INTERNATIONAL STANDARD

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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

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Foreword

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

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

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

ISO 27447 was prepared by Technical Committee ISO/TC 206, *Fine ceramics*.

Introduction

As a result of continuing efforts to provide test methods for photocatalytic materials, this International Standard was developed for antibacterial activity. However, for test pieces with permeable or rough surfaces, etc., the antibacterial activity cannot be measured, 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 the photocatalyst (References [10] and [11] in the Bibliography). 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, paper, etc. 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 and 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 required to provide different evaluation methods for air purification, water decontamination and self-cleaning.

This International Standard applies to testing the antibacterial activity of photocatalytic ceramics and other materials produced by either the coating or 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 International Standard 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 International Standard 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 an antibacterial effect. Other types of performance of photocatalytic materials, i.e. decomposition of water contaminants, self-cleaning, antifogging and air purification, are not determined by this method.

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

2 Normative references

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

ISO 31-0, *Quantities and units — Part 0: General principles*

3 Terms and definitions

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

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, self-cleaning and antifogging actions

3.2
photocatalytic materials
materials in which, or on which, the photocatalyst is added by coating, impregnation, mixing, etc.

NOTE 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 adhesion method
difference between the total number of viable bacteria of photocatalytic treated flat surface materials and non-treated materials after UV irradiation

NOTE This value includes the decrease of the number of bacteria without UV irradiation.

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

NOTE This value includes the decrease of the number of bacteria without UV irradiation.

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

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

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

3.9
glass adhesion method
test method to evaluate the antibacterial performance of photocatalytic cloths

3.10
fluorescent UV lamp
lamp that provides UV-A irradiation within a wavelength range of 300 nm to 400 nm

NOTE A suitable lamp is the so-called black light blue (BLB) fluorescent lamp, with a maximum at 351 nm, as described in ISO 4892-3.

4 Symbols

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

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

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

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

C_L average number of viable bacteria of 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 3 specimens

L_{min} minimum logarithmic value of viable bacteria

M number of viable bacteria with glass adhesion method

M_{BA} average logarithmic value of the number of viable bacteria for 3 non-treated specimens, just after inoculation

M_{BD} average logarithmic value of the number of viable bacteria for 3 non-treated specimens, after being kept in a dark place

M_{BL} average logarithmic value of the number of viable bacteria for 3 non-treated specimens, after UV irradiation of intensity L

M_D average logarithmic value of the number of viable bacteria for 3 photocatalytic treated specimens, after being kept in a dark place

M_L average logarithmic value of the number of viable bacteria for 3 photocatalytic treated specimens, after UV irradiation of intensity L

N number of viable bacteria with film adhesion method

P bacteria concentration

R dilution factor

R_L photocatalyst antibacterial activity value, after irradiation at a constant intensity (L) on a photocatalytic material

ΔR photocatalyst antibacterial activity value with UV irradiation

S_L photocatalyst antibacterial activity value, after UV irradiation of intensity L

ΔS photocatalyst antibacterial activity value with UV irradiation

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

Z average number of colonies in 2 Petri dishes

5 Principle

This International Standard 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 adhesion method is available for flat sheet, board or plate-shaped materials. To avoid warpage in the cloths or textiles, the glass adhesion method is available for cloths or textiles.

The specimen is laid in a Petri dish and the bacterial suspension is dripped onto the specimen. Then the adhesive 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 adhesive film or glass. This washout suspension is measured by the viable bacterial count method.

6 Materials

6.1 Bacteria used and preparation for tests

6.1.1 Film adhesion method

a) *Staphylococcus aureus*

b) *Escherichia coli*

6.1.2 Glass adhesion 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. The slant culture must not be used for further storage after 1 month.

NOTE 1 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.

NOTE 2 If necessary, additional tests with other bacteria can be allowed.

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 1/500 nutrient broth

For 1 000 ml of purified water, take 3,0 g of meat extract, 10,0 g of peptone and 5,0 g of sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly diluted, 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 month ago.

6.2.2 Nutrient broth

For 1 000 ml of purified water, take 3,0 g of meat extract, 10,0 g of peptone and 5,0 g of sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly diluted, 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 6.2.1). After preparation, if 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.

