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Indoor air —

Part 36: Standard method for assessing the reduction rate of culturable airborne bacteria by air purifiers using a test chamber

Air intérieur —

Partie 36: Méthode normalisée d'évaluation du taux d'abattement de bactéries cultivables aéroportées par des purificateurs d'air en utilisant une chambre d'essai

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see www.iso.org/iso/foreword.html.

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.

This corrected version of ISO 16000-36:2018 incorporates the following corrections:

— In 6.3, the values $1,0 \times 10^3$ to $3,5 \times 10^3$ have been changed to $1,0 \times 10^9$ to $9,0 \times 10^9$;

— In 7.2.5, the values $1,0 \times 10^3$ and $3,2 \times 10^3$ have been changed to $1,0 \times 10^4$ and $3,2 \times 10^4$;

— In 8.2, the values $1,0 \times 10^3$ to $3,2 \times 10^3$ have been changed to $1,0 \times 10^4$ to $3,2 \times 10^4$.

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 36: Standard method for assessing the reduction rate of culturable airborne bacteria by air purifiers using a test chamber

WARNING — The test given in this document shall be performed by expert staff trained and certified to handle microorganism-related techniques. The test bacterium *Staphylococcus aureus* is a facultative pathogen for human and animals. National and international safety procedures for working with infectious biomaterials shall be followed to prevent any contamination of apparatus, working place or environment. The examination and preparation of the cultures should be carried out in a microbiological safety cabinet class II.

1 Scope

This document specifies a method to evaluate the capacity of air purifiers to reduce the concentration of airborne culturable bacteria.

The test is applicable to air purifiers commonly used in single room spaces.

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 3696, *Water for analytical laboratory use — Specification and test methods*

ISO 16000-9:2006, *Indoor air — Part 9: Determination of the emission of volatile organic compounds from 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 terminological databases for use in standardization at the following addresses:

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

— IEC Electropedia: available at http://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 *bacteria* (3.3) is expressed

[SOURCE: EN 13098:2000, modified]

3.3
bacteria
procaryotic, single-celled, microscopic organism with peptidoglycan cell wall

3.4
background concentration
concentration of culturable airborne *bacteria* (3.3) inside the test chamber prior to testing

3.5
natural decay rate
reduction rate of culturable *bacteria* (3.3), which is measured by comparing the concentration of bacteria immediately after nebulizing a bacterial 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 per cent.

3.6
bacterial reduction rate
reduction rate of culturable *bacteria* (3.3), which is measured by comparing the concentration of bacteria immediately after nebulizing a bacterial 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: Bacterial reduction rate is expressed in per cent.

3.7
impaction
sampling of airborne culturable *bacteria* (3.3) by inertial separation on a solid agar surface

4 Principle

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

5 Apparatus and materials

5.1 Apparatus

5.1.1 Test chamber

The chamber shall be made from suitable material, i.e. one that emits minimal pollutant is corrosion proof, such as stainless steel. It 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 be 8 m^3 and is typically between 15 m^3 and 30 m^3.

Install a HEPA filter unit for cleaning air by removing particles, an air conditioning unit to control the temperature and humidity, and a system to decontaminate the air inside the test chamber. Particularly for larger test chambers, a fan is needed for homogenous distribution of the bacteria.

The test environment 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 an UV 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);

