INTERNATIONAL STANDARD

ISO 27447

Second edition
2019-07

Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials

Céramiques techniques — Méthode d'essai de l'activité antibactérienne des matériaux photocatalytiques semiconducteurs

Reference number
ISO 27447:2019(E)

ISO copyright office
CP 401 • Ch. de Blandonnet 8
CH-1214 Vernier, Geneva
Phone: +41 22 749 01 11
Fax: +41 22 749 09 47
Email: copyright@iso.org
Website: www.iso.org

Published in Switzerland

Contents

Page

Foreword

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

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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 206, *Fine ceramics*. This second edition cancels and replaces the first edition (ISO 27447:2009), which has been technically revised. The main changes to the previous edition are as follows:

— updating of reference document and cross-references;

— replacement of “adhesive” with “cover” throughout;

— clarification of definition of “photocatalyst antibacterial activity value” (3.4, 3.5, 3.6, 3.7) in Clause 3;

— deletion of a definition of “fluorescent UV lamp” in Clause 3 due to updating of the reference document ISO 10677;

— inclusion of a statement in Clause 5 regarding treatment of results measured by the viable bacterial count method;

— NOTE 1 changed to body text in 6.1.3;

— revision of “storage period of 1/500 nutrient broth” from 1 month ago to 1 week ago in 6.2.2 (formerly 6.2.1);

— addition of a new subclause, 6.2.1, renumbering of subsequent subclauses and updating of cross-references in Clause 6;

— addition of a new subclause, 7.1, and renumbering of subsequent subclauses in Clause 7;

— revision of Figure 1, Figure 4 and Figure 5;

— addition of “paper filter” apparatus in 7.6;

— replacement of “black light fluorescent lamp” with “light source” in 7.7 (formerly 7.5) and revision of a statement in 7.7 that the light source shall be 351BLB or 351BL as specified in ISO 10677;

— replacement of “ultraviolet light radiation mater” with “UV radiometer” in 7.8 (formerly 7.6) and inclusion of a statement in 7.8 that the UV radiometer shall be used as specified in ISO 10677;

— NOTE changed to body text in 8.1;

— addition of a new subclause, 9.1, and renumbering of subsequent subclauses in Clause 9;

— revision of storage time of “the bacteria suspension in case of not using immediately” from 4 h to 2 h in 9.2.1(formerly 9.1.1);

— NOTE 2 changed to body text in 9.2.2 (formerly 9.1.2);

— NOTE 2 and NOTE 3 changed to body text in 9.3.2 (formerly 9.2.2);

— addition of the test environment temperature (25 °C ± 3 °C) in 9.4.1;

— addition of a new subclause, 10.1, and renumbering of subsequent subclauses in Clause 10.

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

This document was developed for antibacterial activity as a result of continuing efforts to provide test methods for photocatalytic materials. However, antibacterial activity cannot be measured for test pieces with permeable or rough surfaces, so other test methods are required.

Under the irradiation of photons, photocatalysts show diverse functions, such as the decomposition of air and water contaminants, as well as deodorization, self-cleaning, antifogging and antibacterial actions. These functions of photocatalysts are generally based on the action of active oxygen species such as hydroxyl (OH) radicals formed on the surface of a photocatalyst (References [14] and [15]). The energy- and labour-saving nature of photocatalysis has attracted keen interest when the photocatalyst is activated by sunlight (or artificial lighting).

Practical applications of photocatalysts for both indoor and outdoor use have rapidly expanded in recent years. Many kinds of photocatalytic materials have been proposed or are already commercialized, based on ceramics, glass, concrete, plastics or paper. Such materials are produced by either the coating or mixing of a photocatalyst; in most cases, titanium dioxide (TiO_2).

However, the effect of photocatalysis is not easily inspected visually, and no appropriate or official evaluation methods have been available to date. Some confusion has thus arisen as photocatalytic materials have been introduced. Furthermore, the above-mentioned diverse functions of photocatalysts cannot be evaluated with a single method; thus, it is necessary to provide different evaluation methods for air purification, water decontamination and self-cleaning.

This document applies to the testing of the antibacterial activity of photocatalytic ceramics and other materials produced by either the coating or the mixing of a photocatalyst. Standards for testing the antifungal activity that use photocatalytic materials will be developed separately.

Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials

WARNING — Handling and manipulation of microorganisms that are potentially hazardous requires a high degree of technical competence. Only personnel trained in microbiological techniques should carry out tests.

1 Scope

This document specifies a test method for the determination of the antibacterial activity of materials that contain a photocatalyst or have photocatalytic films on the surface, by measuring the enumeration of bacteria under irradiation of ultraviolet light.

This document is intended for use with different kinds of semiconducting photocatalytic materials used in construction materials in flat sheet, board, plate shape or textiles that are the basic forms of materials for various applications. It does not include powder, granular or porous photocatalytic materials.

This test method is usually applicable to photocatalytic materials produced for antibacterial effect. Other types of performance of photocatalytic materials, i.e. antifungal activity, antiviral activity, decomposition of water contaminants, self-cleaning, antifogging and air purification, are not determined by this method.

The values expressed in this document are in accordance with the International System of Units (SI).

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 10677, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Ultraviolet light source for testing semiconducting photocatalytic materials*

ISO 80000-1, *Quantities and units — Part 1: General*

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
photocatalyst
substance that carries out many functions based on oxidization and reduction reactions under ultraviolet (UV) irradiation, including decomposition and removal of air and water contaminants, deodorization, and antibacterial, antifungal, antiviral, self-cleaning and antifogging actions

3.2
photocatalytic material
material in which or on which the photocatalyst is added by coating, impregnation or mixing

Note 1 to entry: Photocatalytic materials are to be used for building and road construction materials to obtain the functions mentioned in 3.1.

3.3
antibacterial
condition inhibiting the growth of bacteria on the surface of flat surface materials or cloths

3.4
photocatalyst antibacterial activity value for film cover method
difference between the logarithms of the total number of viable bacteria on photocatalytic treated materials after UV irradiation and on non-treated materials after UV irradiation

Note 1 to entry: This value includes the decrease in the number of bacteria without UV irradiation.

3.5
photocatalyst antibacterial activity value for glass cover method
difference between the logarithms of the total number of viable bacteria on photocatalytic treated cloths after UV irradiation and on standard cloths after UV irradiation

Note 1 to entry: This value includes the decrease in the number of bacteria without UV irradiation.

