INTERNATIONAL STANDARD

ISO 17094

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

Céramiques techniques — Méthode d'essai de l'activité antibactérienne des matériaux photocatalytiques semiconducteurs dans un environnement d'éclairage intérieur

Reference number
ISO 17094:2014(E)

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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 on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is ISO/TC 206, *Fine ceramics*.

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Introduction

A test method for cloths or textiles is not included in this International Standard because of a lack of indoor-light-active photocatalytic cloths or textiles. When indoor-light-active photocatalytic cloths or textiles have been developed, a suitable test method will be proposed with the remediated glass adhesion method given in ISO 27447.

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

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 presents a test method for determining the antibacterial activity of materials that contain an indoor-light-active photocatalytic material or have indoor-light-active photocatalytic films on the surface by measuring the survival of bacteria after illumination with indoor light.

It is intended for use with different kinds of indoor-light-active photocatalytic materials used in construction materials in flat sheet, board or plate shape that are the basic forms of materials for various applications. It does not include powder, granular, or porous indoor-light-active photocatalytic materials, nor is it applicable to cloths or textiles.

It is applicable to indoor-light-active photocatalytic materials produced for antibacterial application. Other types of performance of indoor-light-active photocatalytic materials, i.e. decomposition of water contaminants, self-cleaning, antifogging, and air purification, cannot be determined by this method.

2 Normative references

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

ISO 27447, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials*

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

3 Terms and definitions

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

3.1
photocatalyst
substance that performs one or more functions based on oxidization and reduction reactions under photoirradiation, including decomposition and removal of air and water contaminants, deodorization, and antibacterial, antifungal, self-cleaning, and antifogging actions

3.2
indoor-light-active photocatalyst
photocatalyst that functions under illumination with artificial light used for general lighting purposes

3.3
indoor lighting environment
illumination with artificial light source(s) used for general lighting purposes and excluding sunlight

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3.4
indoor-light-active photocatalytic material
material in which or on which the indoor-light-active photocatalyst is added by coating, impregnation, mixing, etc

3.5
antibacterial
condition inhibiting the growth of bacteria on the surface of flat surface materials

3.6
indoor-light-active photocatalyst antibacterial activity value
numerical difference between the logarithmic values of the total number of viable bacteria on the indoor-light-active photocatalytic treated material and non-treated material after indoor light illumination

Note 1 to entry: This value includes the decrease of number of bacteria without indoor light illumination.

3.7
indoor-light-active photocatalyst antibacterial activity value with indoor light illumination
numerical difference between the logarithmic values of the total number of viable bacteria on the indoor-light-active photocatalytic treated material after indoor light illumination and the same material kept in the dark

4 Symbols

A	average number of viable bacteria on non-treated test pieces, just after inoculation
B_D	average number of viable bacteria on non-treated test pieces, after being kept in a dark place
B_L	average number of viable bacteria on non-treated test pieces, after indoor light illumination of intensity L
C_D	average number of viable bacteria on indoor-light-active photocatalytic treated test pieces, after being kept in dark place
C_L	average number of viable bacteria on indoor-light-active photocatalytic treated test pieces, after indoor light illumination of intensity L
D_F	dilution factor
L	illuminance of indoor light
L_{max}	maximum logarithmic value of viable bacteria
L_{mean}	average logarithmic value of viable bacteria for three test pieces
L_{min}	minimum logarithmic value of viable bacteria
N	number of viable bacteria
P	bacteria concentration
R_L	indoor-light-active photocatalyst antibacterial activity value, after illumination at a constant intensity (L) on an indoor-light-active photocatalytic material
ΔR	indoor-light-active photocatalyst antibacterial activity value with indoor light illumination

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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

The method is used to obtain the antibacterial activity of indoor-light-active photocatalytic materials by contact of a test piece with bacteria, under indoor lighting condition. The film cover method is available for flat sheet, board, or plate-shaped materials.

