(3) Test Method III (2012 Version)
Light exposure film contact method

1. Scope

This test method shall be applied to antibacterial power tests of photocatalytic antibacterial products that exhibit antibacterial power when exposed to light (hereinafter referred to as "photocatalytic products") and hybrid antibacterial products consisting of a combination of a photocatalyst and an antibacterial metal[1] (hereinafter referred to as "hybrid products"). It should be noted, however, that this test method[2] is suitable for antibacterial power tests of test samples in the form of plates, sheets, blocks, films and the like having a smooth surface that prevents the liquid inoculum inoculated to the test sample from being non-uniformly applied or even spilled, and securing good contact with the cover film.

Although test samples to which this test method is applicable may have any surface material, whether hydrophilic or water repellant, including glass, ceramics, plastics, and paints, those showing minimal absorption of water[3] are desirable.

Note that the provisions for this test method concerning the handling of light exposure conditions category (III) and hybrid products are given for the sake of supplementary testing.

2. Test Microorganisms

2.1 Test strains[4]

(1) *Staphylococcus aureus* NBRC 12732 (ATCC 6538P)
(2) *Escherichia coli* NBRC3972 (ATCC 8739)

2.2 Storage of the test microorganisms

Transplant each stock microorganism obtained from the specified microbial strain preservation organization to nutrient agar medium[5] (slant medium), incubate at a temperature of 35 to 37°C for 48 hours, and then store under refrigeration at 5 to 10°C. Maintain the stock microorganisms for 1 month and subculture at intervals of 1 month or less. The subculture generation number shall be up to 10.

3. Procurement of Supplies

The reagents, instruments and other supplies used in this test method shall be in conformity with the Japan Industrial Standards or the Japanese Pharmacopoeia unless otherwise specified.

3.1 Instrumentation, equipment, and materials

(1) Pipettes (milk pipettes and volumetric pipettes allowing dispensing of 10 ml or more)
(2) Constant-temperature chamber (a model that can be operated at an accuracy within ±1°C)
(3) Sterile Petri dishes (inside diameter 80 mm to 100 mm, height 15 mm to 25 mm)
(4) Cover films[6] (prepared by aseptically cutting a sterilized low-density polyethylene film (thickness 30 to 50 μm) into 40±2 mm square pieces)

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[1] Antibacterial products incorporating an inorganic antibacterial agent such as silver, whose antibacterial power is increased by light exposure, shall also be handled as hybrid products. Products incorporating an antibacterial agent as a simple substance such as zinc oxide, and exhibiting binary functions of photocatalytic function and antibacterial function in the dark shall also handled as hybrid products in this test method.

[2] This test method is widely applicable to almost all types of materials, including water repellent materials, enabling the uniform and accurate contact of the liquid inoculum with the test piece by simply placing a cover film on the liquid inoculum inoculated to the test piece. However, the test method is inapplicable to test pieces that are difficult to bring into direct contact with the cover film, such as those having rough surfaces.
Provided that the requirements for test validity are fulfilled, the test may be performed without placing the film on the test piece.

[3] Provided that the requirements for test validity are fulfilled, this test method may be applied to water-absorbing test samples.

[4] These test microorganisms were chosen to represent Gram-positive and -negative bacteria, respectively.

[5] Because the drug susceptibility of the test microorganism is influenced by the degree of drying of the culture medium used, the medium shall, as a rule, be used within 24 hours after preparation.

[6] The cover film used in this test method is desirably a film having high values of ultraviolet permeability and oxygen permeation coefficient. For example, cover films (polyethylene tubes, LDPE manufactured by Asahi Kasei Corporation, γ-ray irradiated) are available from Tochisen Chemical Industry Co., Ltd. (TEL: 81-284-71-2156) and others.