3.6
photocatalyst antibacterial activity value with UV irradiation for film cover method
difference between the logarithms of the total number of viable bacteria on photocatalytic treated materials after UV irradiation and on photocatalytic treated materials kept in a dark place

3.7
photocatalyst antibacterial activity value with UV irradiation for glass cover method
difference between the logarithms of the total number of viable bacteria on photocatalytic treated cloths after UV irradiation and on photocatalytic treated cloths kept in a dark place

3.8
film cover method
test method to evaluate the antibacterial performance of photocatalytic flat surface materials

3.9
glass cover method
test method to evaluate antibacterial performance of photocatalytic cloths

4 Symbols

A	average number of viable bacteria on non-treated specimens, just after inoculation
B_D	average number of viable bacteria on non-treated specimens, after being kept in a dark place
B_L	average number of viable bacteria on non-treated specimens, after UV irradiation of intensity L
C_D	average number of viable bacteria on photocatalytic treated specimens, after being kept in a dark place
C_L	average number of viable bacteria on photocatalytic treated specimens, after UV irradiation of intensity L
F_{BD}	growth value, after being kept in a dark place

F_{BL} growth value, after UV irradiation of intensity L

L UV irradiation intensity

L_{max} maximum logarithmic value of viable bacteria

L_{mean} average logarithmic value of viable bacteria for three specimens

L_{min} minimum logarithmic value of viable bacteria

M number of viable bacteria with glass cover method

M_{BA} average logarithmic value of the number of viable bacteria for three non-treated specimens, just after inoculation

M_{BD} average logarithmic value of the number of viable bacteria for three non-treated specimens, after being kept in a dark place

M_{BL} average logarithmic value of the number of viable bacteria for three non-treated specimens, after UV irradiation of intensity L

M_{D} average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens, after being kept in a dark place

M_{L} average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens, after UV irradiation of intensity L

N number of viable bacteria with film cover method

P bacteria concentration

D_{F} dilution factor

R_{L} photocatalyst antibacterial activity value for film cover method, after irradiation at a constant intensity L

ΔR photocatalyst antibacterial activity value with UV irradiation for film cover method

S_{L} photocatalyst antibacterial activity value for glass cover method, after UV irradiation of intensity L

ΔS photocatalyst antibacterial activity value with UV irradiation for glass cover method

V volume of soybean casein digest broth with lecithin and polysorbate 80 medium for washout

Z average number of colonies in two Petri dishes

5 Principle

This document is for the development, comparison, quality assurance, characterization, reliability and design data generation of photocatalytic materials. The method is used to obtain the antibacterial activity of photocatalytic materials by the contact of a specimen with bacteria, under UV light irradiation. The film cover method is available for flat sheet, board or plate-shaped materials. To avoid warpage in the cloths or textiles, the glass cover method is available for the cloths or textiles.

The specimen is laid in a Petri dish and the bacterial suspension is dripped onto the specimen. Then the cover film or glass is placed on the suspension and the moisture conservation glass is placed on top of the Petri dish. The Petri dish containing the specimen is exposed to light. After exposure, the test bacteria are washed out of the specimen and the cover film or glass. This washout suspension is measured by the viable bacterial count method. The results obtained are compared with those obtained

from inoculated specimens of non-photocatalytic treated material exposed to UV irradiation under identical conditions to the treated material, and to those obtained from inoculated specimens of both photocatalytic treated and non-treated material kept in the dark for the same period of time.

6 Materials

6.1 Bacteria used and preparation for tests

6.1.1 Film cover method

a) *Staphylococcus aureus*

b) *Escherichia coli*

6.1.2 Glass cover method

a) *Staphylococcus aureus*

b) *Klebsiella pneumoniae*

6.1.3 Bacteria preparation

The bacteria strains to be used in the test are equivalent to those described in Table 1 and are stored by entities that are registered under the World Federation for Culture Collections or the Japan Society for Culture Collections.

Aseptic manipulations using microorganisms can be performed in an adequate safety cabinet. Inoculate each strain into a slant culture medium (nutrient agar medium), incubate for 16 h to 24 h at 37 °C ± 1 °C, and then store in a refrigerator at 5 °C to 10 °C. Repeat subcultures within 1 month by replicating this process. The maximum number of subcultures from the original strain transferred by culture collection is 10 times. In the case of bacteria stored in a deep freezer, the maximum number of subcultures from the original strain transferred by culture collection is 10. The slant culture shall not be used for further storing after 1 month.

NOTE If necessary, additional tests with other bacteria can be carried out.

Table 1 — Bacteria strains to be used in test

Bacteria species	Strain number	Organization for the collection
Staphylococcus aureus	ATCC 6538P	American Type Culture Collection
	DSM 346	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 12732	NITE Biological Resource Center
Escherichia coli	ATCC 8739	American Type Culture Collection
	DSM 1576	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 3972	NITE Biological Resource Center
Klebsiella pneumoniae	ATCC 4352	American Type Culture Collection
	DSM 789	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 13277	NITE Biological Resource Center

6.2 Chemicals and implements

6.2.1 General

Commercial media with the components described as follows may be used.

The volume of prepared media may be adjusted in accordance with the number of specimens.

6.2.2 1/500 nutrient broth

For 1 000 ml of purified water, take 3,0 g meat extract, 10,0 g peptone and 5,0 g sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly dissolved, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,1 ± 0,1) at 25 °C. Dilute this medium 500 times using purified water, and set the pH to (7,0 ± 0,2) using hydrochloric acid solution or sodium hydroxide solution. Sterilize in an autoclave at 121 °C ± 2 °C for at least 15 min. After preparation, if 1/500 nutrient broth is not used immediately, store it at 5 °C to 10 °C. Do not use 1/500 nutrient broth made more than 1 week ago.

6.2.3 Nutrient broth

For 1 000 ml of purified water, take 3,0 g meat extract, 10,0 g peptone and 5,0 g sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly dissolved, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,1 ± 0,1) at 25 °C. If necessary, dispense the contents in a test tube, add a cotton plug and sterilize in an autoclave (see 6.2.2). After preparation, if the nutrient broth is not used immediately, store it at 5 °C to 10 °C. Do not use nutrient broth made more than 1 month ago.

6.2.4 Nutrient agar

For 1 000 ml of purified water, take 3,0 g meat extract and 5,0 g peptone, put them in a flask and dissolve them thoroughly. When the contents are thoroughly dissolved, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (6,8 ± 0,2) at 25 °C. Add 15,0 g agar powder to this medium and heat the flask in boiling water to dissolve agar powder thoroughly. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After preparation, if nutrient agar is not used immediately, store it at 5 °C to 10 °C. Do not use nutrient agar made more than 1 month ago. Keep the medium temperature between 45 °C and 48 °C when mixing with a bacterial suspension.

6.2.5 Soybean-casein digest broth with lecithin and polysorbate 80 (SCDLP)

For 1 000 ml of purified water, take 17,0 g casein peptone, 3,0 g soybean peptone, 5,0 g sodium chloride, 2,5 g dipotassium hydrogenphosphate, 2,5 g glucose and 1,0 g lecithin, put them in a flask and dissolve them. Add 7,0 g non-ionic surfactant and dissolve it. Use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,0 ± 0,2) at 25 °C. If necessary, dispense it in a test tube, add a cotton plug and sterilize in an autoclave (see 6.2.2). After preparation, if SCDLP is not used immediately, store it at 5 °C to 10 °C. Do not use SCDLP made more than 1 month ago.

6.2.6 Physiological saline solution

For 1 000 ml of purified water, take 8,5 g sodium chloride, put it in a flask and dissolve it thoroughly. If necessary, dispense it in a test tube and sterilize in an autoclave (see 6.2.2). After preparation, if physiological saline solution is not used immediately, store it at 5 °C to 10 °C. Do not use physiological saline solution made more than 1 month ago.

6.2.7 Physiological saline solution for washout

For 1 000 ml of purified water, take 8,5 g sodium chloride, put it in a flask and dissolve it thoroughly. Add 2,0 g non-ionic surfactant and dilute. If necessary, dispense 20 ml of the solution in a test tube or Erlenmeyer flask and sterilize in an autoclave (see 6.2.2). After preparation, if physiological saline

solution for washout is not used immediately, store it at 5 °C to 10 °C. Do not use physiological saline solution for washout made more than 1 month ago.