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

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

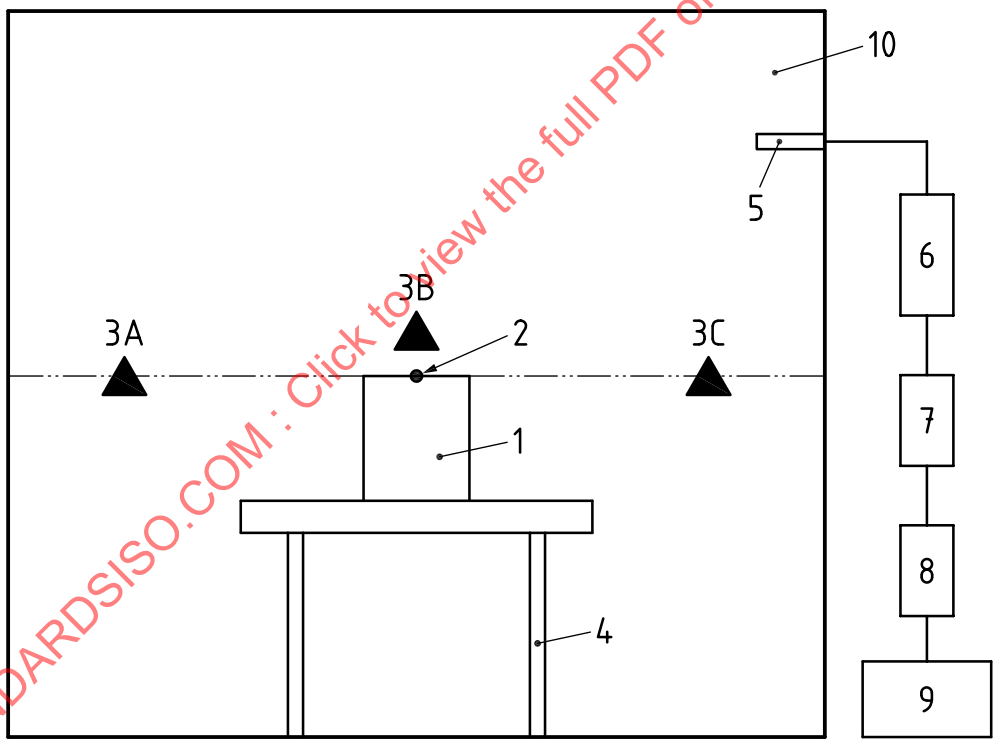
— an air conditioning system inside the chamber capable of controlling temperature and relative humidity in a stable and precise manner; the air conditioning system shall be switched off during the test;

— 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 filter to prevent contamination from the outside during ventilation.

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



Key

1	air purifier	6	dehumidifier
2	air intake of air purifier	7	nebulizer
3	3A, 3B, 3C position of impactors	8	filter (to supply clean air)
4	stand for the air purifier	9	pressure pump
5	the inlet of spray	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 16000-9:2006, 8.1:

— the test temperature and acceptable range of variation shall be (23 ± 2) °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 an 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 culture medium into particles (0,05 µm to 5 µm) to produce, as far as possible, individual bacterial particles. It typically comprises a pump to generate a defined air pressure to nebulize, a clean air supplying unit and a dehumidifier to remove excess water from the generated culture medium.

5.1.3 Impactor for sampling of bacteria.

The impaction method described in this document is only applicable for relatively low concentrations of culturable bacteria and small chambers, e.g. 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 100 l or 50 l, the upper detection limit is approximately 1,6 × 10^4 cfu/m^3 or approximately 3,2 × 10^4 cfu/m^3, respectively (299 of 300 possible colonies).

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 (36 ± 2) °C.

5.1.7 Deep freezer, thermostatically controlled at (–70 ± 10) °C.

5.1.8 Microbiological safety cabinet class II.

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.

5.2 Materials

5.2.1 Test bacteria

5.2.1.1 *Staphylococcus aureus* ATCC 6538

5.2.1.2 *Micrococcus luteus* ATCC 10240

For specific questions, other bacteria 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 reagents, 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 bacterial growth inhibiting substances. Alternatively, use complete media and follow strictly the manufacturer's instructions.

5.2.2.2 Nutrient broth

Beef extract	3,0 g
Peptone	10,0 g
Sodium chloride	5,0 g
Water	1 000 ml

Dissolve ingredients in 1 000 ml of distilled or deionized water. 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 ± 3) °C for 15 min. Store at (5 ± 3) °C for not longer than one month.

5.2.2.3 Nutrient agar

Beef extract	3,0 g
Peptone	10,0 g
Sodium chloride	5,0 g
Water	1 000 ml
Agar	15,0 g

Dissolve ingredients in 1 000 ml of distilled or deionized water by heating. Adjust the 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 ± 3) °C for 15 min. Store at (5 ± 3) °C for not longer than one month.

5.2.2.4 Physiological saline solution

Sodium chloride	8,5 g
Water	1 000 ml

Prepare physiological saline solution by dissolving 8,5 g of sodium chloride in 1 000 ml of distilled or deionized water. Sterilize by autoclaving at (121 ± 3) °C for 20 min. Store for no longer than 12 months.

