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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

Céramiques techniques — Détermination de l'activité antivirale des matériaux photocatalytiques semi-conducteurs dans un environnement d'éclairage intérieur — Méthode d'essai utilisant un bactériophage Q-béta

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

Introduction

This International 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 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. The International Standard for determination of antiviral activity of semiconducting photocatalytic materials has also been published as ISO 18061.

The test method for cloths or textiles is not included in this International Standard 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, a test method for indoor-light-active photocatalytic cloths or textiles will be proposed with the glass adhesion method in ISO 27447.

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 International Standard 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 a test method that measures the infectivity titre of bacteriophage Q-beta after illumination with indoor light.

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

This International Standard 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 International Standard 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.

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

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

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

3 Terms and definitions

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

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

3.3
indoor lighting environment
environment with artificial light source for general lighting service

Note 1 to entry: Does 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 1 to entry: The bacteriophage used in this International Standard 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 1 to entry: 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 a dark place

4 Symbols

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
$B_{F\text{-}L}$	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

$C_{F\text{-}L}$	average of titre of bacteriophage on indoor-light-active photocatalytic treated specimens, after indoor light illumination of intensity L under condition F
D_F	dilution factor
F	type of UV cut-off condition (condition A or condition B)
L	illuminance of indoor light
Logmax	maximum logarithmic value of titre of bacteriophage
Logmean	average logarithmic value of titre of bacteriophage for three non-treated specimens
Logmin	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
$V_{F\text{-}L}$	indoor-light-active photocatalyst antiviral activity value after indoor light illumination at a constant intensity (F-L) on an indoor-light-active photocatalytic material
ΔV	indoor-light-active photocatalyst antiviral activity value with indoor light illumination
Z	average number of plaques in two Petri dishes

5 Principle

The 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.

NOTE This International Standard is adapted from the common methodological concept for ISO 18061. Namely, the same apparatus without light source (see 7.6), UV sharp cut-off filter (see 7.7), and test piece size, similar procedure and calculation are adapted between this International Standard and ISO 18061. Therefore, ISO 18061 is recommended to be used as reference during actual test of this International Standard.

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
Escherichia coli	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 the same origin.

6.1.2 Bacteria preparation

a) Inoculate *E. coli* strain into a slant culture medium (6 ml to 10 ml of LB agar; see 6.2.6), incubate for 16 h to 24 h at (37 ± 1) °C and then store in the refrigerator at 5 °C to 10 °C.

b) Repeat subcultures within 1 month by replicating this process.

c) The slant culture shall not be used for further storing after 1 month.

d) The maximum number of subcultures from the original strain transferred by culture collection is 10.

NOTE 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

a) 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.

b) Incubate for (18 ± 2) h at (37 ± 1) °C while shaking at (110 ± 10) min^{-1}.

c) Pre-warm 25 ml of LB broth with calcium (see 6.2.4) in a 300 ml conical flask to 35 °C to 37 °C and inoculate with 0,025 ml of the culture prepared under b).

d) Incubate as above condition until a bacterial concentration will be reached at (2,0 ± 1,0) × 10^8 cfu/ml.

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.

e) Inoculate the bacterial culture with Q-beta from a stock solution to a final concentration of approximately 2 × 10^7 pfu (plaque forming unit)/ml [multiplicity of infection (m.o.i.) is approximately 0,1].

f) Incubate the inoculated bacterial culture for 4 h as under b).

g) Store the culture overnight at (4 ± 2) °C.

h) Pour the culture into centrifuge tubes and centrifuge for 20 min at (4 ± 2) °C at 10 000*g*.

i) Pipette the supernatant carefully to a sterilized tube.

j) Filter bacteriophage containing supernatant suspension through a sterilized syringe filter unit to purify the bacteriophage solution.

k) Determine the titre of the bacteriophage stock solution (see 9.6) and store at (4 ± 2) °C.

l) To check bacterial contamination, mix 1 ml of the bacteriophage stock solution with LB agar (see 6.2.6) and incubate for 24 h at (37 ± 1) °C. Discard the bacteriophage stock solution if any colonies are detected.

m) Do not use the bacteriophage stock solution with less than $1,0 \times 10^{10}$ pfu/ml or contaminated stock solution.

The titre of the phage suspension should be above $1,0 \times 10^{11}$ pfu/ml and might reach up to $1,0 \times 10^{13}$ pfu/ml.

