by preparing and handling the test fungus in a Class II Biological Safety Cabinet (5.1.8).

The test is performed in two steps. In step 1 (see 7.2) the concentration of the test fungus is measured without operating the air purifier, then in step 2 (see 7.3) the concentration is measured again with operation of the air purifier (see Annex D).

The test is only valid if the conditions in 8.2 are met and the test (step 2) is performed in the time period when the decay rate step 1 remains below 50 % (see Annex B). If these conditions are not met, the test (step 1 and step 2) shall be repeated.

The test is subsequently repeated with the test fungus. The suspension of the respective test fungus used in step 2 is the same as the suspension used in step 1.

7.2 Step 1 — Measurement of the concentration of culturable test fungi, C_i, without operating the air purifier

7.2.1 General

In step 1, the concentration of the test fungi is measured without operating the air purifier.

7.2.2 Preparation of the air purifier and the test chamber

Place the air purifier in the middle of the chamber. Gently clean the front surface of the air purifier two or three times with a piece of gauze or cotton ball soaked in 70 % ethanol or isopropanol (5.1.12) and dry it completely. If ethanol is not suitable for the surface materials of the air purifier or causes other destruction that can affect the test results, use another decontamination method.

The temperature and relative humidity inside the test chamber shall be maintained at:

— temperature: (23 ± 2) °C;

— humidity: (50 ± 5) % RH.

Before the test, decontaminate the interior space of the chamber, e.g. by using a UV lamp.

Insert three or more impactors (5.1.3) containing the agar plates with potato dextrose agar (5.2.2.2) into the test chamber. Decontaminate the impactors with 70 % ethanol or isopropanol (5.1.12) or using another appropriate method.

NOTE Using more than three measurement points can be useful to demonstrate the homogenous distribution of the fungi in the test chamber with higher volumes.

7.2.3 Measurement of fungal background concentration in the test chamber

Measure the background concentration after placing the air purifier and prior to nebulizing test fungi into the chamber. Measure the concentration of culturable fungi in the middle of the chamber using the impactor (5.1.3) in which the prepared agar plate is placed. Use a sampling volume of 1 000 l. Remove the agar plate from the impactor and count the colonies after incubating the plates at (25 ± 1) °C for 5 days.

The fungal background concentration shall be maintained at < 1 cfu/m^3. If higher concentrations are detected, ventilate and decontaminate the chamber, e.g. by using a UV lamp, and repeat the measurement.

7.2.4 Nebulizing test fungal suspension

Add a defined amount of fungal suspension (6.3) into the nebulizer (5.1.2). The amount of the fungal suspension can vary depending on the nebulizer used. Spray the fungal suspension using a nebulizer at a pressure of 3 bar[1)] (i.e. around 50 l/min). Use a nebulizer nozzle size of 0,3 mm. The nebulizing time varies depending on the volume of the chamber. Use a stirring fan to secure homogeneous distribution of the test fungi inside the chamber.

NOTE 1 If the fungal suspension volume is less than 100 ml, nebulizing is difficult (depending on the size of nebulizer).

NOTE 2 More information on the homogeneity of airborne culturable fungi in the test chamber is given in Annex C.

Clean and decontaminate/sterilize the nebulizer according to the manufacturer's instructions.

7.2.5 Measurement of the initial concentration of culturable fungi inside the test chamber after nebulizing

Measure the initial concentration of culturable fungi inside the chamber after nebulizing the test fungal suspension (6.3) using the three impactors (5.1.3) with inserted potato dextrose agar (5.2.2.2) plates. Decontaminate the impactors with 70 % ethanol, isopropanol (5.1.12) or with the aid of another appropriate method before use. Measurement time and volume vary depending on the expected fungal concentration. The initial concentration shall be between 1,0 × 10^3 cfu/m^3 and 9,0 × 10^3 cfu/m^3.

NOTE Potato dextrose agar plates are removed from the impactors using the glove box. For measuring the fungal concentration after a defined time, new potato dextrose agar plates are inserted into the impactors using the glove box. The changed agar plates (with closed lids) are kept in the chamber until the end of the experiment.

1) 1 bar = 0,1 MPa = 10^5 Pa; 1 MPa = 1 N/mm^2.

7.2.6 Measurement of the concentration of culturable fungi inside the test chamber after a defined time

To determine the natural decay rate of the fungi, measure the concentration of airborne culturable fungi inside the test chamber after a defined time period without operating the air purifier. Choose the time period based on the intended operation time of the air purifier. Measure the natural decay rate more than three times.