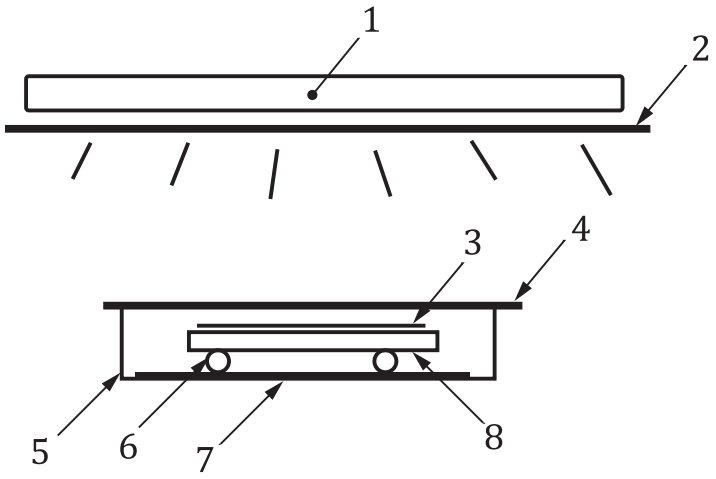
6.2.8 Non-ionic surfactant

Polyoxyethylene sorbitan monooleate (Polysorbate 80).

7 Apparatus

7.1 Test equipment

The testing equipment enables a photocatalytic material to be examined for its antibacterial activity by providing UV irradiation to activate the photocatalyst. It consists of a light source and a chamber with a test piece. An example of a test system is shown in Figure 1.



Key
1 light source
2 punched metal
3 cover film
4 moisture preservation glass
5 petri dish
6 glass tube or glass rod
7 paper filter
8 test piece

Figure 1 — Schematic diagram of the test equipment

7.2 Cover film

The cover film is inert and non-water absorbent with good sealing properties, with a transparency rate over 85 % for the range 340 nm to 380 nm. The sheets are cut with dimensions of (40 ± 2) mm.

NOTE Reference data for cover films are given in Annex B.

7.3 Cover glass

The cover glass consists of glass panes with a thickness less than or equal to 1,1 mm, with a transparency rate over 85 % for the range 340 nm to 380 nm. The panes are cut with dimensions of (50 ± 2) mm.

NOTE Reference data for cover films are given in Annex B.

7.4 Moisture preservation glass

The moisture preservation glass consists of glass panes with a thickness less than or equal to 1,1 mm, with a transparency rate over 85 % for the range 340 nm to 380 nm. The panes are cut to fully cover Petri dishes.

7.5 Glass tube or glass rod

The glass tube or glass rod is prepared by cutting a tube or rod to a 10 cm to 15 cm length and bending it into a u-shape or v-shape.

7.6 Paper filter

The cellulose paper filter should be prepared by cutting it to approximately 85 mm in diameter.

NOTE The number of round-shaped cellulose paper filters per Petri dish is between one and four pieces depending on the amount of water absorption.

7.7 Light source

The light source shall be 351BLB or 351BL as specified in ISO 10677.

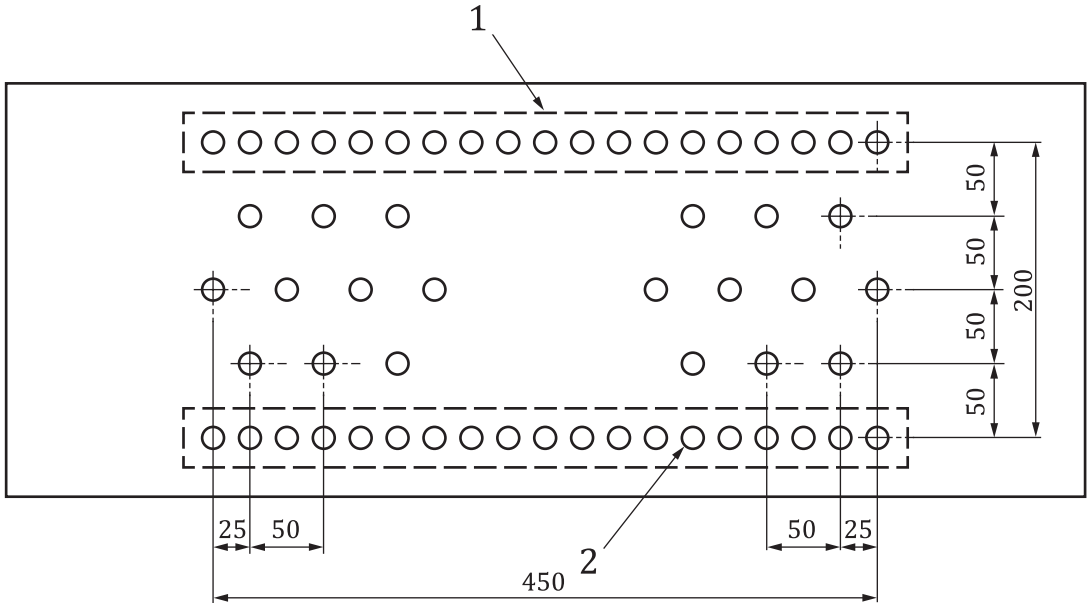
7.8 UV radiometer

The UV radiometer shall be used as specified in ISO 10677. The irradiation intensity shall be measurable at the test sample position. The UV radiation meter shall be calibrated for the light source to be used or corrected to ascertain sensitivity within the wavelength range to be absorbed by the photocatalytic test piece.

7.9 Punched metal sheet

When the prescribed intensity cannot be obtained by tuning the light source height, attenuate the intensity by using a punched metal sheet (see Figure 2 and Figure 3) directly below the lamp.

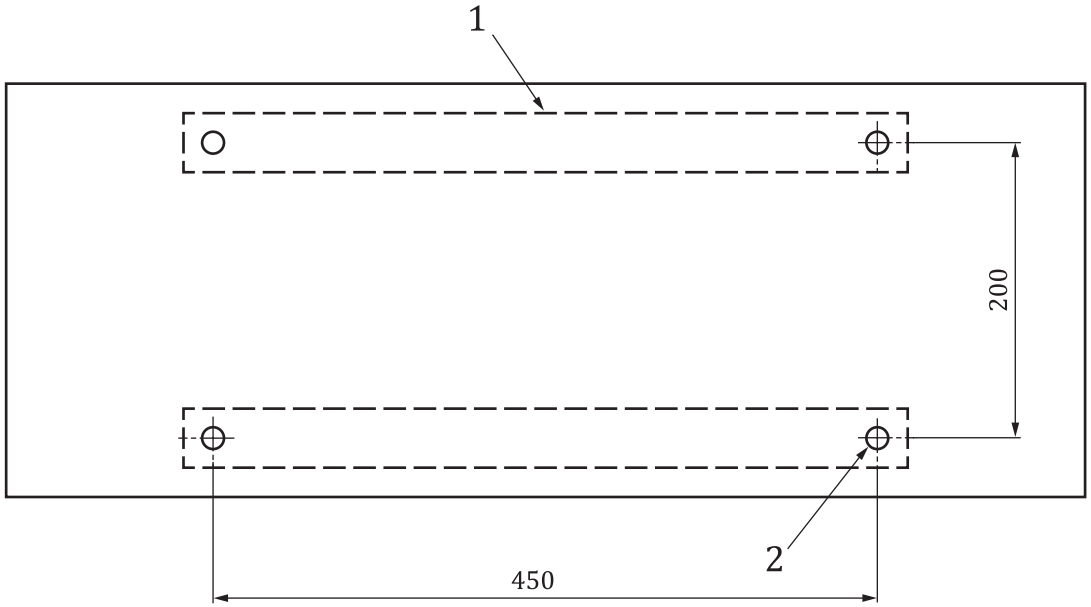
Dimensions in millimetres



Key
1 lamp position
2 bore (⌀5 ~⌀15)

Figure 2 — Punched metal sheet for 0,01 mW/cm^2 light intensity

Dimensions in millimetres



Key
1 lamp position
2 bore (⌀5 ~⌀15)

Figure 3 — Punched metal sheet for 0,001 mW/cm^2 light intensity

8 Test piece

8.1 Film cover method

Cut a flat portion of the material in a (50 ± 2) mm × (50 ± 2) mm square. The materials should be up to 10 mm in thickness. Use this as the standardized shaped specimen. Prepare nine pieces of non-treated specimens and six pieces of photocatalytic treated specimens. When non-treated specimens cannot be provided, use glass panes instead. Take great care to avoid microbial contamination and cross-contamination among specimens.