NOTE The titre of the phage stock suspension will slowly decrease over time.

6.2 Media

6.2.1 General

Commercial media of same components described below may be used.

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

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

For 100 ml of purified water, take 0,30 g of meat extract, 1,0 g of peptone and 0,50 g of sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly dissolved, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,0 ± 0,2) at 25 °C. Dilute 1,0 ml of this medium by 500 times adding purified water and set the pH to (7,0 ± 0,2) at 25 °C using hydrochloric acid solution or sodium hydroxide solution. Sterilize in an autoclave [at (121 ± 2) °C for at least 15 min]. After preparation, if 1/500 nutrient broth is not used immediately, store at 5 °C to 10 °C. Do not use 1/500 nutrient broth made more than a week ago.

6.2.3 Calcium solution

For 100 ml of purified water, take 3,0 g of calcium chloride dihydrate, put it in a flask and dissolve it thoroughly. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After preparation, if calcium solution is not used immediately, store at 5 °C to 10 °C. Do not use the calcium solution made more than 6 months ago.

6.2.4 LB broth with calcium

For 1 000 ml of purified water, take 10,0 g of peptone, 5,0 g of yeast extract and 10,0 g of sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly dissolved, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,0 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After autoclaving, add 10 ml of calcium solution to medium and mix well. After preparation, if LB broth with calcium is not used immediately, store at 5 °C to 10 °C. Do not use the broth made more than 1 month ago.

6.2.5 Agar powder

Use agar powder of microbiological grade. The gel strength measured using 1,5 % agar is between 400 g/cm^2 and 600 g/cm^2.

6.2.6 LB agar

For 1 000 ml of purified water, take 10,0 g of peptone, 5,0 g of yeast extract, and 10,0 g of sodium chloride and 10,0 g of agar powder (see 6.2.5), 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 (7,0 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After preparation, if

nutrient agar is not used immediately, store at 5 °C to 10 °C. Do not use nutrient agar made more than 1 month ago.

6.2.7 Bottom agar plate (LB agar plate with calcium)

For 1 000 ml of purified water, take 10,0 g of peptone, 5,0 g of yeast extract, and 10,0 g of sodium chloride and 15,0 g of agar powder (see 6.2.5), 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 (7,0 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After autoclaving, add 10 ml of calcium solution to medium and mix well. After preparation, pour 15 ml to 20 ml of medium into 90 mm diameter Petri dish, store at 5 °C to 10 °C. Do not use nutrient agar made more than 2 weeks ago.

6.2.8 Top agar

For 1 000 ml of purified water, take 15,0 g of peptone, 7,5 g of yeast extract, and 15,0 g of sodium chloride and 10,0 g of agar powder (see 6.2.5), 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 (7,0 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.2). After autoclaving, add 15 ml of calcium solution to medium and mix well. After preparation, if top agar is not used immediately, store at 5 °C to 10 °C. Do not use nutrient agar made more than 1 month ago.

NOTE When the top agar is remelted, heat the flask in boiling water, but not autoclaving.

6.2.9 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 dipotassium hydrogenphosphate, 2,5 g of glucose and 1,0 g of lecithin, put them into 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 of (7,0 ± 0,2) at 25 °C. If necessary, dispense it in a test tube, add a cotton plug and sterilize in an autoclave (see 6.2.2). After preparation, if SCDLP is not used immediately, store at 5 °C to 10 °C. Do not use SCDLP medium made more than 1 month ago.

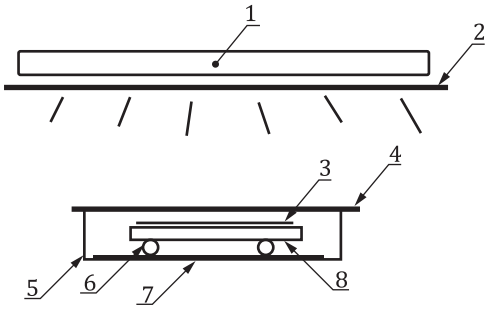
6.2.10 Peptone saline solution

For 1 000 ml of purified water, take 10,0 g of peptone and 8,5 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 (7,0 ± 0,1) at 25 °C. If necessary, dispense it in a test tube and sterilize in an autoclave (see 6.2.2). After preparation, if peptone saline solution is not used immediately, store at 5 °C to 10 °C. Do not use peptone saline solution made more than 1 month ago.

