

DRAFT INTERNATIONAL STANDARD

ISO/DIS 18071

ISO/TC 206

Voting begins on:

2015-11-26

Secretariat: JISC

Voting terminates on:

2016-02-26

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

Titre manque

ICS: 81.060.30

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Reference number
ISO/DIS 18071:2015(E)

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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.

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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.

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ISO 18071 was prepared by Technical Committee ISO/TC 206, *Fine ceramics*.

This second/third/... edition cancels and replaces the first/second/... edition (), [clause(s) / subclause(s) / table(s) / figure(s) / annex(es)] of which [has / have] been technically revised.

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Introduction

This standard applies to testing the antiviral activity of indoor-light-active photocatalytic ceramics and other materials produced by either coating or mixing of a light-active photocatalyst. The standard for testing the antibacterial activity of photocatalytic materials have been published as ISO 27447 and the standard for testing the antibacterial activity of indoor-light-active photocatalytic materials have been published as ISO17094. The standard for determination of antiviral activity of semiconducting photocatalytic materials have also been published as ISO18061.

The test method for cloths or textiles is not included in this draft, because of lack of indoor-light-active photocatalytic cloths or textiles. When the indoor-light-active photocatalytic cloths or textiles with antiviral activity using indoor-light-active photocatalytic activity have been developed, test method for indoor-light-active photocatalytic cloths or textiles will be proposed with the glass adhesion method in ISO27447.

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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

WARNING –Only personnel trained in microbiological techniques should carry out tests.

1 Scope

This test method specifies the determination of the antiviral activity of materials that contain indoor-light-active photocatalytic materials or have indoor-light-active photocatalytic films on the surface, by measuring the infectivity titre of bacteriophage Q-beta after illumination with indoor light.

NOTE In this test method, the surrogate microbe is bacteriophage Q-beta, intended as a model for Influenza viruses.

This test method 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.

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

The values expressed in this 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 80000-1, *Quantities and units -- Part 0: General principles*

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) -- Visible light source of testing semiconducting photocatalytic materials*

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 photoirradiation, including decomposition and removal of air and water contaminants, deodorization, and antiviral, antibacterial, antifungal, self-cleaning and antifogging actions.

**3.2
indoor-light-active photocatalyst**

of the photocatalyst, substance that reacts with artificial light source for general lighting service (i.e. indoor lighting environment).

**3.3
indoor lighting environment**

the environment with artificial light source for general lighting service. Not include sunlight.

**3.4
indoor-light-active photocatalytic materials**

materials in which or on which the indoor-light-active photocatalyst is added by coating, impregnation, mixing, etc.

**3.5
antiviral**

condition decreasing the infectivity of viruses on the surface of materials

**3.6
bacteriophage**

type of virus which infects bacteria

NOTE The bacteriophage used in this test method is Q-beta that is one of F-specific RNA phages. The bacteriophage Q-beta is not pathogenic to humans and animals, but serves to simulate Influenza viruses that are pathogenic to humans.

**3.7
plaque**

visible, clear area which is theoretically the result of infection and lysis of host cells by a single viable bacteriophage

**3.8
indoor-light-active photocatalyst antiviral activity value**

difference between the logarithms of the total number of bacteriophage plaques on photocatalytic treated materials after indoor light illumination and on non-treated materials after indoor light illumination

NOTE This value includes the decrease of number of bacteriophage plaques without Indoor light illumination.

**3.9
indoor-light-active photocatalyst antiviral activity value for Indoor light illumination**

difference between the logarithms of the total number of bacteriophage plaques on photocatalytic treated materials after indoor light illumination and on photocatalytic treated materials kept in the dark place

4 Symbols (and abbreviated terms)

- A average of titre of bacteriophage on non-treated specimens, just after inoculation
- B_D average of titre of bacteriophage on non-treated specimens, after being kept in a dark place
- B_{F-L} average of titre of bacteriophage on non-treated specimens, after indoor light illumination of intensity L under condition F