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

6 Materials

6.1 Bacteria strains and preparation for tests

6.1.1 Bacteria strains

The bacteria strains to be used in the test shall be the same as or equivalent to those described in Table 1 and supplied by an entity that is registered under the World Federation for Culture Collections or the Japan Society for Culture Collections.

Table 1 — Bacteria strains to be used in test

Bacteria species	WDCM code
Staphylococcus aureus	WDCM 00195
Escherichia coli	WDCM 00196
NOTE Refer to WDCM (World Data Centre for Microorganisms) and its website: http://www.wdcm.org/.	

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

6.1.2 Bacteria preparation

Aseptic manipulations using microorganisms should be performed in an adequate safety cabinet. Inoculate each strain into 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 one month by replicating this process. The maximum number of subcultures from the original strain transferred by culture collection is 10. A slant culture shall not be stored for more than one month.

NOTE 1 In the case of bacteria stored in deep freezer, the maximum number of subcultures from original strain transferred by culture collection is 10.

NOTE 2 If activity of used bacteria is maintained, agar plates can be used.

6.2 Chemicals and implements

6.2.1 General

Commercial media of same components described below can be used.

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Volume of prepared media should be adjusted in accordance with the number of test pieces.

6.2.2 1/500 nutrient broth (1/500 NB)

For 100 ml of purified water, take 0,3 g meat extract, 1,0 g peptone, and 0,5 g sodium chloride, put them into 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. Take 2 ml of this medium and dilute it by 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.

6.2.3 Nutrient agar

For 1 000 ml of purified water, take 3,0 g meat extract, 5,0 g peptone, put them into 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 one 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 casein peptone, 3,0 g soybean peptone, 5,0 g sodium chloride, 2,5 g dipotassium hydrogenphosphate, 2,5 g glucose, 1,0 g lecithin, put them into a flask and dissolve them thoroughly. Add 7,0 g polyoxyethylene sorbitan monooleate and dissolve it. Use a solution of sodium hydroxide or hydrochloric acid to bring the pH of (7,0 ± 0,2) at 25 °C. Sterilize in an autoclave (see 6.2.2). 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 one month ago.

6.2.5 Physiological saline solution

For 1 000 ml of purified water, take 8,5 g sodium chloride, put it into a flask and dissolve it thoroughly. Sterilize in an autoclave (see 6.2.2). 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 one month ago.

7 Apparatus

7.1 General

The testing equipment enables an indoor-light-active photocatalytic material to be examined for its antibacterial activity by providing indoor light illumination to activate the indoor-light-active photocatalyst. It consists of a light source and a chamber containing the test piece. A schematic of a testing system is shown in Figure 1.

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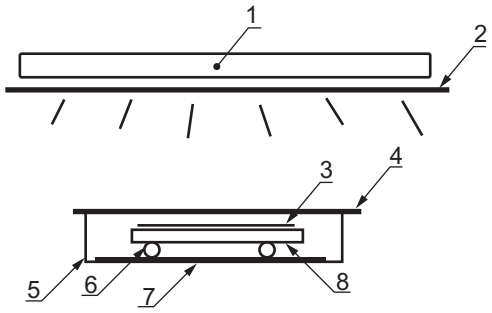

Key

1 light source
2 UV sharp cut-off filter
3 cover film
4 moisture preservation glass plate
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 shall be inert and non-water absorbent with good sealing properties, with an optical transmittance over 85 % for the 380 nm to 780 nm range. The sheets should be cut in a (40 ± 2) mm × (40 ± 2) mm square.

7.3 Moisture preservation glass plate

The moisture preservation glass consists of a glass plate with a thickness less than 1,1 mm, with an optical transmittance over 85 % for the 380 nm to 780 nm range. The glass plates should be sufficient to fully cover Petri dishes.

7.4 Glass tube or glass rod

The glass tube or glass rod is specified in ISO 27447. The glass tube or glass rod should be prepared by cutting it to 10 cm to 15 cm length and bending it into a U-shape or V-shape.