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

6.1 Preparation and maintenance of stock culture

Rehydrate the lyophilised reference culture of the test bacteria according to the manufacturer's instructions. Transfer the rehydrated reference culture to a conical flask containing a defined volume of nutrient broth medium (5.2.2.2). Incubate for (18 ± 2) h at (36 ± 2) °C in an incubator (5.1.6) while gently shaking. Add the same volume of 20 % (volume fraction) sterile glycerol or 10 % (volume fraction) dimethylsulphur oxide (DMSO), to the bacterial suspension to attain 10 % (volume fraction) glycerol or 5 % (volume fraction) DMSO suspension and mix well. Distribute the aliquots into plastic tubes of approximately 0,5 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 bacteria on agar plates

Prepare a working culture of the test bacteria from the stock culture (6.1). Equilibrate the frozen stock culture to room temperature (15 to 30) °C and inoculate the bacterial suspension to a nutrient agar plate (5.2.2.3) in such a way that single colonies are obtained. After cultivation, store the plates at (5 ± 3) °C for not longer than one month.

6.3 Preparation of working culture suspensions

Inoculate (50 ± 5) ml of nutrient broth media (5.2.2.2) in a conical flask of 300 ml with one to five colonies from the working culture agar plates (6.2) and incubate for (18 ± 2) h at (36 ± 2) °C while gently shaking.

Obtain a concentration of about $1{,}0 \times 10^9$ to $9{,}0 \times 10^9$ cfu/ml (equivalent to 300 "full holes" in a 300 impactor lid, if 100 l were impinged) for testing. If the concentration of the test bacterial suspension is more than $1{,}0 \times 10^9$ to $9{,}0 \times 10^9$ cfu/ml, dilute this suspension with physical saline solution (5.2.2.4) through 10-fold dilutions. Store the culture suspension at (5 ± 3) °C and use within 4 h.

To test the concentration of the bacteria in the suspension, inoculate the prepared suspension on nutrient agar media through 10-fold dilutions and, after incubating at (36 ± 2) °C for (18 to 24) h, count the colonies on the plates.

7 Procedures

7.1 General

Prevent any bacterial contamination by preparing and handling the test bacteria and use a microbiological safety cabinet (5.1.8).

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

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

The test is performed subsequently with both test bacteria. The suspension of the respective test bacterium 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 bacteria, c_i, without operating the air purifier

7.2.1 General

In step 1, the concentration of the test bacteria 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. Clean gently the front surface of the air purifier two or three times with a piece of gauze or cotton ball soaked in 70 % ethanol (5.1.12) and dry it completely. If ethanol is not suitable for the surface materials of the air conditioner, or causes other destruction that might 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 an UV lamp.

Insert three or more impactors containing the agar plates with nutrient agar into the test chamber. Decontaminate the impactors with 70 % ethanol (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 bacteria in test chambers with higher volumes.

7.2.3 Measurement of bacterial background concentration in the test chamber

Measure the background concentration after placing the air purifier and prior to nebulizing test bacteria into the chamber. Measure the concentration of culturable bacteria in the middle of the chamber using the impactor 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 (36 ± 2) °C for (18 to 24) h.

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

7.2.4 Nebulizing test bacterial suspension

Add a defined amount of bacterial suspension (6.2) into the nebulizer. The amount of the bacterial suspension can vary depending on the nebulizer used. Spray the bacterial suspension using a nebulizer at a pressure of 3 bar (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 bacteria inside the chamber.

NOTE 1 If the bacterial 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 bacteria in the test chamber are 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 bacteria inside the test chamber after nebulizing

Measure the initial concentration of culturable bacteria inside the chamber after nebulizing the test bacterial suspension (6.3) using the three impactors with inserted nutrient agar plates. Decontaminate

the impactors with 70 % ethanol or other appropriate method before use. Measurement time and volume vary depending on the expected bacterial concentration. The initial concentration shall be between $1{,}0 \times 10^4$ cfu/m^3 and $3{,}2 \times 10^4$ cfu/m^3.

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

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

To determine the natural decay rate of the bacteria, measure the concentration of culturable bacteria 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 nutrient agar plates at (36 ± 2) °C for (18 to 24) h and calculate the number of culturable bacteria in accordance with 8.1.

7.2.7 Post-test actions

Decontaminate the interior space of the test chamber using an 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 bacteria, c_t, after operating the air purifier

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

Nebulize the test bacterial suspension (see 7.2.4) and measure the initial bacteria 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 30 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 nutrient plates at (36 ± 2) °C for (18 to 24) h and calculate the number of culturable bacteria in accordance with 8.1.

