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**Fine ceramics (advanced ceramics,  
advanced technical ceramics) —  
Determination of bacterial reduction  
rate by semiconducting photocatalytic  
materials under indoor lighting  
environment — Semi-dry method  
for estimating antibacterial activity  
on the actual environmental bacteria  
contamination surface**

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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](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

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## 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](http://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](http://www.iso.org/patents)).

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

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

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](http://www.iso.org/members.html).

## Introduction

This document applies to testing the antibacterial activity of indoor-light-active photocatalytic ceramics and other materials, produced by either coating or mixing of a light-active photocatalyst in a simulated indoor lighting environment. The International Standard for testing the antibacterial activity of photocatalytic materials has been published as ISO 27447 and the International Standard for testing the antibacterial activity of indoor-light-active photocatalytic materials has been published as ISO 17094.

Recently, it was discovered that antibacterial activities by indoor-light-active photocatalyst showed different results between the ISO 17094 testing in a laboratory and the evaluation in a real environment. Thus, the ISO 17094 testing method should be complemented by a testing method that simulates an indoor lighting environment.

ISO 17094 is one of the best methods for measuring the potential of antibacterial activity using photocatalysts. This document can measure the reduction rate of living bacteria by testing with impure substances similar to an indoor lighting environment.

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# Fine ceramics (advanced ceramics, advanced technical ceramics) — Determination of bacterial reduction rate by semiconducting photocatalytic materials under indoor lighting environment — Semi-dry method for estimating antibacterial activity on the actual environmental bacteria contamination surface

**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 establishes a test method for determining the antibacterial activity of materials containing an indoor-light-active photocatalytic material on the surface. The antibacterial reduction rate is determined by measuring the survival of bacteria after illumination with indoor light. This test assumes a surface with high potential of possible person contact with bacteria. This test is designed to evaluate the suppression of contact infection of bacteria using an indoor-light-active photocatalytic material under indoor lighting environment.

It is intended for use with different kinds of indoor-light-active photocatalytic materials used in construction, for example, flat sheets, board or plate shapes, which are the basic forms of materials for various applications. It is not applicable to powder, granular, or porous indoor-light-active photocatalytic materials, as well as cloths or textiles.

It is applicable to indoor-light-active photocatalytic materials produced for antibacterial application. Other types of indoor-light-active photocatalytic materials applications, i.e. decomposition of water contaminants, self-cleaning, antifogging, and air purification, are non-applicable by this method.

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

ISO 17094:2014, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials under indoor lighting environment*

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

**3.2  
indoor-light-active photocatalyst**

*photocatalyst* (3.1) 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

**3.4  
indoor-light-active photocatalytic material**

material in which or on which the *indoor-light-active photocatalyst* (3.2) is added by, for example, coating, impregnation or mixing

**3.5  
reduction rate of living bacteria**

reduction of the viable bacteria counts on the surface of *indoor-light-active photocatalytic materials* (3.4) versus that of non-treated materials after treatment under illumination or in a dark condition for a designated time, expressed as a proportion of the bacteria in percentage per unit of inoculating mass

**3.6  
dispersion medium**

medium used for the preparation of a test bacterial solution, comprising a chemical component, which simulates human sebum

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**4 Principle**

The testing method is used to obtain the antibacterial activity of indoor-light-active photocatalytic materials under indoor lighting condition by contact of a test piece with bacteria suspended in a dispersion medium simulating human sebum. This method is applicable to materials with smooth surfaces, such as flat sheet, board or plate-shaped materials.

The surface of the test piece is uniformly inoculated with bacterial suspension. The inoculated test piece is placed on a Petri dish and a glass plate 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. This washout suspension is measured by the viable bacterial count method.

**5 Materials**

**5.1 Bacteria strains and preparation for tests**

**5.1.1 Bacteria strains**

The bacterial strains to be used in the test shall be the same as or equivalent to those described in [Table 1](#) and supplied by an entry registered under the World Federation for Culture Collections or the Japan Society for Microbial Resources and Systematics.



Table 1 — Bacteria strains to be used in the test

Bacteria species	WDCM code
<i>Staphylococcus epidermidis</i>	WDCM 00132
<i>Escherichia coli</i>	WDCM 00012

### 5.1.2 Subculture of bacteria strains

Testing is performed in a biosafety level testing room equipped for the required type of microorganisms to be used. A nutrient agar slant medium (NA slant medium) is inoculated with *Staphylococcus epidermidis* or *Escherichia coli* and streak cultured for 24 h to 48 h at  $(37 \pm 1) ^\circ\text{C}$ . The cultured bacteria can be stored at  $5 ^\circ\text{C}$  to  $10 ^\circ\text{C}$  for one month. Using this material as a stock strain, the cultured bacteria can be subcultured up to 10 times under the same conditions.