7 Apparatus and equipment

7.1 Test equipment

The testing equipment enables an indoor-light-active photocatalytic material to be examined for its antiviral 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 equipment is shown in Figure 1.



Key

1	light source	5	Petri dish
2	UV sharp cut-off filter	6	glass tube or glass rod
3	cover film	7	paper filter
4	moisture preservation glass	8	test piece

Figure 1 — Schematic of the testing equipment

7.2 Cover film

The cover film shall be inert and non-water absorbent with good sealing properties, with an optical transparency 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

The moisture preservation glass consists of a glass pane with a thickness less than 1,1 mm, with an optical transparency over 85 % for the 380 nm to 780 nm range. The dimensions of 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 in approximately 4 mm to 6 mm in diameter should be prepared by cutting it to a 10 cm to 15 cm in length and bending it into a u-shape or v-shape.

NOTE The glass tube/rod supports the test piece in the Petri dish.

7.5 Paper filter

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

NOTE The round-shaped cellulose paper filter per Petri dish needs to be one to four pieces depending on the amount of water absorption.

7.6 Light source

The light source for indoor lighting condition is specified in ISO 14605. A halophosphate fluorescent lamp, with a correlated colour temperature 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 between 3 800 K to 4 500 K and a colour rendering index (Ra) higher than 80, is recommended as a substitute.

7.7 UV sharp cut-off filter

A UV sharp cut-off filter specified in ISO 14605 shall be used under UV cut-off condition.

7.7.1 Condition A (under 400 nm cut-off condition)

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

7.7.2 Condition B (under 380 nm cut-off condition)

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

The sharp cut-off filter shall be mounted immediately below the lamp.

7.8 Illuminance meter

The illuminance meter is specified in ISO 14605. The irradiance shall be measured in illuminance (lx), by using an illuminance meter that has been calibrated by a calibration laboratory and traceability with the national standard have to be maintained.

7.9 Centrifuge

The centrifuge shall be capable of applying an acceleration of 10 000*g* at (4 ± 2) °C.

7.10 Sterilized syringe filter unit

The filter of the syringe filter units (pore size is 0,2 µm to 0,45 µm) shall consist of a polyethersulfone (PES) or polyvinylidine fluoride (PVDF).

NOTE Because of the adsorption, cellulose filter is not adequate.

8 Test piece

a) Cut a flat portion of the material to be tested in a (50 ± 2) mm × (50 ± 2) mm square. The thickness of materials for test should exceed 10 mm. Use it as the standardized shaped specimen.

b) Prepare nine pieces of non-treated specimens and six pieces of indoor-light-active photocatalytic treated specimens. When non-treated specimens cannot be provided, use glass plates instead. Take great care to avoid microbial contamination and cross-contamination among specimens.

NOTE 1 When it is difficult or impossible to cut (50 ± 2) mm long (up to 10 mm thickness) squares, it is acceptable to use a test piece to be reduced to a size of 1/2 (up to 10 mm thickness).

When test piece size is reduced, the film size shall be reduced. The size of film shall not be less than 800 mm^2 and the edges of the cover film shall be 2,5 mm to 5,0 mm inside the edge of the test piece.

NOTE 2 When the specimen surface is stained with organic contaminant, it is acceptable to first eliminate 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. wiping with ethanol or 70 % ethanol in water).

9 Procedure

9.1 General

The test method flowchart is shown in Figure 2.