When it is difficult or impossible to cut (50 ± 2) mm long (up to 10 mm thickness) squares, it is acceptable to use a different specimen size, as long as the specimen surface can be covered with a 400 mm^2 to 1 600 mm^2 film. When the specimen surface is stained with organic contaminant, it is acceptable to first eliminate the contaminant by exposure to a 1,0 mW/cm^2 light source within the limit of 24 h. If necessary, specimens can be disinfected prior to testing (e.g. by wiping with ethanol or 70 % ethanol in water).

8.2 Glass cover method

Cut the material into (50 ± 2) mm × (50 ± 2) mm squares and use them as specimens. Prepare nine pieces of standard cloth and six pieces of photocatalytic treated specimens. Take great care to avoid microbial contamination and cross-contamination among specimens.

Put each of the specimens in a glass Petri dish. Put the dishes in a wire mesh basket, cover the upper part with aluminium foil and sterilize them in an autoclave. After autoclaving, take off the aluminium foil, move the cover of the dishes to a clean bench and dry the specimens for about 60 min.

9 Procedure

9.1 General

The flowcharts of test methods are shown in Figures 4 and 5.

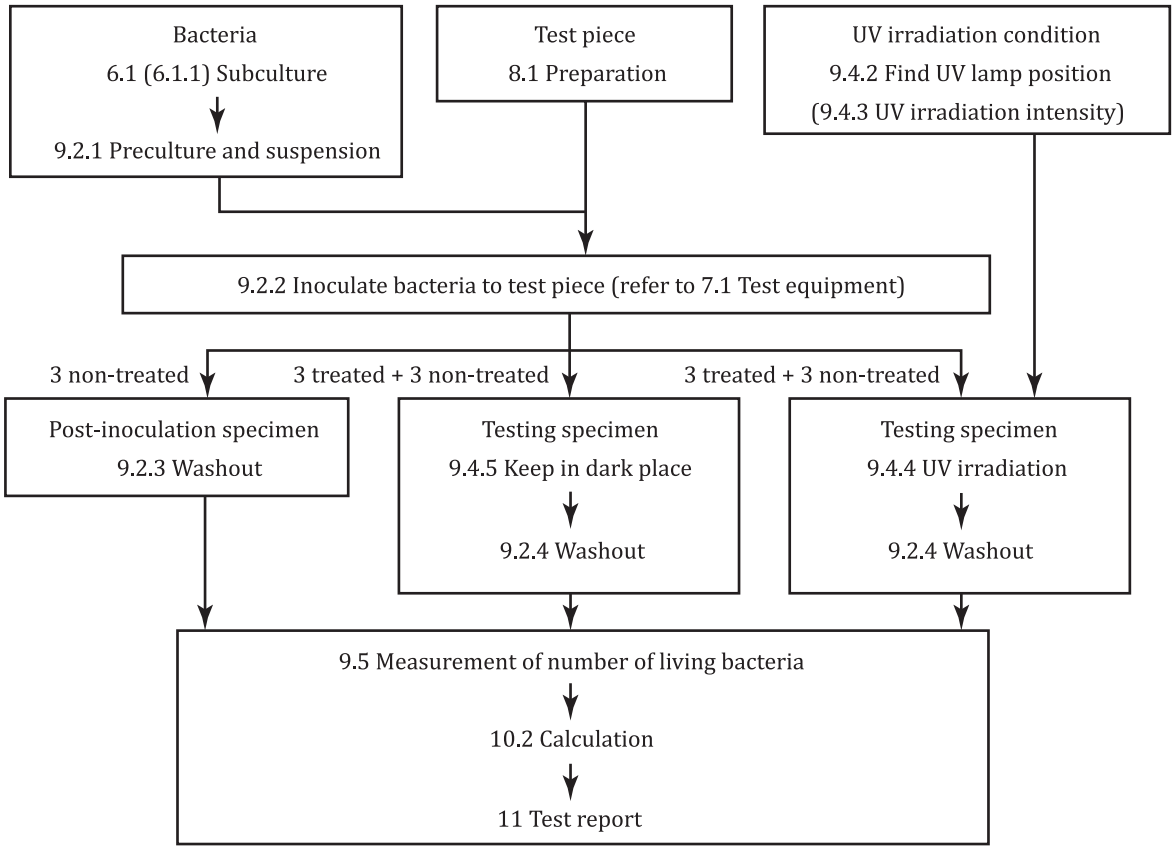


Figure 4 — Flowchart of film cover method

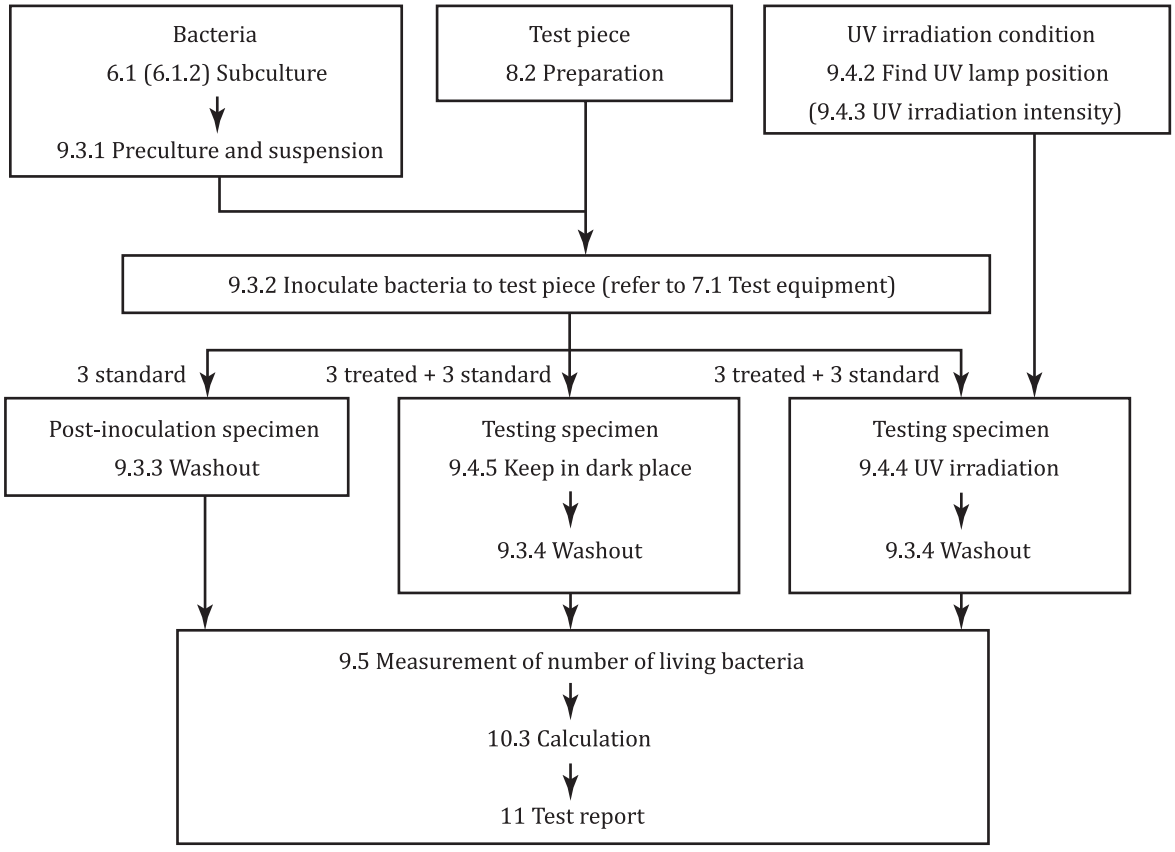


Figure 5 — Flowchart of glass cover method

9.2 Film cover method

9.2.1 Transfer the stored bacteria to the nutrient agar slant using a platinum loop and incubate at (37 ± 1) °C for 16 h to 24 h. Transfer the bacteria to a new nutrient agar slant and incubate at (37 ± 1) °C for 16 h to 20 h. Uniformly disperse a small quantity of test bacteria in 1/500 nutrient broth (NB) with a platinum loop, and measure the bacteria count using the optical microscope observation method or any other adequate method. Suitably dilute this bacteria suspension with 1/500 NB to obtain a count of $6{,}7 \times 10^5$ cells/ml to $2{,}6 \times 10^6$ cells/ml and use the result as the bacterial suspension for the test. If the test bacteria suspension is not to be used immediately, store it at 0 °C and use it within 2 h.

9.2.2 Lay a sterilized moisture control paper filter in the bottom of a sterilized Petri dish, add an adequate quantity of sterilized water, intercalate a glass tube or glass rod in order to avoid contact between the test piece and the paper filter, and place the test piece on it with the photocatalytic treated surface up. Collect exactly 0,15 ml of test bacterial suspension with a sterilized pipette and drip it on each test piece. Put a film on top of the dripped suspension and lightly push to get the suspension to spread to the whole film surface while taking care that no suspension leaks out of the film edge. Then place a moisture conservation glass on the top of Petri dish. Just after the test bacterial suspension is inoculated, perform a measurement of the number of viable cells with regard to three standard cloth pieces. With regard to the remaining six pieces of standard cloths and six pieces of photocatalytic treated specimens, proceed with the irradiation test described in 9.4.

NOTE 4 ml to 6 ml sterilized water added per Petri dish is adequate.

The regulated suspension quantity can create leakage of suspension from the film edge or might not be enough to spread suspension uniformly. In such cases, it is acceptable to reduce down to half the quantity of suspension or increase to twice the quantity of suspension. However, even when the bacterial suspension quantity for inoculation has been changed, the count per specimen shall be the same as with standard size specimen, with $1{,}0 \times 10^5$ cells to $4{,}0 \times 10^5$ cells.

The quantity of test bacterial suspension for inoculation in the case of non-standard size specimens shall be proportional to the film area used.

9.2.3 For the three non-treated bacterial suspension inoculated specimens for the test (post-inoculation specimen of test bacteria), put the adherence film and non-treated test piece in a Stomacher bag using sterilized tweezers, taking care to avoid bacterial suspension leakage from the film and non-treated test piece. Add 10 ml of SCDLP, rub the specimens and the film well from outside the Stomacher bag by hand and wash out the test bacteria. Quickly use this washout solution to perform a measurement of the number of viable cells.