NOTE 1 For long-term storage such as cryopreservation, appropriate measures are taken.

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

## 5.2 Chemicals and implements

The chemicals, materials and implements used in this testing are as follows, unless otherwise designated. The commercial media of the same components described below can be used.

### 5.2.1 Dispersion medium for *Staphylococcus epidermidis*

Add 49 ml of sterile water and 50 g of weighed triol-type polypropylene glycol (alpha, alpha', alpha"-1,2,3-propanetriyltris [omega-hydroxy-poly[oxy(methyl-1,2-ethanediyl)]], average molecular weight 300 (PPG 300)) to a flask with a magnetic stirring bar. Immediately after addition, stir with the bar. Then, add 1 g of polyethylene glycol [average molecular weight 500 000 (PEG 500K)] under continuous stirring, and stir for 1 h. Sterilize in an autoclave at  $(121 \pm 2) ^\circ\text{C}$  for at least 15 min. After dissolving, use sodium hydroxide or hydrochloric acid to adjust the solution pH to  $(7,1 \pm 0,2)$  at  $25 ^\circ\text{C}$ . Adjust to 100 ml using sterile water. Sterilize in an autoclave at  $(121 \pm 2) ^\circ\text{C}$  for at least 15 min and store at room temperature. Do not use dispersion medium made more than one month previously.

### 5.2.2 Dispersion medium for *Escherichia coli*

Add 30 ml of sterile water and 0,1 g of weighed mucin to a flask with a magnetic stirring bar. Immediately after addition, stir with the bar. Then, add 0,2 g of sodium alginate under continuous stirring. After dissolving, add 20 ml of glycerol. Store at room temperature. Do not use dispersion medium made more than one month previously.

### 5.2.3 1/5-concentration nutrient broth medium (1/5 NB)

For 100 ml of sterile water, take 0,3 g of meat extract, 1,0 g of peptone and 0,5 g of 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  $(7,1 \pm 0,1)$  at  $25 ^\circ\text{C}$ . Dilute this solution fivefold using sterile water, and adjust the pH to  $(7,0 \pm 0,2)$  at  $25 ^\circ\text{C}$  using sodium hydroxide or hydrochloric acid. Sterilize in an autoclave at  $(121 \pm 2) ^\circ\text{C}$  for at least 15 min. After preparation, if 1/5 NB is not used immediately, store it at  $5 ^\circ\text{C}$  to  $10 ^\circ\text{C}$ . Do not use 1/5 NB made more than one month previously.

### 5.2.4 1/2-concentration luris-bertani broth medium (1/2 LB)

For 100 ml of sterile water, take 0,5 g yeast extract, 1 g tryptone and 1 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  $(7,1 \pm 0,1)$  at  $25 ^\circ\text{C}$ . Dilute this solution twofold

using sterile water, and adjust the pH to  $(7,0 \pm 0,2)$  at 25 °C using sodium hydroxide or hydrochloric acid. Sterilize in an autoclave at  $(121 \pm 2)$  °C for at least 15 min. After preparation, if 1/2 LB is not used immediately, store it at 5 °C to 10 °C. Do not use 1/2 LB made more than one month previously.

**5.2.5 Nutrient agar medium (NA medium)**

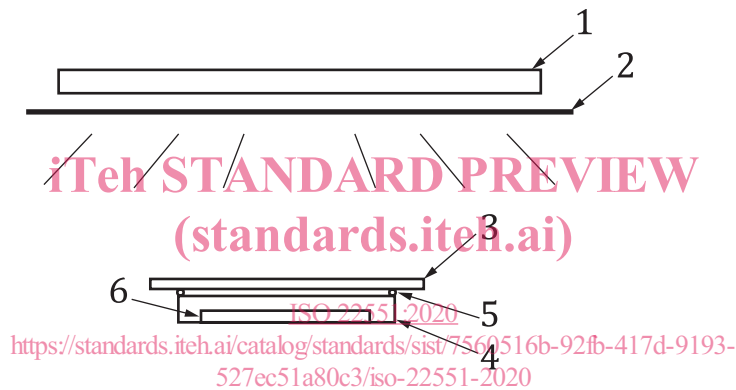
The preparation of the NA medium shall follow ISO 17094:2014, 6.2.3.

**5.2.6 Soybean-casein digest broth with lecithin and polysorbate 80 (SCDLP medium)**

The preparation of the SCDLP medium shall follow ISO 17094:2014, 6.2.4.

**6 Apparatus**

The testing equipment enables examination of antibacterial activity using an indoor-light-active photocatalytic material 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 representation of the testing system is shown in [Figure 1](#).