7.5 Light source

The light source for indoor lighting condition is specified in ISO 14605. A halophosphate fluorescent lamp with a correlated colour temperature of between 3 800 K to 4 500 K shall be used for the light source.

When a halophosphate fluorescent lamp is not available, a three band fluorescent lamp, with a correlated colour temperature of between 3 800 K and 4 500 K and a colour rendering index (Ra) higher than 80, can be used as a substitute.

7.6 UV sharp cut-off filter

An UV sharp cut-off filter specified in ISO 14605 shall be used under UV cut-off condition (condition A or condition B). The UV sharp cut-off filter shall be mounted immediately below the lamp.

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Condition A (under 400 nm cut-off condition)

Type A UV sharp cut-off filter specified in ISO 14605

Condition B (under 380 nm cut-off condition)

Type B UV sharp cut-off filter specified in ISO 14605

7.7 Illuminance meter

The illuminance meter is specified in ISO 14605.

8 Test piece

Cut a flat portion of the material in a (50 ± 2) mm × (50 ± 2) mm square. The thickness of materials should be not exceed 10 mm. Use it as the standardized shaped test piece. Prepare nine pieces of non-treated test pieces and six pieces of indoor-light-active photocatalytic treated test pieces. When non-treated test pieces cannot be provided, use glass plates instead. Take great care to avoid microbial contamination and cross-contamination among test pieces.

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 test piece size as long as the test piece surface can be covered with a 400 mm^2 to 1 600 mm^2 cover film. When the test piece surface is stained with organic contaminant, it is acceptable to first eliminate contaminant in accordance with ISO 27447. If necessary, test pieces can be disinfected prior to testing (e.g. by wiping with ethanol or 70 % ethanol in water).

9 Procedure

9.1 General

The flowchart of test method is shown in Figure 2.

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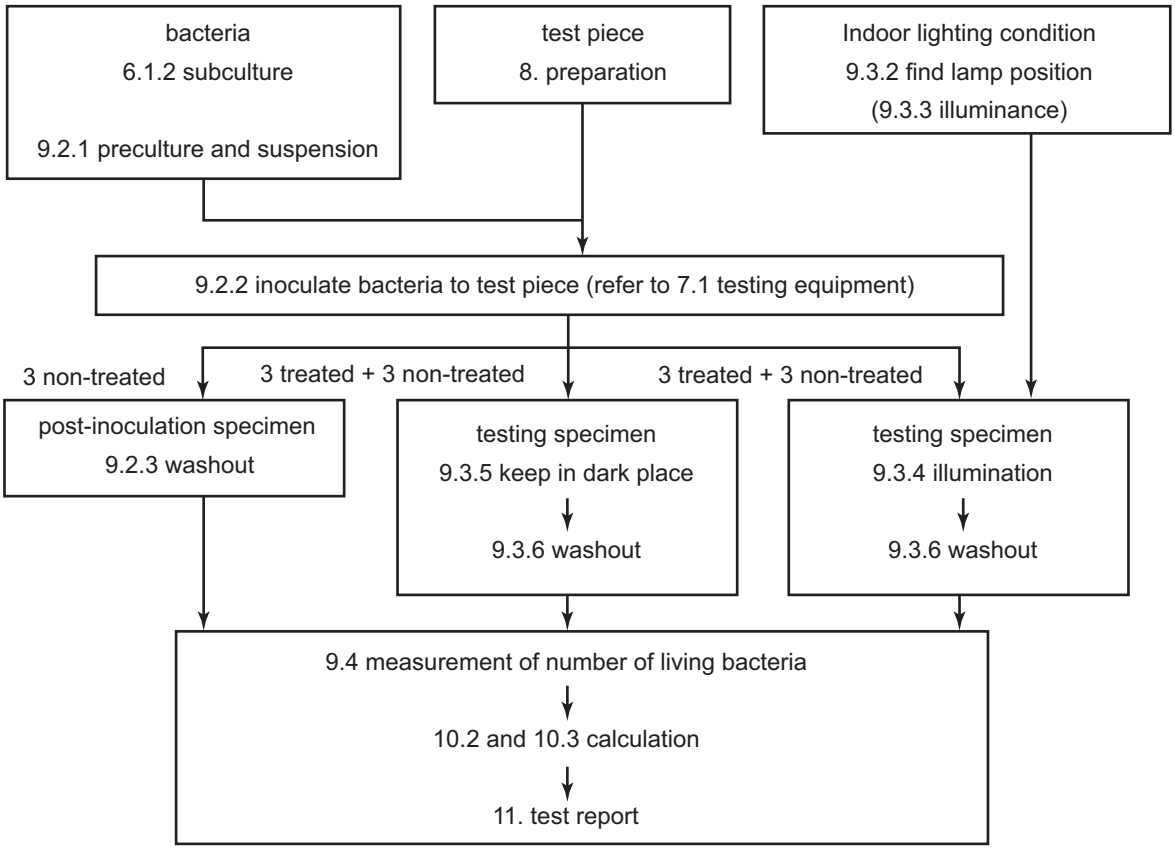