(5) Underlay films (prepared by aseptically cutting a “polyethylene bag for stomacher-400” commercially available for microbial testing (Organo Corporation: 180 mm x 300 mm x 0.09 mm)” etc.) into 50 mm square pieces or larger)
(6) Light exposure apparatus (a white fluorescent lamp[1] or black light fluorescent lamp used according to the conditions; output power and number of units of the light source are not specified because light irradiation conditions are determined by illuminance or ultraviolet intensity.)
(7) Ultraviolet intensity tester[2] (measuring wavelength band: 310 - 400 nm)
(8) Illuminometer (specified in JIS C-1609^{-1993})

3.2 Culture media etc.

(1) Nutrient broth medium (NB medium)

Meat extract	5.0 g
Peptone	10.0 g
Sodium chloride	5.0 g
Purified water	1,000 ml
pH	7.0 to 7.2

(2) Nutrient agar medium (NA medium)
NB medium (1) supplemented with 1.5% of agar

(3) Standard agar medium (SA medium)

Yeast extract	2.5 g
Trypton	5.0 g
Glucose	1.0 g
Agar	15.0 g
Purified water	1,000 ml
pH	7.1±0.1

(4) SCDLP medium

Casein peptone	17.0 g
Soybean peptone	3.0 g
Sodium chloride	5.0 g
Potassium monohydrogen phosphate	2.5 g
Glucose	2.5 g
Lecithin	1.0 g
Polysorbate 80	7.0 g
Purified water	1,000 ml
pH	6.8 to 7.2

(5) Ethanol (purity not less than 99%)

(6) Phosphate-buffered physiological saline
Dissolve 34 g of KH_2PO_4 in 500 ml of purified water, adjust the solution to pH 7.2 with 1N NaOH, and then add purified water to make a total volume of 1,000 ml. Dilute 1.25 ml of this solution 800 fold with physiological saline (0.85% NaCl) to make a total volume of 1,000 ml.

[1] A fluorescent lamp specified as a white light source in JIS C-7601^{-1989}.
[2] A periodically calibrated ultraviolet intensity tester shall be used. Generally, ultraviolet intensity testers from different manufacturers have somewhat different measuring wavelength bands, sensitivities and the like; they often produce inconsistent measured values. In the case of ultraviolet intensity testers with a dome-shaped or flat light receiving port, in particular, special attention should be paid to the possibility that measured values can differ up to about 3 times.

4. Test Samples

The test sample subjected to the antibacterial power test shall, as a rule, be an actual supply of the product as is. However, provided that it is prepared using the same method of treatment, and is judged to produce similar levels of antibacterial power even if it has a different shape from that of the product, it may be used as the test sample.

5. Test Procedures

5.1 Incubation of the test microorganisms

(1) Transplant[1] each test microorganism to NA medium[2] and incubate at a temperature of 35 to 37°C for 16 to 24 hours (pre-pre-culture).

(2) Transplant a platinum loopful of the test microorganism pre-pre-cultured in (1) to NA medium[3] and incubate at a temperature of 35 to 37°C for 16 to 20 hours (pre-culture)[4].

5.2 Preparation of liquid inoculum

Dilute NB medium 500 fold with sterile purified water[5] and adjust the dilution to a pH of 7.0±0.2[6] to obtain a "1/500 NB medium". Uniformly disperse the pre-cultured microorganism in the "1/500 NB medium" to obtain a given cell count and use this liquid as the liquid inoculum[7].

5.3 Preparation of test pieces[8]

Provide a 50±2 mm square (thickness not more than 10 mm[9]) standard test sample[10]. As required, gently wipe the entire surface of each test sample two or three times with gauze or absorbent cotton of Japanese Pharmacopoeia grade, previously impregnated with ethanol, and dry. Subsequently, expose the test sample to ultraviolet rays using a black light fluorescent lamp for 12 hours or more[11], and cool to room temperature. Provide a total of eight such samples (2 samples (N = 2) × 2 bacterial species × 2 sets of conditions (dark conditions and bright conditions)) and use these as the photocatalytic antibacterial test pieces.