Alternative equivalents to a Stomacher bag may be used if they can be shown to lead to the same results.

9.2.4 For the specimens in 9.4.4 and 9.4.5, perform the washout in the same manner as in 9.2.3.

9.3 Glass cover method

9.3.1 The test bacterial suspension cultivation is processed in the following way.

a) Inoculate stock strain to nutrient agar medium with a platinum loop. Incubate for 24 h to 48 h at (37 ± 1) °C (incubation A). Stock the medium at 5 °C to 10 °C and use within 1 week.

b) Put 20 ml of nutrient culture in a 100 ml Erlenmeyer flask. Collect a colony with a platinum loop from incubation A, inoculate and incubate with agitation (110 min^{-1} with about 3 cm of amplitude) for 18 h to 24 h at (37 ± 1) °C (incubation B).

c) Put 20 ml of nutrient culture in a 100 ml Erlenmeyer flask. Add 0,4 ml of bacterial suspension of incubation B with a 1×10^8 cells/ml to 2×10^8 cells/ml bacteria concentration and incubate with agitation (110 min^{-1} with about 3 cm of amplitude) for (3 ± 1) h at (37 ± 1) °C to reach 10^7 cells/ml (incubation C).

d) Estimate the bacteria concentration of incubation C using the optical density absorbance method or the optical microscope observation method. Dilute the nutrient broth by 20 times at room temperature using purified water, cool it and use it to tune the bacteria concentration of incubation C at (1 ± 0,3) × 10^5 cells/ml. Use the resulting suspension as test material. If the test bacteria suspension is not to be used immediately, store it at 0 °C and use it within 4 h.

9.3.2 Lay a sterilized moisture control paper filter in the bottom of a sterilized Petri dish, add an adequate quantity of sterilized water, intercalate a glass tube or glass rod in order to avoid contact between the test piece and the paper filter, place the sterilized glass on it and place the test piece on the sterilized glass with the photocatalyst treated surface up. Collect exactly 0,2 ml of test bacterial suspension with a sterilized pipette and drip it on each test piece. Put a glass on top of the dripped suspension and lightly push to get the suspension to spread to the whole glass surface while taking care that no suspension leaks out of the glass edge. Then place a moisture conservation glass on the top. Just after the test bacterial suspension is inoculated, perform a measurement of the number of viable cells with regard to three standard cloth pieces. With regard to the remaining six pieces of standard cloths and six pieces of photocatalytic treated specimens, proceed with the irradiation test described in 9.4.

NOTE 4 ml to 6 ml sterilized water added per Petri dish is adequate.

The regulated suspension quantity can create leakage of suspension from the edge or might not be enough to soak the suspension uniformly. In such cases, it is acceptable to reduce down to half the quantity of suspension or to increase to twice the quantity of suspension. However, even when the bacterial suspension quantity for inoculation has been changed, the count per specimen shall be the same as with the standard size specimen, with 1,4 × 10^4 cells to 2,6 × 10^4 cells.

In order to permeate the sample well with the test bacterial suspension, it is acceptable to use a test bacterial suspension containing 0,05 % of non-ionic surfactant. When using non-ionic surfactant in the test bacterial suspension, record the information in the output report.

9.3.3 For the three standard cloth pieces (post-inoculation specimen of test bacteria), put the adherence sterilized glass, non-treated cloths and glass pane in a Stomacher bag using sterilized tweezers, taking care to avoid bacterial suspension leakage. Add 20 ml of physiological saline for washout, rub the Stomacher bag well by hand, as well as the non-treated cloths and the glasses, and wash out the test bacteria. Quickly use this washout solution to perform a measurement of the number of viable cells.

Alternative equivalents to a Stomacher bag may be used if they can be shown to lead to the same results.