Figure 2 — A flowchart of test method

9.2 Cover film 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 incubated 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 bacteria count using 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.

9.2.2 Lay a sterilized moisture control paper filter in the bottom of a sterilized Petri dish, add an adequate quantity of sterilized water, in order to avoid contact between the test piece and the wetted paper filter, place a bent glass tube or rod, as described in 7.4, on the paper filter and place the test piece on it with the photocatalytic treated surface up. Collect 0,15 ml test bacterial suspension with a sterilized pipette and drop it onto the test piece. Put a piece of cover film on top of the dripped suspension and lightly push to get the suspension spread to the whole cover film surface, while taking care that no suspension leaks out of the cover film edge. Then place a moisture conservation glass on the top of Petri dish. Repeat this procedure for each of the photocatalytic treated and non-photocatalytic treated test pieces used in the test (six of the former and nine of the latter). Except for three non-treated test pieces for viable cell count performed just after test bacterial suspension is inoculated, proceed with illumination test of 9.3.

NOTE 1 In order to prevent the moisture conservation glass misting over, 4 ml to 6 ml sterilized water added per Petri dish (90 mm in diameter) is adequate.

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NOTE 2 The regulated suspension quantity can create leakage of suspension from the cover film edge or might not be enough to spread 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 test piece must be the same as with standard size test piece, 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 test pieces (for different sized test pieces than described in Clause 8) shall be proportional to the cover film area used.

9.2.3 For the three non-treated bacterial suspension inoculated test pieces for the test (post-inoculation test pieces of test bacteria), put the cover film and non-treated test piece in a Stomacher® bag[1)] using sterilized tweezers, taking care to avoid bacterial suspension leakage from cover film and non-treated test piece. Add 10 ml of SCDLP, rub the test pieces and the cover film well from outside the Stomacher bag by hands and washout the test bacteria. Quickly bring this washout solution to perform measurement of number of viable cells.

9.3 Indoor lighting condition

9.3.1 Depending on the real conditions where the indoor-light-active photocatalyst material is used, choose the UV cut-off condition(s) from the two conditions referred to in 7.6.

Maintain the temperature around test pieces at 25 °C ± 5 °C throughout a period of time in 9.3.4 and 9.3.5.

9.3.2 Set the illuminance meter on the base of the illumination apparatus. Place the cover film and glass plate used for testing on top of the sensor.

9.3.3 Adjust the intensity of the lamp to give an illuminance of 1 000 lx ± 50 lx at surface of the test pieces.

NOTE This illuminance can be altered between 100 lx ± 5 lx and 3 000 lx ± 150 lx to take into account the real conditions where the indoor-light-active photocatalyst material is effectively used.

9.3.4 Expose to light the Petri dishes containing the test pieces (three non-treated test pieces and 3 indoor-light-active photocatalytic treated test pieces) with bacterial suspension for 8 h.