**Key**

- |   |                         |   |            |
|---|-------------------------|---|------------|
| 1 | light source            | 4 | Petri dish |
| 2 | UV sharp cut-off filter | 5 | spacer     |
| 3 | glass plate             | 6 | test piece |

**Figure 1 — Schematic diagram of the test equipment**

**6.1 Light source**, as specified in ISO 14605, for indoor lighting condition. A halophosphate fluorescent lamp with a correlated colour temperature of 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 3 800 K to 4 500 K and a colour rendering index (Ra) higher than 80 can be used as a substitute.

**6.2 UV sharp cut-off filter**, as specified in ISO 14605, used under UV cut-off condition (condition B). The UV sharp cut-off filter shall be mounted immediately below the lamp. Condition B is under 380 nm cut-off condition. Type B UV sharp cut-off-filter is specified in ISO 14605.

**6.3 Moisture preservation glass plate**, as specified in ISO 17094:2014, 7.3, to prevent contamination by floating bacteria.

**6.4 Illuminance meter**, as specified in ISO 14605.

## 7 Test piece

Cut a  $(50 \pm 2)$  mm  $\times$   $(50 \pm 2)$  mm flat portion of the material. The thickness of the material should not exceed 10 mm. Use this as the standardized shaped test piece. A film-like material may be attached to non-treated glass to maintain its strength. 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, a material as similar as possible to that of the indoor-light-active photocatalytic treated test pieces can be used. Take great care to avoid microbial contamination and cross-contamination among test pieces. If the surface of the test piece is contaminated by organic material, the test piece may be UV irradiated for up to 24 h using approximately  $1,0 \text{ mW/cm}^2$ , or wiped clean with ethanol. If a non-treated test piece cannot be provided, it is necessary to use a material that would produce a similar bacteria recovery rate to that of an indoor-light-active photocatalytic treated test piece.

## 8 Procedure

### 8.1 Preparation of test bacterial solution

#### 8.1.1 Culturing of test bacteria

Transfer bacteria (5.1.2) to the NA medium (slant or plate) using a platinum loop and incubate at  $(37 \pm 1) \text{ }^\circ\text{C}$  for 24 h to 48 h. Transfer the incubate bacteria to a new NA medium (slant or plate) and incubate at  $(37 \pm 1) \text{ }^\circ\text{C}$  for 24 h to 48 h. This cultivated bacteria shall be used as test bacteria.

#### 8.1.2 Preparation of test bacterial solution

Scrape the test bacteria cultured in 8.1.1 from the NA medium using a platinum loop, suspend the material in a glass reagent tube containing 5 ml of 1/5 NB for *Staphylococcus epidermidis*, 10 ml of 1/2 LB for *Escherichia coli*, and agitate thoroughly. The obtained solution shall be used as the original bacterial solution. Add the dispersion medium described in 5.2.1 (for *Staphylococcus epidermidis*) or 5.2.2 (for *Escherichia coli*) to the glass reagent tube. Then add 1/100 (for *Staphylococcus epidermidis*) or 1/10 (for *Escherichia coli*) of the amount of the original bacterial solution and agitate thoroughly. Adjust the test bacterial solution to a concentration of  $5,0 \times 10^2 \text{ cfu/mg}$  to  $2,0 \times 10^3 \text{ cfu/mg}$ . After adjustment, use immediately. If the test bacterial solution is not to be used immediately, store it on ice and use within 2 h. In all cases, use glass reagent tubes for preparation of the test bacterial solution. *Staphylococcus epidermidis* adheres readily to the surface of plastic materials.

**NOTE** The high viscosity of the dispersion medium impedes blending of the dispersion medium and the original bacterial solution. One way to achieve a nearly homogenous test bacterial solution is to repeat the process three times or more (inverting the tube five times and mixing the tube by vortex mixer for 5 s).

### 8.2 Application of test bacterial solution

#### 8.2.1 Measurement of test piece mass

Measure the mass of all test pieces to a precision of 0,1 mg and record.

#### 8.2.2 Application of test bacterial solution

Apply  $(2,5 \pm 0,5)$  mg of the test bacterial solution as prepared in 8.1.2 to test pieces (nine non-treated test pieces and six indoor-light-active photocatalytic treated test pieces) uniformly. 8.2.3 provides an example of the application method. However, other similar application methods may be employed.

#### 8.2.3 Direct application method

Transfer 0,2 ml of the bacterial solution to the tip of a sterilized cellulose non-woven folded cloth as shown in Figure 2. Prepare a piece of aluminium foil by wiping clean with alcohol and inoculate an area of approximately  $15 \text{ cm} \times 15 \text{ cm}$  to eliminate excess bacterial solution. Then apply the solution on the