9.3.4 For the specimens in 9.4.4 and 9.4.5, perform the washout in the same manner as in 9.3.3.

9.4 UV irradiation condition

9.4.1 Keep the temperature around the specimens at 25 °C ± 3 °C throughout the period of time given in 9.4.4 and 9.4.5.

9.4.2 Set the photoelectric sensor of a UV radiometer on the base of the irradiation apparatus. Place the film and glass plate used for testing on top of the sensor. Find the positions where the indicated value conforms with the UV intensity referred to in 9.4.3.

9.4.3 Test the UV intensity condition, depending on the circumstances where the materials are used. In order to select the UV intensity for the test, the UV intensity in representative place is described in

Table 2. When the prescribed UV intensity cannot be obtained by tuning the height of the light source, attenuate the intensity by using a punched metal sheet.

Table 2 — UV irradiation intensity to be referred to in the test

UV intensity	Example
0,25 mW/cm^2	Beside a window in the daytime, beside the assistant lamp for photocatalytic reaction (e.g. BLB)
0,10 mW/cm^2	In a room (inside, about 1,5 m from a window) in the daytime, by a window in the early morning or before sunset
0,01 mW/cm^2	In a room (inside, about 3 m from a window) in the daytime
0,001 mW/cm^2	In a room without windows (only indoor light), in a room at night (only indoor light)

NOTE The maximum UV intensity is 0,25 mW/cm^2 to avoid damage from UV irradiation only. The minimum UV intensity of the photoelectric sensor at present is 0,001 mW/cm^2. Reference data for damage caused by UV irradiation to bacteria is given in Annex C.

9.4.4 Expose to light the Petri dishes containing the specimens (three non-treated specimens and three photocatalytic treated specimens) with bacterial suspension for 8 h.

NOTE This exposure time could be reduced to 4 h to take into account the real conditions where the photocatalytic material is effectively used.

9.4.5 Keep the Petri dishes containing the specimens (three non-treated specimens and three photocatalytic treated specimens) with bacterial suspension in a dark place for the same time as in 9.4.4.

9.5 Measurement of the number of living bacteria

1 ml of washout solution is taken with a sterilized pipette and added to (9 ± 0,1) ml of physiological saline solution in a test tube and thoroughly agitated. 1 ml of the solution is extracted with a new sterilized pipette and added to another test tube containing (9 ± 0,1) ml of physiological saline and thoroughly agitated again. This process is repeated to obtain a series of dilutions, in conformity with the 10-times dilution method. 1 ml of the solution from the tubes of each series is extracted with new sterilized pipettes and placed in two separate Petri dishes. 15 ml to 20 ml of nutrient agar kept at 45 °C to 48 °C is added to each Petri dish; allow them to stand for 15 minutes at room temperature. When the agar medium solidifies, the Petri dishes are placed upside down and incubated for 40 h to 48 h at (37 ± 1) °C. Colony numbers are counted in the series of Petri dishes with 30 colonies to 300 colonies. The bacteria concentration of washout liquid is obtained by Formula (1) and expressed to two significant digits.

$$P = Z \times D_F \tag{1}$$

where

P is the bacteria concentration (cells/ml);

Z is the average number of colonies in two Petri dishes;

D_F is the dilution factor.

When the number of viable bacteria is less than 30 in the Petri dishes with 1 ml of washout solution, the cell number is used to calculate the average number. When the number of viable bacteria is less than one in the Petri dishes with 1 ml of washout solution, the average number is taken as one.

10 Calculation

10.1 General

The test results are calculated as follows. The calculated values are usually rounded to the second decimal place in accordance with ISO 80000-1.

NOTE Examples of text results are given in Annex A.

10.2 Film cover method

10.2.1 Test requirement fulfilment validation

Use the bacteria concentration obtained in 9.5 and apply Formula (2) to calculate the number of viable bacteria.

$$N = P \times V \tag{2}$$

where

N is the number of viable bacteria;

P is the bacteria concentration obtained in 9.5 (cells/ml);

V is the volume of SCDLP medium for washout (ml).

A test is considered valid if it fulfils all of the following four items. If one or more of these items are not fulfilled, the test is considered as not valid and shall be performed again.

1) The logarithmic value of the number of viable bacteria of non-treated specimens after inoculation is derived from Formula (3).

$$\left(L_{\max} - L_{\min}\right) / \left(L_{\text{mean}}\right) \leq 0{,}2 \tag{3}$$

where

$L_{\max}$ is the maximum logarithmic value of viable bacteria;

$L_{\min}$ is the minimum logarithmic value of viable bacteria;

L_{mean} is the average logarithmic value of viable bacteria for three specimens.

2) The logarithmic value of viable bacteria of non-treated specimens after inoculation shall be within $1{,}0 \times 10^5$ cells to $4{,}0 \times 10^5$ cells range.

3) The viable bacteria of non-treated specimens after light exposure shall be more than $1{,}0 \times 10^3$ cells for all three specimens. However, when a glass pane is used as the non-treated specimen, the number of viable bacteria after light exposure shall be more than $1{,}0 \times 10^4$ cells.

4) After being kept in a dark place, the viable bacteria of non-treated specimens shall be more than $1{,}0 \times 10^3$ cells for all three specimens. However, when a glass pane is used as the non-treated specimen, the number of viable bacteria after light exposure shall be more than $1{,}0 \times 10^4$ cells.

10.2.2 Photocatalyst antibacterial activity value calculation

Use Formulae (4) and (5) to calculate the photocatalyst antibacterial activity value after the test is completed.

Delete the second decimal and express the value with one decimal.

$$R_L = \left[\log\left(B_L / A\right) - \log\left(C_L / A\right)\right] = \log\left(B_L / C_L\right) \tag{4}$$

where

R_L is the photocatalyst antibacterial activity value for film cover method, after UV irradiation of intensity L;

L is the UV irradiation intensity (mW/cm^2);

A is the average number of viable bacteria of non-treated specimens, just after inoculation;

B_L is the average number of viable bacteria of non-treated specimens, after UV irradiation of intensity L;

C_L is the average number of viable bacteria of photocatalytic treated specimens, after UV irradiation of intensity L.

$$\Delta R = \log\left(B_L / C_L\right) - \left[\log\left(B_D / A\right) - \log\left(C_D / A\right)\right] = \log\left(B_L / C_L\right) - \log\left(B_D / C_D\right) \tag{5}$$

where

ΔR is the photocatalyst antibacterial activity value with UV irradiation for film cover method;

B_D is the average number of viable bacteria of non-treated specimens, after being kept in a dark place;

C_D is the average number of viable bacteria of photocatalytic treated specimens, after being kept in a dark place.

10.3 Glass cover method

10.3.1 Test requirement fulfilment validation

Use the bacteria concentration obtained in 9.5 and apply Formula (6) to calculate the number of viable bacteria.

$$M = P \times 20 \tag{6}$$

where

M is the number of viable bacteria (cells);

P is the bacteria concentration obtained in 9.5 (cells/ml);

20 is the quantity of physiological saline for washout (ml).