NOTE This exposure time can be altered between 4 h and 24 h to take into account the real conditions where the indoor-light-active photocatalyst material is effectively used.

9.3.5 Keep the Petri dishes containing the test pieces (three non-treated test pieces and three indoor-light-active photocatalytic treated test pieces) with bacterial suspension, in a dark place for the same length of time as used as in 9.3.4.

9.3.6 For the test pieces of 9.3.4 and 9.3.5, perform the washout in the same manner as in 9.2.3.

9.4 Measurement of number of living bacteria

1) Take 1 ml of washout solution (see 9.2.3) with a sterilized pipette. Add to (9 ± 0,1) ml of physiological saline solution in a test tube and agitate thoroughly.

2) Take 1 ml of the solution [see item 1)] with a new sterilized pipette. Add to another test tube containing (9 ± 0,1) ml of physiological saline solution and agitate thoroughly again.

   This process is repeated to obtain a series of dilutions, in compliance with the 10- times dilution method.

1) Stomacher® bag is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

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3) Take 1 ml of the solution from the tubes of each series [see 9.2.3 and items 1) and 2)] with new sterilized pipettes and place 1 ml of the solution in two Petri dishes each.

4) Add 15 ml to 20 ml of nutrient agar kept at 45 °C to 48 °C in each Petri dish [see item 3)]. Allow them to stand for 15 min at room temperature.

5) Place the Petri dishes upside down, when the agar medium solidifies. Incubate for 40 h to 48 h at (37 ± 1) °C.

6) Count colony numbers in the series Petri dishes with 30 colonies to 300 colonies.

The bacterial concentration of washout liquid is obtained by Formula (1) at two significant digits.

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

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 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 1.

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.

10.2 Test requirement fulfilment validation

Use the bacteria concentration obtained in 9.4 and apply Formula (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 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) Logarithmic value of number of viable bacteria of non-treated test pieces after inoculation is derived from Formula (3).

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$$(L_{max} - L_{min}) / (L_{mean}) \leq 0,2 \quad (3)$$

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 of three test pieces.

2) The number of viable bacteria of non-treated test pieces after inoculation shall be within $1,0 \times 10^5$ to $4,0 \times 10^5$ cells range.

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

10.3 Indoor light-active photocatalyst antibacterial activity value calculation

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

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

$$R_L = \left[\log_{10}(B_L/A) - \log_{10}(C_L/A)\right] = \log_{10}(B_L/C_L) \quad (4)$$

where

R_L is the indoor-light-active photocatalyst antibacterial activity value, after indoor light illumination of intensity L;

L is the indoor light illumination intensity (lx);

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

B_L is the average number of viable bacteria of non-treated test pieces, after indoor light illumination of intensity L;

C_L is the average number of viable bacteria of indoor-light-active photocatalyst treated test pieces, after indoor light illumination of intensity L.

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$$\Delta R = \log_{10}[B_L/C_L] - [\log_{10}(B_D/A) - \log_{10}(C_D/A)] = \log_{10}[B_L/C_L] - \log_{10}[B_D/C_D] \quad (5)$$

where

ΔR is the indoor-light-active photocatalyst antibacterial activity value with indoor light illumination;

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

C_D is the average number of viable bacteria of indoor-light-active photocatalyst treated test pieces, after being kept in a dark place.

11 Test report

The test report shall include the following information.

a) a reference to this International Standard (i.e. ISO 17094);

b) description of the type, size, shape, thickness of indoor-light-active photocatalyst, and non-treated test pieces;

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

d) type of test bacteria, bacteria strain number;

e) manufacturer of fluorescent lamp, product number;

f) type of UV sharp cut-off filter (manufacturer, product number);

g) manufacturer of illuminance meter, product number;

h) lighting conditions including illuminance and light exposure duration;

i) type and size of cover film and moisture preservation glass;

j) quantity of inoculated test bacterial suspension, number of viable bacteria of test suspension;

k) values of A, B_L, C_L, R_L, B_D, C_D, and ΔR in 10.3.

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