6.2.3 Nutrient agar

For 1 000 ml of purified water, take 3,0 g of meat extract, 5,0 g of peptone and 15,0 g of agar powder, put them in a flask and mix. Heat the flask in boiling water to dissolve the contents thoroughly. Use a 0,1 mol/l solution of sodium hydroxide to bring the pH to (6,8 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.1). 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.

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

For 1 000 ml of purified water, take 17,0 g of casein peptone, 3,0 g of soybean peptone, 5,0 g of sodium chloride, 2,5 g of phosphoric acid monopotassium dehydrogenate, 2,5 g of glucose and 1,0 g of lecithin, put them in a flask and dissolve them. Add 7,0 g of 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 6.2.1). After preparation, if SCDLP is not used immediately, store it at 5 °C to 10 °C. Do not use SCDLP medium made more than 1 month ago.

6.2.5 Physiological saline solution

For 1 000 ml of purified water, take 8,5 g of 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 6.2.1). 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.

6.2.6 Physiological saline solution for washout

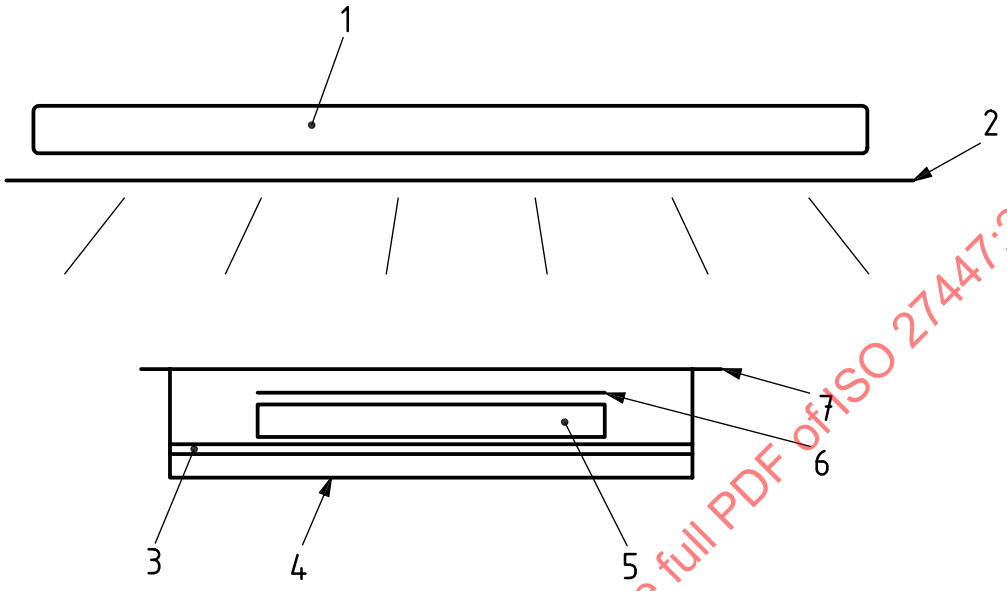
For 1 000 ml of purified water, take 8,5 g of sodium chloride, put it in a flask and dissolve it thoroughly. Add 2,0 g of non-ionic surfactant and dilute. If necessary, dispense 20 ml of the solution in a test tube or Erlenmeyer flask and sterilize in an autoclave (see 6.2.1). After preparation, if physiological saline solution for washout is not used immediately, store it at 5 °C to 10 °C. Do not use physiological saline solution made more than 1 month ago for washout.

6.2.7 Non-ionic surfactant

Polyoxyethylene sorbitan monooleate (polysorbate 80).

7 Apparatus

The test 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 glass stick
4 paper filter
5 test piece
6 adhesive film or glass
7 moisture preservation glass

Figure 1 — Schematic diagram of the test equipment

7.1 Adhesive film

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

NOTE Reference data for adhesive films is given in Annex B.

7.2 Adhesive glass

The adhesive glass consists of glass panes with a thickness less than or equal to 1,1 mm, with a transparency rate over 85 % for the 340 nm to 380 nm range. The panes are cut with dimensions of (40 ± 2) mm.

NOTE Reference data for adhesive glasses is given in Annex B.

7.3 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 340 nm to 380 nm range. The panes are cut to fully cover Petri dishes.

7.4 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.