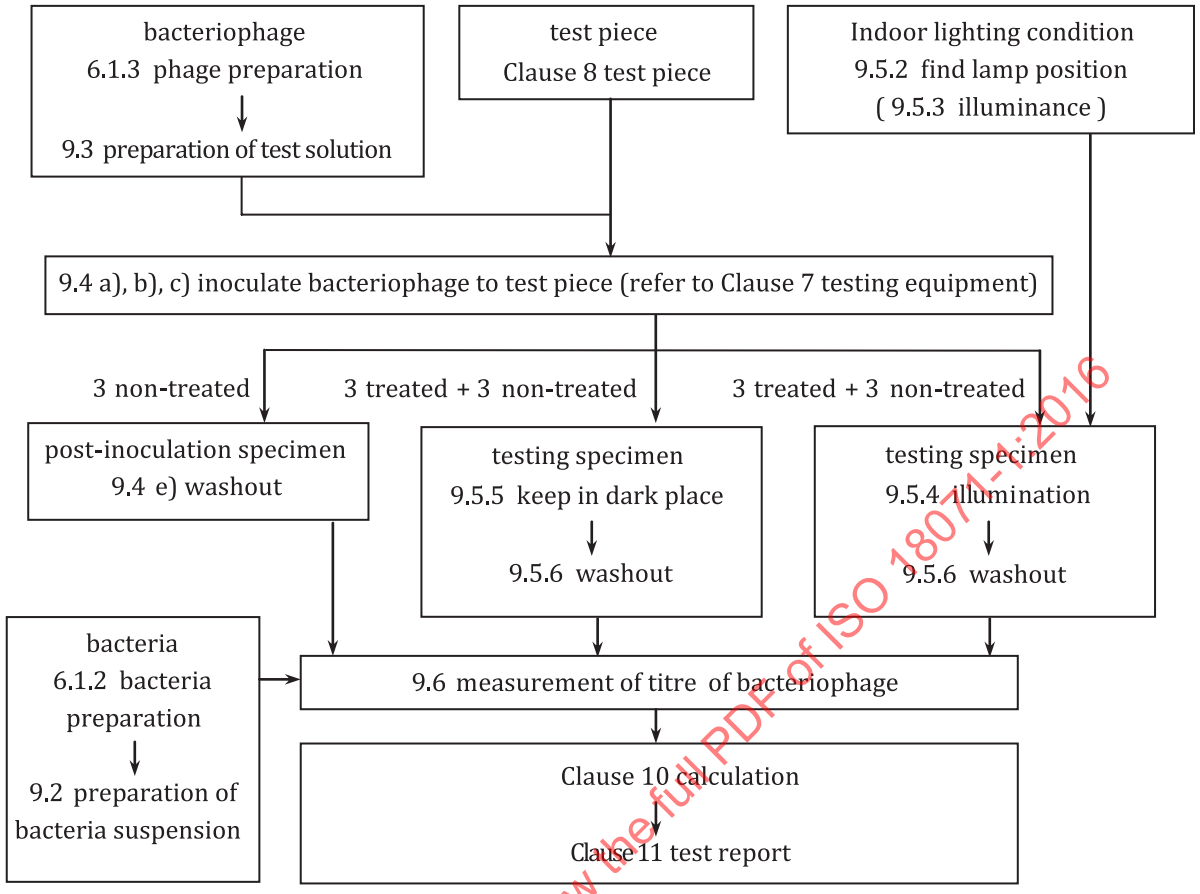


Figure 2 — Flowchart of firm adhesion method

9.2 Procedure for preparation of bacteria suspension

a) Transfer the stored bacteria (see 6.1.2) to 3 ml of LB with calcium using a platinum loop.

b) Incubate at (37 ± 1) °C for 16 h to 24 h.

c) Transfer approximately 1/1 000 of the incubated bacteria culture prepared under b) to LB with calcium.

NOTE The volume of LB with calcium depends on the number of Petri dishes (see 9.6 g)).

d) Incubate at (37 ± 1) °C to a cell density of $5{,}0 \times 10^8$ cfu/ml to $2{,}0 \times 10^9$ cfu/ml.

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.

9.3 Procedure of preparation of test bacteriophage solution

a) Dilute a bacteriophage stock solution (see 6.1.3) with 1/500 NB to obtain a titre of $1{,}0 \times 10^7$ pfu/ml to $4{,}0 \times 10^7$ pfu/ml.

NOTE The volume of LB with calcium depends on the number of Petri dishes [see 9.6 g)].

This procedure should be carried out without using vortex mixer.

b) If the test bacteriophage suspension is not to be used immediately, store it at 0 °C and use it within 2 h.

9.4 Procedure of test for indoor-light-active photocatalytic antiviral activity

a) Lay a sterilized moisture control paper filter in the bottom of a sterilized Petri dish. Add an adequate quantity of sterilized water. Place a glass tube or glass rod on paper filter in order to avoid contact between the test piece and the paper filter, and place the test piece on it with the indoor-light-active photocatalytic treated surface up. Repeat this procedure for each of the indoor-light-active photocatalytic treated and non-photocatalytic treated samples used in the test (six of the former and nine of the latter).