Test requirement validation uses the propagation value. The propagation value is obtained by Formulae (7) and (8). The last two digits are rounded off. If the propagation value is over 0, the test is validated. If it is under 0, the test is not validated and shall be performed again.

$$F_{BL} = M_{BL} - M_{BA} \tag{7}$$

where

F_{BL} is the growth value, after UV irradiation of intensity L;

L is the UV irradiation intensity (mW/cm^2);

M_{BL} is the average logarithmic value of the number of viable bacteria for three non-treated specimens, after UV irradiation of intensity L;

M_{BA} is the average logarithmic value of the number of viable bacteria for three non-treated specimens, just after inoculation.

$$F_{BD} = M_{BD} - M_{BA} \tag{8}$$

where

F_{BD} is the growth value, after being kept in a dark place;

M_{BD} is the average logarithmic value of the number of viable bacteria for three non-treated specimens, after being kept in a dark place;

10.3.2 Photocatalyst antibacterial activity value calculation

For the completed test, the photocatalyst antibacterial activity value is obtained with up to two digits using Formulae (9) and (10).

$$S_L = M_{BL} - M_L \tag{9}$$

where

S_L is the photocatalyst antibacterial activity value for glass cover method, after UV irradiation of intensity L;

M_L is the average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens, after UV irradiation of intensity L.

$$\Delta S = \left(M_{BL} - M_L\right) - \left(M_{BD} - M_D\right) \tag{10}$$

where

ΔS is the photocatalyst antibacterial activity value with UV irradiation for glass cover method;

M_D is the average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens, after being kept in a dark place.

11 Test report

The test report shall include the following information:

a) description of the type, size, shape and thickness of the photocatalyst and non-treated specimens;

b) description of conditions of pre-exposure when applied;

c) type of test bacteria and bacteria strain number;

d) manufacturer of fluorescent UV lamp and product number;

e) manufacturer of ultraviolet light radiometer and product number;

f) light exposure conditions, including UV irradiation intensity and light exposure duration;

g) in the film cover method, type and size of cover film and moisture preservation glass; quantity of inoculated test bacterial suspension; number of viable bacteria in the test suspension; values of A, B_L, C_L, R_L, B_D, C_D and ΔR in 10.2.2;

h) in the glass cover method, type and size of cover glass and moisture preservation glass; type of test bacteria; bacteria strain number; inoculated bacteria concentration; values of S_L and ΔS in 10.3.2;

i) in the glass cover method, the information when using non-ionic surfactant in the test bacterial suspension.

Annex A
(informative)

Examples of test results

Examples of test results with the film cover method are shown in Table A.1 and Table A.2.

Table A.1 — Test result example for *Staphylococcus aureus*

Laboratory	0,01 mW/cm^2,8 h	
	$R_{0,01}$	ΔR
A	3,4	2,9
B	2,5	2,0
C	2,3	2,3
D	3,4	3,0
Average	2,90	2,55
σ_{n-1}	0,58	0,48

Table A.2 — Test result example for *Escherichia coli*

Laboratory	0,01 mW/cm^2,8 h	
	$R_{0,01}$	ΔR
E	5,1	4,1
F	4,5	3,8
G	2,6	2,2
H	4,9	4,4
Average	4,28	3,63
σ_{n-1}	1,14	0,98

Examples of test results with the glass cover method are shown in Table A.3 and Table A.4.

Table A.3 — Test result example for *Staphylococcus aureus*

Laboratory	0,01 mW/cm^2,8 h	
	$S_{0,01}$	ΔS
I	0,9	0,2
J	0,7	−1,0
K	0,3	−0,4
Average	0,63	−0,40
σ_{n-1}	0,31	0,60

Table A.4 — Test result example for *Klebsiella pneumoniae*

Laboratory	0,01 mW/cm^2,8 h	
	$S_{0,01}$	ΔS
L	2,2	1,0
M	3,1	1,3
N	2,2	1,3

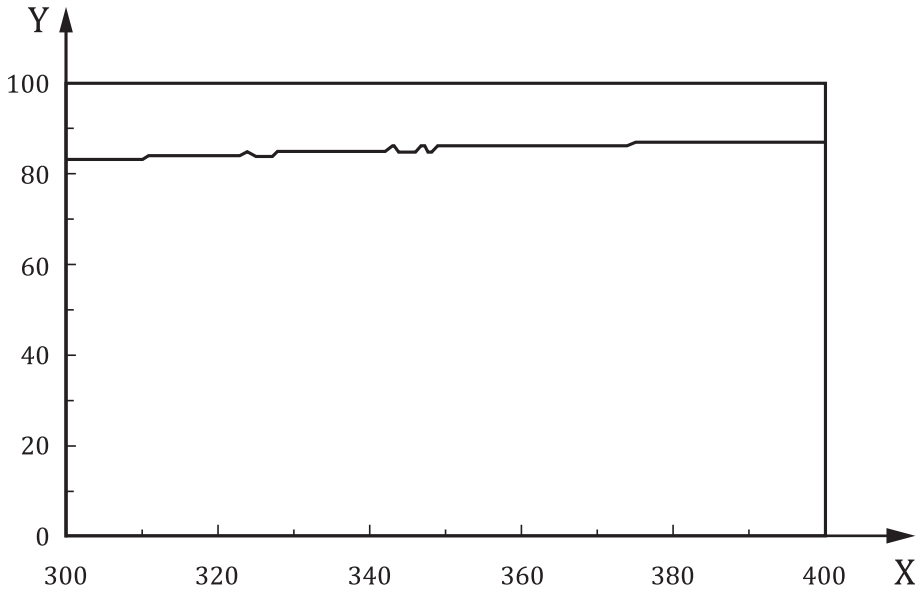
Table A.4 *(continued)*

Laboratory	0,01 mW/cm^2,8 h	
	$S_{0,01}$	ΔS
Average	2,50	1,20
σ_{n-1}	0,52	0,17

Annex B
(informative)

Reference data of cover films and cover glasses

The transmittance data for a suitable cover film made by polypropylene [VF-15[1)] by KOKUYO Co., Ltd, Japan] is shown in Figure B.1.



Key

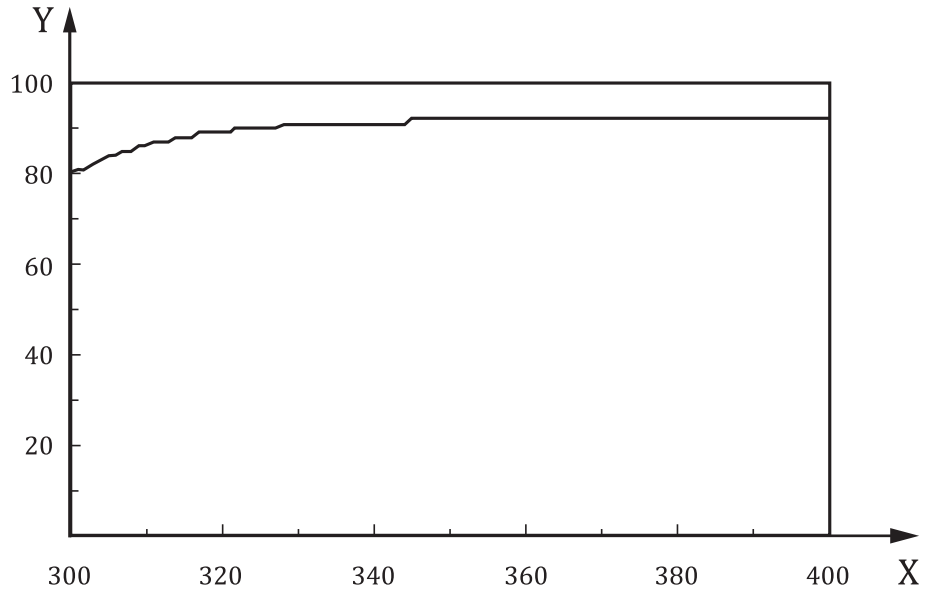
X wavelength (nm)

Y transmittance (%)

Figure B.1 — Transmittance data for a suitable cover film

The transmittance data for a suitable cover glass made by borosilicate [TEMPAX[1)] by SCHOTT AG or Pyrex7740[1)] by CORNING] is shown in Figure B.2.