7.5 Black light fluorescent lamp

The fluorescent lamp shall be a BLB (black light blue) lamp that has a peak wavelength of 351 nm with blue glass for absorbing visible light.

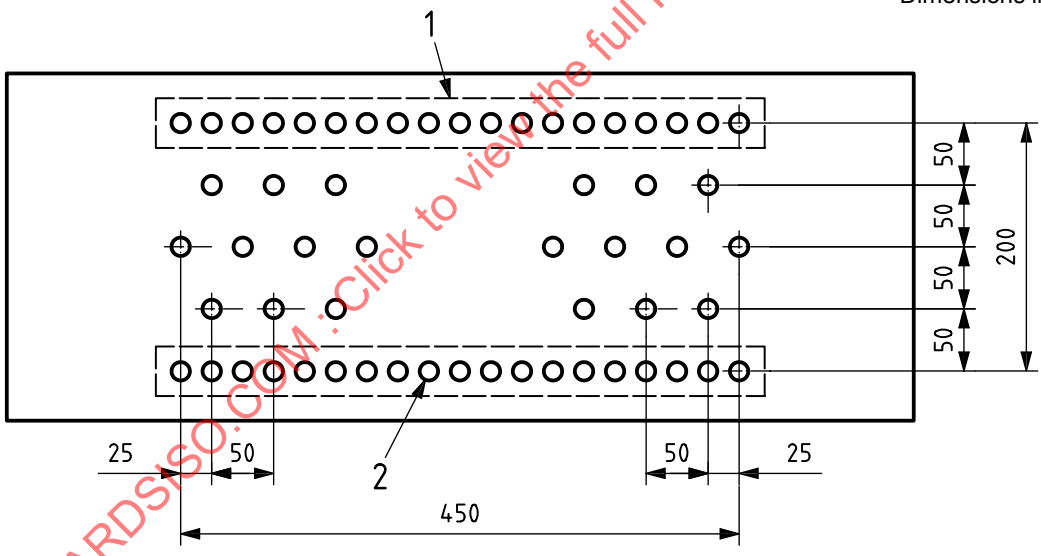
7.6 Ultraviolet light radiation meter

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.

7.7 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 Figures 2 and 3) directly below the lamp.

Dimensions in millimetres



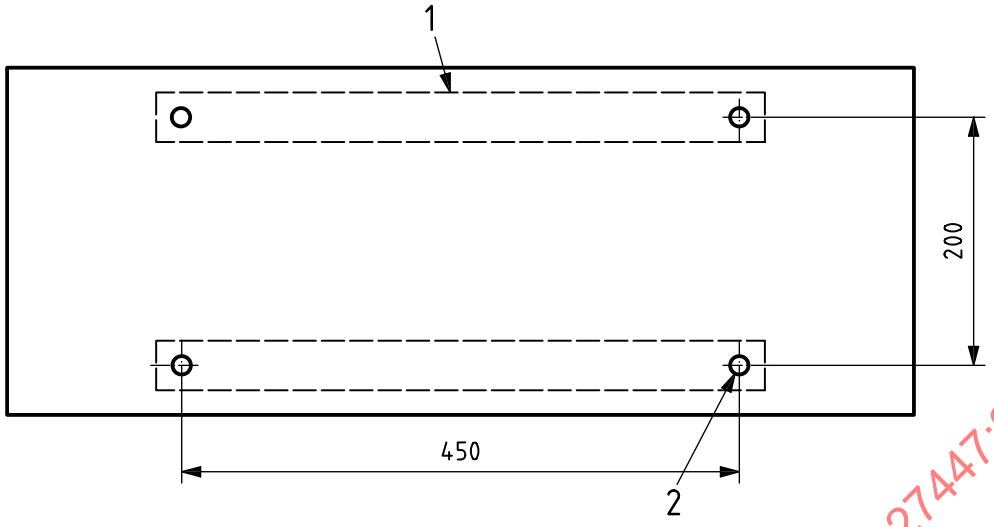
Key

1 lamp position

2 bore (diameter approximately 5 to 15)

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

Dimensions in millimetres



Key

1 lamp position

2 bore (diameter approximately 5 to 15)

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

8 Test piece

8.1 Film adhesion 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 it as the standardized shaped specimen. Prepare 9 pieces of non-treated specimens and 6 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.