Incubate the potato dextrose agar plates at (25 ± 1) °C for 5 days and calculate the number of culturable fungi in accordance with 8.1.

7.2.7 Post-test actions

Decontaminate the interior space of the test chamber using a UV lamp, spraying 70 % ethanol (5.1.12) or adopting another decontamination method in order to remove any contamination after a test.

7.3 Step 2 — Measurement of the concentration of culturable test fungi, C_t, after operating the air purifier

Prepare the test chamber and measure the fungal background concentration as described in 7.2.2 and 7.2.3.

Nebulize the test fungal suspension (see 7.2.4) and measure the initial fungi concentration inside the chamber with the impactors (see 7.2.5).

Operate the air purifier after measuring the initial concentration. The operation time can be changed according to the air purifier's characteristics. The operation time of the air purifier should be less than 10 min.

Measure at the height of the air intake of the air purifier with the impactors, at least at three different positions (see 7.2.6).

Incubate all potato dextrose agar plates (5.2.2.2) at (25 ± 1) °C for 5 days and calculate the number of airborne culturable fungi in accordance with 8.1.

8 Calculation and expression of results

8.1 Calculation of the concentration of airborne culturable fungi

Calculate the concentration of airborne culturable fungi by counting the fungal colonies on the incubated agar plates and by applying the compensation factor for the respective impactor and collected air volume according to Formula (1):

$$C = N \cdot \frac{1}{V} \tag{1}$$

where

C is the concentration of culturable fungi recovered per m^3 (cfu/m^3);

N is the average colony number on each of the three plates with compensation factor, if applicable, in cfu;

V is the sample volume in m^3.

8.2 Conditions for a valid test

The initial concentration inside the chamber immediately after spraying and prior to operating the test unit shall be 1,0 × 10^3 cfu/m^3 to 9,0 × 10^3 cfu/m^3. In addition, Formula (2) shall be applied to the initial fungi count and fungi count after operating the air purifier:

$$(L_{max} - L_{min}) / (L_{mean}) \leq 0,2 \quad (2)$$

where

L_{max} is the maximum logarithm number of fungi count;

L_{min} is the minimum logarithm number of fungi count;

L_{mean} is the average value of logarithm numbers of the measured fungi count.

8.3 Reduction rate of fungi

The fungal reduction rate, R, shall be calculated according to Formula (3);

$$R = \frac{C_i^* - C_t^*}{C_i^*} = 1 - \frac{C_t^*}{C_i^*} \quad (3)$$

where

C_i^* is the normalized concentration of culturable fungi after i hours without operating the air purifier, and defined as $C_i^* = C_i / C_{i,t=0}$

C_t^* is the normalized concentration of culturable fungi after t hours with operating the air purifier, and defined as $C_t^* = C_t / C_{t,t=0}$

9 Test report

The test report shall include the following:

— the date of the test;

— a reference to this document, i.e. ISO 16000-43:2025;

— the fungi used;

— volume of test chamber, used impactor name and flow rate;

— test conditions, including the air purifier operating mode and test time;

— the reduction rate of airborne fungi;

— the result(s), including a reference to the clause which explains how the results were calculated;

— the fungal reduction, which shall be stated down to 0,1 % (round up to one decimal place);

— any deviations from the procedure;

— all details necessary for the identification of the test laboratory;

— the name(s) and signature(s) of the persons(s) in charge of testing;

— product related information (client, model, etc.);

— any unusual features observed.

10 Quality assurance

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

Annex A
(informative)

Test chamber

Figures A.1, A.2 and A.3 show examples of a test chamber.