C_D	average of titre of bacteriophage on indoor-light-active photocatalytic treated specimens, after being kept in a dark place
C_{F-L}	average of titre of bacteriophage on indoor-light-active photocatalytic treated specimens, after indoor light illumination of intensity L under condition F
D_i	dilution factor
F	type of UV cut-off condition (condition A or condition B)
L	illuminance of indoor light
Log_{max}	maximum logarithmic value of titre of bacteriophage
Log_{mean}	average logarithmic value of titre of bacteriophage for 3 non-treated specimens
Log_{min}	minimum logarithmic value of titre of bacteriophage
N	titre of bacteriophage (plaque forming unit)
V_D	antiviral activity value without indoor-light-active photocatalyst, after being kept in a dark place on a testing material
V_{F-L}	indoor-light-active photocatalyst antiviral activity value, after indoor light illumination at a constant intensity ($F-L$) on a indoor-light-active photocatalytic material
ΔV	indoor-light-active photocatalyst antiviral activity value with indoor light illumination
Z	average number of plaques in 2 Petri dishes

5 Principle

This test method is suitable for use in development, comparison, quality assurance, characterization, reliability, and design data generation of indoor-light-active photocatalytic materials. The method is used to obtain the antiviral activity of indoor-light-active photocatalytic materials by the contact of a specimen with bacteriophage under indoor lighting condition. The method is suitable for use with flat sheet, board or plate shaped materials.

The specimen of indoor-light-active photocatalytic treated material is inoculated with bacteriophage suspension and exposed to light for a specified period. Following exposure, the test suspension is removed and measured by the plaque forming method with *Escherichia coli* which is sensitive to bacteriophage Q-beta. The results obtained are compared with those obtained from inoculated specimens of non-photocatalytic treated material exposed to light 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 Strains and preparation for tests

6.1.1 Strains

The 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. Aseptic manipulations using microorganisms can be performed in an appropriate safety cabinet.

Table 1 — Bacteriophage and bacteria strains to be used in test

Species	Strain number	Organization for the collection
Bacteriophage Q-beta	ATCC 23631-B1	American Type Culture Collection
	DSM 13768	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 20012	NITE Biological Resource Center
<i>Escherichia coli</i>	ATCC 23631	American Type Culture Collection
	DSM 5210	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 106373	NITE Biological Resource Center

NOTE ATCC23631-B1 and NBRC20012 are not strictly same, but they are from same origin.

6.1.2 Bacteria preparation

- Inoculate *E. coli* strain into a slant culture medium (6 to 10 ml of LB agar, see 6.2.6), incubate for 16 h to 24 h at $(37 \pm 1) ^\circ\text{C}$, and then store in refrigerator at $5 ^\circ\text{C}$ to $10 ^\circ\text{C}$.
- Repeat subcultures within 1 month by replicating this process.
- The slant culture must not be used for further storing after 1 month.
- The maximum number of subcultures from the original strain transferred by culture collection is 10.

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

6.1.3 Bacteriophage preparation

- Introduce 25 ml of LB broth with calcium (see 6.2.4) into a conical flask of 300 ml and inoculate with *E. coli* strain.
- Incubate for $18 \text{ h} \pm 2 \text{ h}$ at $(37 \pm 1) ^\circ\text{C}$ while shaking at $110 \text{ min}^{-1} \pm 10 \text{ min}^{-1}$.
- Pre-warm 25 ml of LB broth with calcium (see 6.2.4) in a 300 ml conical flask to $35 ^\circ\text{C}$ to $37 ^\circ\text{C}$ and inoculate with 0,025 ml of the culture prepared under b).
- Incubate as above condition until a bacterial concentration will be reached at $2,0 \pm 1,0 \times 10^8 \text{ cfu/ml}$.

NOTE This procedure should be carried out several times to establish the relationship between turbidity measurements and colony counts. If sufficient data have been obtained, further work can be based on turbidity measurements only.

- Inoculate the bacterial culture with Q-beta from a stock solution to a final concentration of approximately $2 \times 10^7 \text{ pfu}$ (plaque forming unit)/ml [multiplicity of infection (m.o.i.) is approximately 0,1].
- Incubate the inoculated bacterial culture for 4 h as under b).
- Store the culture for overnight at $4 ^\circ\text{C} \pm 2 ^\circ\text{C}$.
- Pour the culture into centrifuge tubes and centrifuge for 20 min at $4 ^\circ\text{C} \pm 2 ^\circ\text{C}$ at 10 000 g.
- Pipette the supernatant carefully to a sterilized tube.