NOTE 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 adhesion method

Cut the material into (50 ± 2) mm × (50 ± 2) mm squares and use them as specimens. Prepare 9 pieces of standard cloth and 6 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 aluminum foil and sterilize them in an autoclave. After autoclaving, take off the aluminum foil, move the cover of the dishes to a clean bench and dry the specimens for about 60 min.

9 Procedure

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

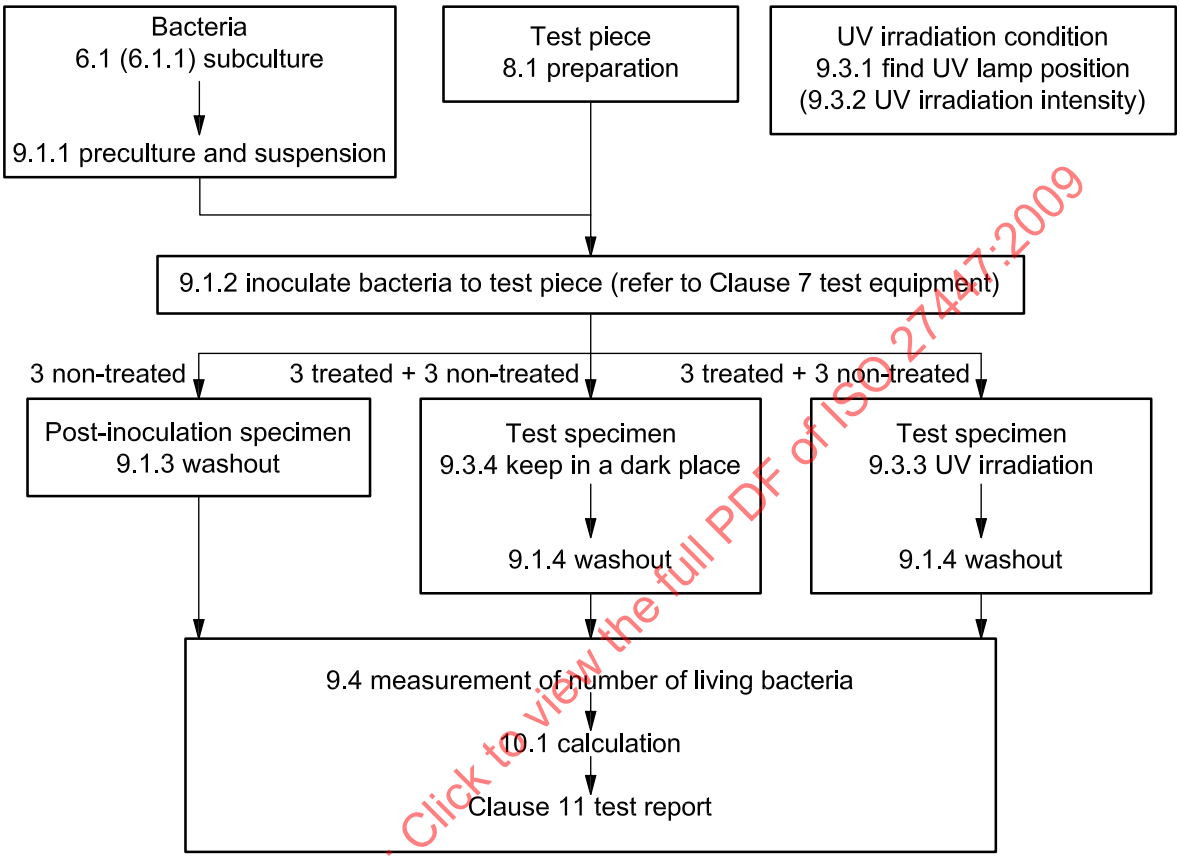


Figure 4 — Flowchart of film adhesion method

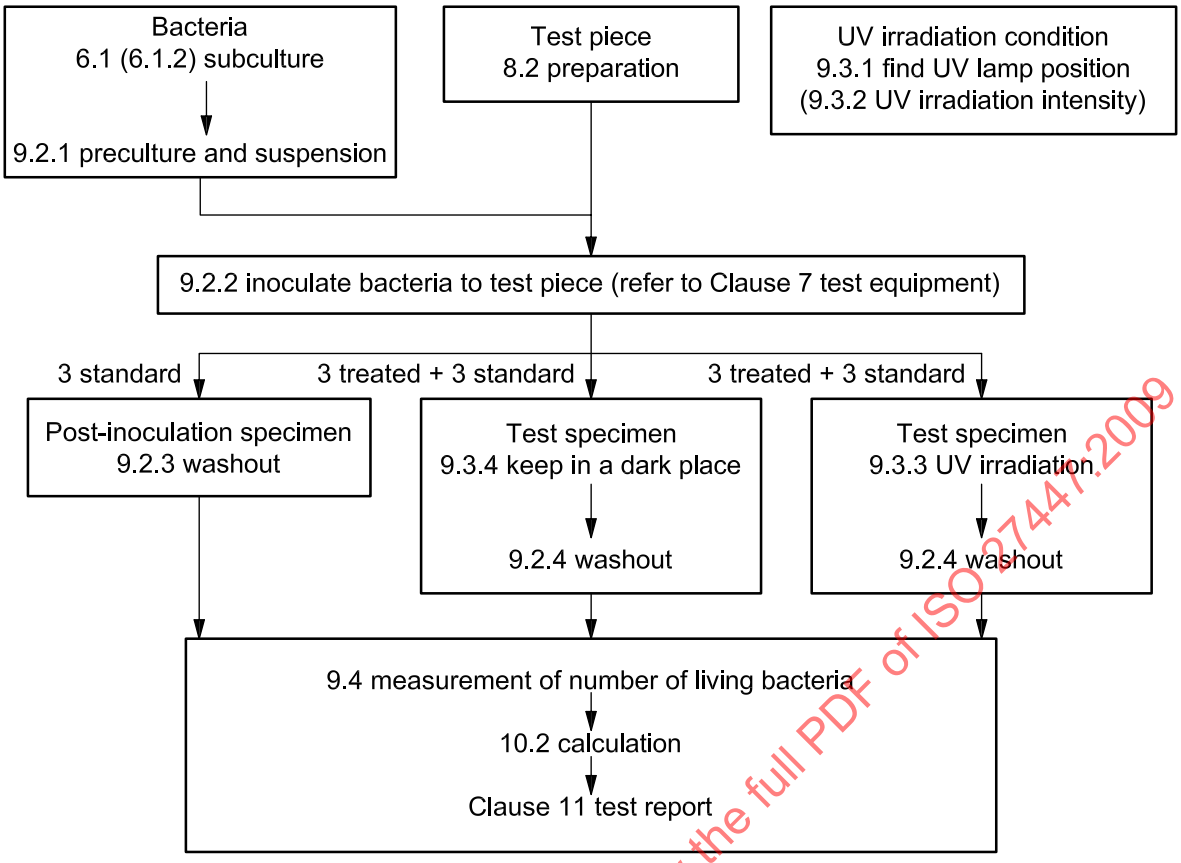


Figure 5 — Flowchart of glass adhesion method

9.1 Film adhesion method

9.1.1 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 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 4 h.

9.1.2 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 onto 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 the Petri dish. Except for 3 non-treated specimens for a viable cell count performed just after the test bacterial suspension is inoculated, proceed with the irradiation test described in 9.3.

NOTE 1 4 ml to 6 ml sterilized water added per Petri dish is adequate.

NOTE 2 The regulated suspension quantity can create leakage of suspension from the film edge or might not be enough to spread the suspension uniformly. In such a case, 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 must be the same as with the 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.

9.1.3 For the 3 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 washout the test bacteria. Quickly use this washout solution to perform a measurement of the number of viable cells.

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

9.1.4 For the specimens of 9.3.3 and 9.3.4, perform the washout in the same manner as in 9.1.3.

9.2 Glass adhesion method

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

a) Inoculate stock strain to the 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) during 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 rpm with about 3 cm of amplitude) during (3 ± 1) h at (37 ± 1) °C to reach 10^7 cells/ml (incubation C).

d) Estimate the bacteria concentration of the 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 $(1 \pm 0{,}3) \times 10^5$ 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.

9.2.2 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, 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. Except for 3 standard cloth pieces for viable cell count performed just after the test bacterial suspension is inoculated, proceed with the irradiation test described in 9.3.

NOTE 1 4 ml to 6 ml sterilized water added per Petri dish is adequate.