1) VF-15, TEMPAX® and Pyrex7740® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.



Key

X wavelength (nm)

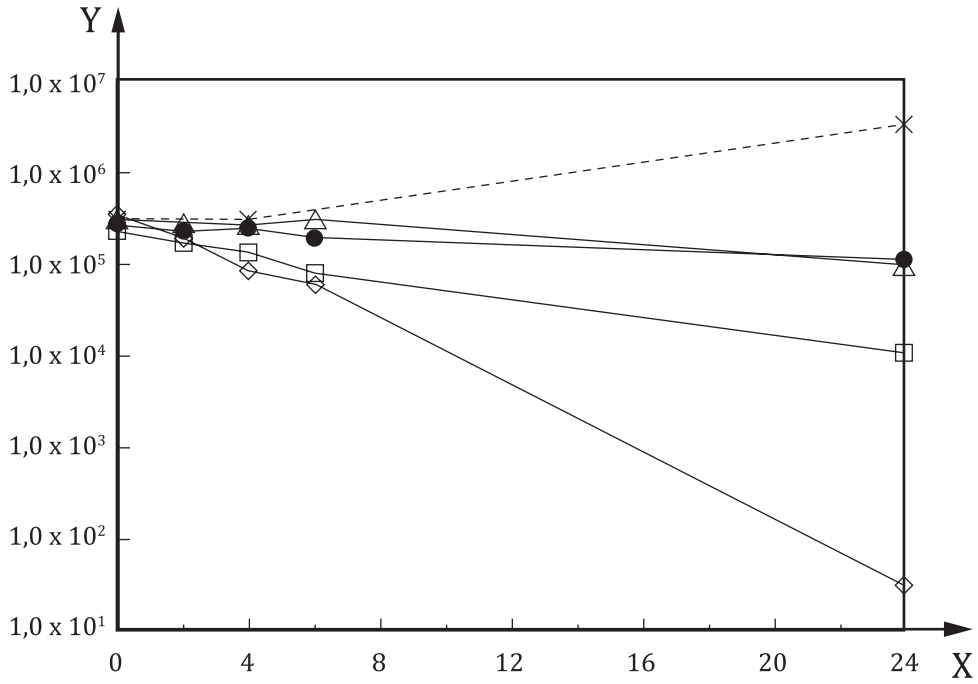
Y transmittance (%)

Figure B.2 — Transmittance data for a suitable cover glass

Annex C
(informative)

Reference data of damage caused by ultraviolet to bacteria

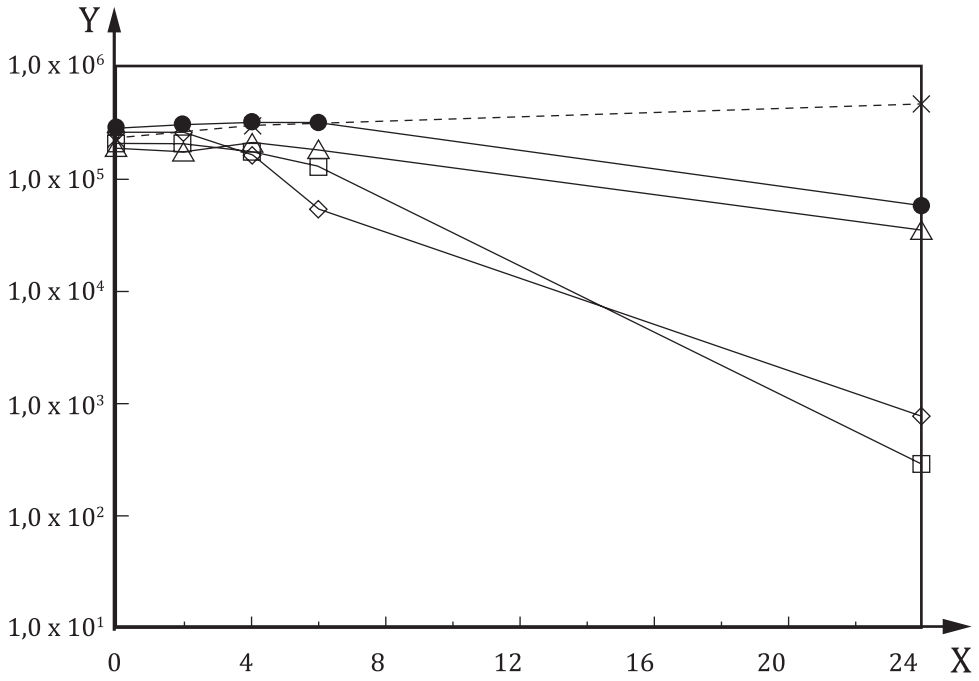
The damage caused by ultraviolet irradiation to bacteria is evaluated with a glass plate (without TiO_2). The results are shown in Figure C.1 for *Escherichia coli* and in Figure C.2 for *Staphylococcus aureus.*



Key

X irradiation time (h)
Y survival bacteria (LOG)
—△— 0,10 mW/cm^2
—●— 0,25 mW/cm^2
—□— 0,30 mW/cm^2
—◇— 0,50 mW/cm^2
---×--- in the dark

Figure C.1 — UV effects for *Escherichia coli*



Key

X irradiation time (h)

Y survival bacteria (LOG)

—△— 0,10 mW/cm²

—●— 0,25 mW/cm²

—□— 0,30 mW/cm²

—◇— 0,50 mW/cm²

---×--- in the dark

Figure C.2 — UV effects for *Staphylococcus aureus*

Bibliography

[1] ISO 554, *Standard atmospheres for conditioning and/or testing — Specifications*

[2] ISO 835, *Laboratory glassware — Graduated pipettes*

[3] ISO 4892-3, *Plastics — Methods of exposure to laboratory light sources — Part 3: Fluorescent UV lamps*

[4] ISO 6353-2, *Reagents for chemical analysis — Part 2: Specifications — First series*

[5] ISO 6353-3, *Reagents for chemical analysis — Part 3: Specifications — Second series*

[6] ISO 10523, *Water quality — Determination of pH*

[7] ISO 10677, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Ultraviolet light source for testing semiconducting photocatalytic materials*

[8] ISO 14605, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Light source for testing semiconducting photocatalytic materials used under indoor lighting environment*

[9] ISO 17094, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials under indoor lighting environment*

[10] ISO 18061, *Fine Ceramics (Advanced Ceramics, Advanced Technical Ceramics) — Determination of antiviral activity of semiconducting photocatalytic materials — Test method using bacteriophage Q-beta*

[11] ISO 18071, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Determination of antiviral activity of semiconducting photocatalytic materials under indoor lighting environment — Test method using bacteriophage Q-beta*

[12] ISO 20743, *Textiles — Determination of antibacterial activity of textile products*

[13] ISO 22196, *Measurement of antibacterial activity on plastics and other non-porous surfaces*

[14] Sunada K., Watanabe T., Hashimoto K., Studies on photokilling of bacteria on TiO_2 thin film. *J. Photochem. Photobiol. Chem.* 2003, **156** p. 227

[15] Sunada K., Watanabe T., Hashimoto K., Bactericidal activity of copper-deposited TiO_2 thin film under weak UV light illumination. *Environ. Sci. Technol.* 2003, **37** p. 4785

ICS 81.060.30

Price based on 24 pages