8 Calculation and expression of results

8.1 Calculation of the concentration of airborne culturable bacteria

Calculate the concentration of airborne culturable bacteria by counting the bacterial 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 bacteria 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.

If no colony appears on the agar plates, the result is expressed as "< 1 cfu" in the sampled air volume. For example, "< 10 cfu/m^3" indicates that there was no bacterial colony in the incubated agar plates after sampling 100 l of air.

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 $1{,}0 \times 10^4$ cfu/m^3 to $3{,}2 \times 10^4$ cfu/m^3. In addition, Formula (2) shall be applied to the initial bacteria count and bacteria count after operating the air purifier:

$$\frac{(L_{max} - L_{min})}{L_{mean}} \leq 0{,}2 \tag{2}$$

where

L_{max} is the maximum bacteria count;

L_{min} is the minimum bacteria count;

L_{mean} is the average value of the measured bacteria counts.

8.3 Reduction rate of bacteria

The bacterial reduction rate, R, shall be calculated according to Formula (3):

$$R = \frac{C_i^* - C_t^*}{C_i^*} = 1 - \frac{C_t^*}{C_i^*} \tag{3}$$

where

C_i^* is the normalized concentration of culturable bacteria 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 bacteria after i 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:

— standard name of the test;

— the bacteria used;

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

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

— the reduction rate of airborne bacteria;

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

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

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

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

10 Quality assurance

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

Annex A
(informative)

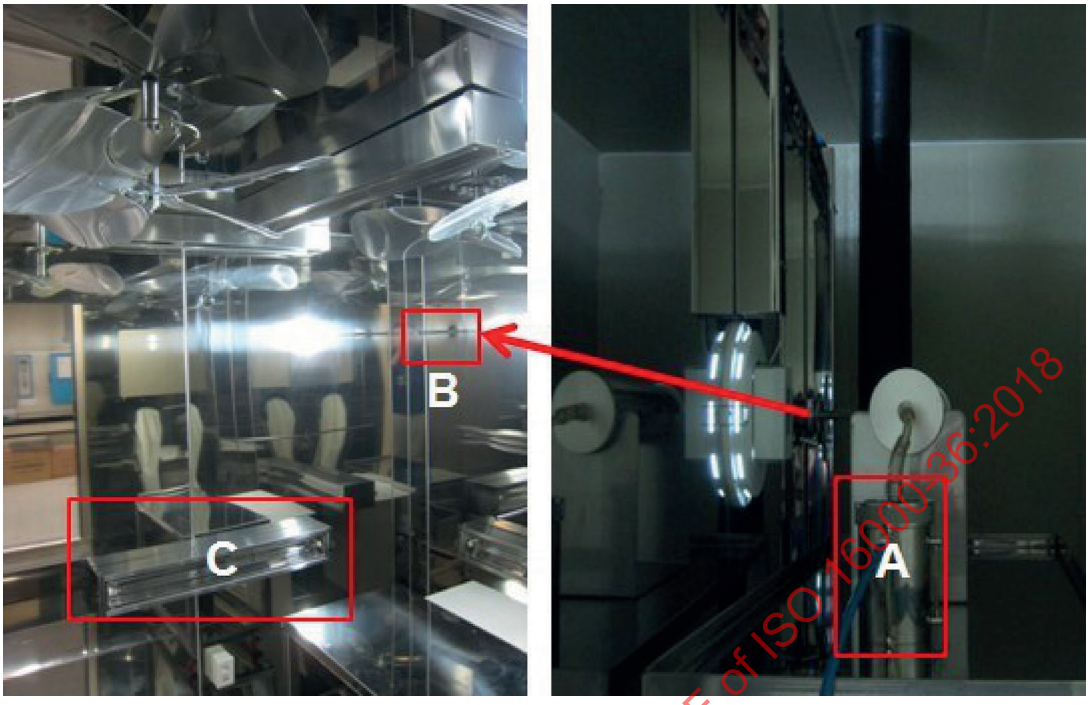
Test chamber

Key

A glove box

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

Figure A.2 — Outside of the test chamber



Key

A nebulizer
B inlet of spray
C UV lamp

Figure A.3 — Example of the test chamber system