Key

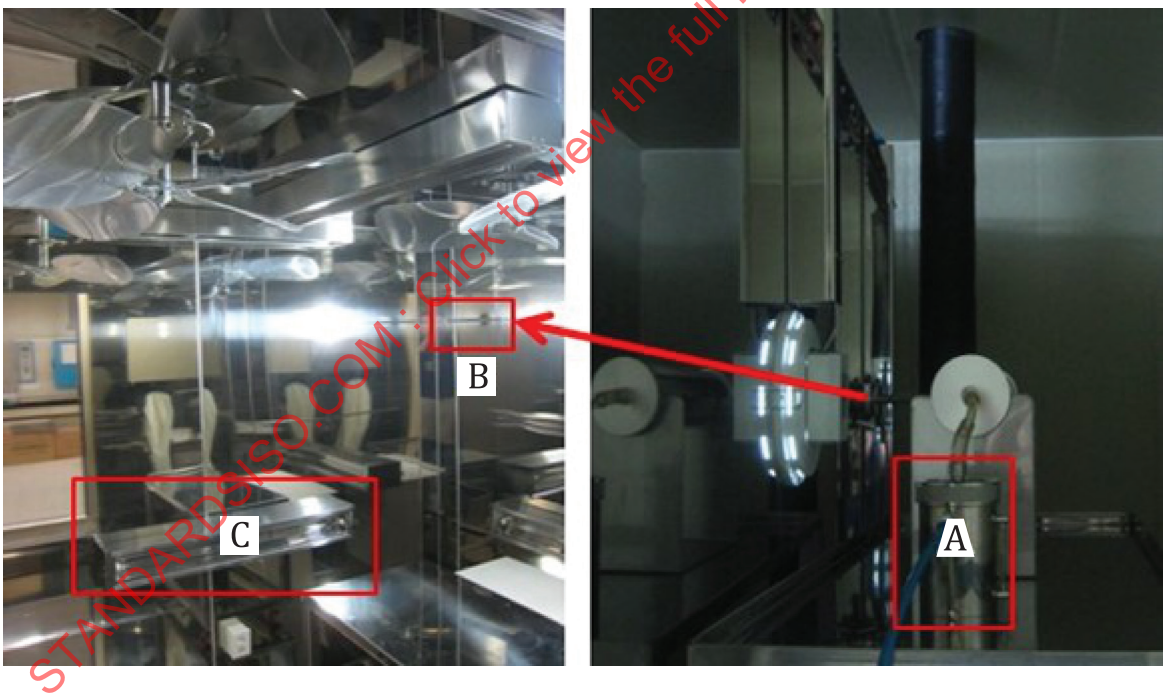
A glove box

SOURCE: Korean Conformation Laboratories (KCL), reproduced with the permission of the authors.

Figure A.1 — Main chamber with a glove box (red box) for external operation

SOURCE: Korean Conformation Laboratories (KCL), reproduced with the permission of the authors.

Figure A.2 — Outside of the test chamber



Key

A nebulizer

B inlet of spray

C UV lamp

SOURCE: Korean Conformation Laboratories (KCL), reproduced with the permission of the authors.

Figure A.3 — Example of the test chamber system

Annex B
(informative)

Natural decay rate depending on the operating mode of the air purifier

The natural decay rate according to the operating mode of air purifier was measured to find valid test conditions for the air purifier in an 8 m^3 test chamber. In general, the operating modes of air purifiers are divided into three types (see Table B.1). The spores of a test fungus *Aspergillus brasiliensis* ATCC 9642 were used as a bioaerosol. The air purifier is tested without filters. When the operating mode of air purifier is strong mode, it is not appropriate to test, because the natural decay rate is more than 50 % after 10 min (this test method prescribes the operating time of the air purifier within 10 min; see Table B.4). When the operating mode of air purifier is weak and medium mode, it is appropriate to test, because the natural decay rate is less than 50 % within 10 min (see Tables B.2, B.3).

Therefore, the operating mode of an air purifier should be carried out in medium mode below in an 8 m^3 test chamber.

Table B.1 — Example of the flow rate of the air purifiers according to operating mode

Operating mode	Flow rate (m/sec)
Weak	1,1 to 1,6
Medium	2,8 to 3,7
Strong	3,8 to 7,3

Table B.2 — The fungal concentration and natural decay rate (air purifier operating mode: weak)

Time (min)	Concentration (cfu/m^3)	Natural decay rate (%)
0	$(5,5 \pm 0,1) \times 10^3$	-
5	$(4,0 \pm 0,2) \times 10^3$	27,3
10	$(3,0 \pm 0,2) \times 10^3$	45,5
15	$(2,0 \pm 0,3) \times 10^3$	63,6
20	$(1,1 \pm 0,3) \times 10^3$	80,0

Table B.3 — The fungal concentration and natural decay rate (air purifier operating mode: medium)

Time (min)	Concentration (cfu/m^3)	Natural decay rate (%)
0	$(5,7 \pm 0,1) \times 10^3$	-
5	$(4,1 \pm 0,2) \times 10^3$	28,0
10	$(3,0 \pm 0,2) \times 10^3$	47,3
15	$(1,8 \pm 0,3) \times 10^3$	68,4
20	$(0,98 \pm 0,3) \times 10^2$	82,8