NOTE 2 The regulated suspension quantity can create leakage of suspension from the edge or might not be enough to soak the suspension uniformly. In such a case, 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 must be the same as with the standard size specimen, with $1{,}4 \times 10^4$ cells to $2{,}6 \times 10^4$ cells.

NOTE 3 In order to well permeate the sample 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.

9.2.3 For the 3 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 solution for washout, rub the

Stomacher bag well by hand and also the non-treated cloths and the glasses, and washout the test bacteria. Quickly use this washout solution to perform measurement of the number of viable cells.

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

9.2.4 For the specimens of 9.3.3 and 9.3.4, perform the washout in the same manner as in 9.2.3.

9.3 UV irradiation condition

9.3.1 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 UV intensity referred to 9.3.2 is complied with by reading the indicator value.

9.3.2 Test the UV intensity condition, depending on the circumstances where the materials are used. 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 test

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

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

9.3.3 Expose to light the Petri dishes containing the specimens (3 non-treated specimens and 3 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.

9.3.4 Keep the Petri dishes containing the specimens (3 non-treated specimens and 3 photocatalytic treated specimens) with bacterial suspension in a dark place, for the same time as in 9.3.3.

9.4 Measurement of 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 solution and thoroughly agitated again. This process is repeated to obtain a series of dilutions, in compliance 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 Petri dishes each. 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 min 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 the washout liquid is obtained by Equation (1) and expressed to two significant digits.

$$P = Z \times R \quad (1)$$

where

P is the bacteria concentration (cells/ml);

Z is the average number of colonies in 2 Petri dishes;

R 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 1 in the Petri dishes with 1 ml of wash-out solution, the average number is taken as 1.

10 Calculation

The test results are calculated as follows. The calculated values are usually rounded to the second decimal place in accordance with ISO 31-0.

NOTE Examples of text results are given in Annex A.

10.1 Film adhesion method

10.1.1 Test requirement fulfilment validation

Use the bacteria concentration obtained in 9.4 and apply Equation (2) to calculate the number of viable bacteria.

$$N = P \times V \quad (2)$$

where

N is the number of cells of viable bacteria;

P is the bacteria concentration obtained in 9.4 (cells/ml);

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

A test is considered valid if it fulfils all of the following 4 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 Equation (3)

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

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 3 specimens.

2) The logarithmic value of viable bacteria of non-treated specimens after inoculation shall be within the $1{,}0 \times 10^5$ 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 3 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 3 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.

10.1.2 Photocatalyst antibacterial activity value calculation

Use Equations (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.

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

where

R_L is the photocatalyst antibacterial activity value, 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.

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

where

ΔR is the photocatalyst antibacterial activity value with UV irradiation;

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.

10.2 Glass adhesion method

10.2.1 Test requirement fulfilment validation

Use the bacteria concentration obtained in 9.4 and apply Equation (6) to calculate the number of viable bacteria.

$$M = P \times 20 \quad (6)$$

where

M is the number of cells of viable bacteria;

P is the bacteria concentration obtained in 9.4 (cells/ml);

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

Test requirement validation uses the propagation value. The propagation value is obtained by Equations (7) and (8). The last 2 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 3 non-treated specimens, after UV irradiation of intensity L;

M_{BA} is the average logarithmic value of the number of viable bacteria for 3 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 3 non-treated specimens, after being kept in a dark place.

10.2.2 Photocatalyst antibacterial activity value calculation

For the completed test, the photocatalyst antibacterial activity value is obtained with up to 2 digits using Equations (9) and (10).

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

where

S_L is the photocatalyst antibacterial activity value, after UV irradiation of intensity L;

M_L is the average logarithmic value of the number of viable bacteria for 3 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;

M_D is the average logarithmic value of the number of viable bacteria for 3 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 adhesion method, type and size of adhesive 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, ΔR in 10.1.2;

h) in the glass adhesion method, type and size of adhesive glass and moisture preservation glass; type of test bacteria; bacteria strain number; inoculated bacteria concentration; values of S_L, ΔS in 10.2.2;

i) in the glass adhesion 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 adhesion method are shown in Tables A.1 and 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 adhesion method are shown in Tables A.3 and 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
Average	2,50	1,20
σ_{n-1}	0,52	0,17