NOTE 1 In order to prevent the moisture conservation glass misting over, addition of 4 ml to 6 ml sterilized water per Petri dish is adequate.

b) Collect 0,15 ml of test bacteriophage solution with a sterilized pipette and drip it onto each test piece. Put a film on top of the dripped solution and lightly push to get the solution spread to the whole film surface, while taking care that no solution leaks out of the film edge. In case of irregularly sized test piece (see Clause 8 b) Note 1), adjust the inoculate volume of test bacteriophage solution to proportional to film size (see 7.2).

NOTE 2 The regulated solution 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 bacteriophage solution quantity for inoculation has been changed, the number of phages per specimen will be kept the same as with the standard size specimen, within a range of $1,0 \times 10^6$ pfu to $4,0 \times 10^6$ pfu.

This procedure should be carried out without using a vortex mixer to avoid disappearing phage infectivity. Agitation with hands is recommended.

c) Place a moisture conservation glass plate on the top of Petri dish.

d) Except for three non-treated specimens for measurement of titre of bacteriophage (see 9.6) performed just after the test bacteriophage solution is inoculated, proceed with irradiation test described in 9.5.

e) For the three non-treated test bacteriophage solution inoculated specimens for the test (post-inoculation specimen of test bacteriophage solution), put the cover film and non-treated test piece in a Stomacher bag using sterilized tweezers, taking care to avoid test bacteriophage solution leakage from the film and non-treated test piece. Add 10 ml of SCDLP, rub the specimens and the film well from outside of the Stomacher bag by hands and washout the test bacteriophage solution. Quickly use this washout solution to perform a measurement of titre of bacteriophage (see 9.6).

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

9.5 Indoor lighting condition

9.5.1 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 7.7.

Maintain the temperature around the specimens at (25 ± 5) °C throughout a period of time in 9.5.4 and 9.5.5.

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

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

This illuminance may 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.

9.5.4 Expose to light the Petri dishes containing the specimens (three non-treated specimens and three indoor-light-active photocatalytic treated specimens) with bacterial suspension for 4 h.

This exposure time may be altered between 2 h and 8 h to take into account the real conditions where the indoor-light-active photocatalyst material is effectively used.

9.5.5 Keep the Petri dishes containing the specimens (three non-treated specimens and three indoor-light-active photocatalytic treated specimens) with bacterial suspension in a dark place for the same length of time as used in 9.5.4

9.5.6 As for the specimens in 9.5.4 and 9.5.5, perform the washout in the same manner in 9.4 e).

9.6 Measurement of titre of bacteriophage

a) Melt bottles of top agar, cool to approximately 50 °C and distribute 2,0 ml aliquots into culture tubes with caps placed in a water bath at (45 ± 1) °C.

b) Pre-warm the bottom agar plate at (37 ± 1) °C.

c) Take 1 ml of washout solution [or stock solution, see 9.3 a)] with a sterilized pipette. Add to (9 ± 0,1) ml of peptone saline solution in a test tube. Agitate thoroughly and gently without using vortex mixer.

This procedure should be carried out without using vortex mixer to avoid disappearing phage infectivity. Agitation with hands is recommended.

NOTE If the washout solution and diluted solution are not to be infected with *E. coli* immediately, store washout solution at 0 °C. Dilute and infect them with *E. coli* within 2 h.

d) Extract 1 ml of the solution [above c)] with a new sterilized pipette. Add to another test tube containing (9 ± 0,1) ml of peptone saline solution. Agitate thoroughly and gently again.

e) Repeat to obtain series of dilutions in compliance with the 10-time dilution method.

f) Pre-warm the solution [wash out solution, above c), d) and e)] at (37 ± 1) °C for 10 min.

g) Add 0,1 ml of bacteria suspension [see 9.2 d)] into the culture tube [above a)] and mix gently without using vortex mixer.

This procedure should be carried out without using vortex mixer to avoid disappearing phage infectivity. Agitation with hands is recommended.

h) Add 1 ml of solution [above f)] into the culture tube [above a)] and mix gently without using vortex mixer.

This procedure should be carried out without using vortex mixer to avoid disappearing phage infectivity. Agitation with hands is recommended.

i) Pour the contents [above h)] over the surface of a pre-warmed bottom agar plate [above b)]. Examine each washout solution or diluted solution in duplicate.

j) Allow them to stand for 15 min to 30 min at room temperature.

k) Incubate for 18 h ± 2 h at (37 ± 1) °C, after the top agar solidifies.

l) Count the number of plaques appearing on the series of Petri dishes with 30 colonies to 300 colonies.