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1 Because the drug susceptibility of the test microorganism is influenced by the degree of drying of the culture medium used, the medium shall, as a rule, be used within 24 hours after preparation.

2 Because liquid culture involves a risk of contamination of the liquid inoculum with liquid medium ingredients that can affect the test results, NA medium must be used for pre-culture.

3 Exclusively for pre-culture, pre-pre-cultured samples may be used within 3 days of refrigerated storage at 5 to 10°C.

4 Slant medium shall be used for pre-pre-culture and pre-culture.

5 If there is a deviation of pH from the specified range due to sterilization after pH adjustment, the liquid may be diluted with phosphate-buffered solution (dissolve 34 g of KH_2PO_4 in 500 ml of purified water, adjust the solution to pH 7.2 with 1N NaOH, then add purified water to make a total volume of 1,000 ml; dilute 1.25 ml of this solution 800 fold with purified water to make a total volume of 1,000 ml).

6 Use after adjustment to a pH of 7.0±0.2 with hydrochloric acid or sodium hydroxide and sterilization.

7 As with the film contact method, 1/500 NB medium was adopted as the bacterial dispersion. The bacterial dispersion may be of a dilution rate smaller than that for 1/500 NB medium. In this case, however, the dilution rate shall be indicated in the Test Methods section. The cell count shall be adjusted to fulfill the requirements for test validity.

8 If the test piece is judged as not to undergo deterioration or not to have its antibacterial power etc. affected, the test piece may be subjected to sterilizing treatments such as dry heat sterilization, high-pressure steam sterilization, and gas sterilization after being wiped clean by ethanol to remove oil and other stains. In the case of porous materials such as cement and composites of porous materials such as pottery, spores of bacteria of the genus *Bacillus* etc. can remain viable. Hence, the test piece shall be sterilized with dry heat (at 160 to 180°C for 120 minutes or more) after being cleaned with ethanol to remove oil and other stains. In the case of ceramics such as pottery, enamelware and glass, as well as metals, cement, etc., it is desirable to perform such treatments.

9 The thickness of the test piece shall be up to 10 mm to allow easy entry into a Petri dish.

10 Regarding the size of the test piece, the test piece may be rectangular, provided that its area is constant. However, the test piece size should not be less than a quarter of the area of the standard test piece. If a standard test piece area cannot be assured because of the size of the product, the size of the test piece shall be indicated in the Test Results section. The area of the control underlay film must be matched with the area of the test piece.

11 Ultraviolet exposure is performed to decompose the organic matter and other stains adhering to the test piece surface, so as to assure a stable reproducible state on the test piece surface. Use a black light fluorescent lamp of an output power of about 20 W (FL20S•BLB). Irradiate ultraviolet rays at a distance of about 10 mm from the lamp in the case of a test piece of sufficient heat resistance. In the case of a test piece considered not to require ultraviolet exposure, this operation may be omitted.

In evaluating a hybrid product, test an additional four untreated pieces (sample pieces incorporating neither a photocatalyst nor an antibacterial metal) (2 test pieces (N = 2) × 2 bacterial species x 1 set of conditions (dark conditions)).

5.4 Test piece storage

(1) Dark conditions control plot

Provide four sterile Petri dishes[1] (2 dishes × 2 bacterial species), place an underlay film on each dish, inoculate 0.4 ml of the liquid inoculum[2] on the film, place a cover film, and store at 20 to 25°C[3] under shading (dark conditions)[4].

(2) Bright conditions control plot

Provide four sterile Petri dishes (2 dishes × 2 bacterial species), place an underlay film on each dish, inoculate 0.4 ml of the liquid inoculum on the film, place a cover film, expose to light[5] with reference to Table 1, and store at 20 to 25°C (bright conditions)[6].

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[1] Provided that the moisture retention container is sterilizable, the lids of the sterile Petri dishes may not be used during storage.

[2] In the case of a test piece of non-standard size, the volume of liquid inoculum shall be determined proportionally to the area of the cover film. However, the number of cells inoculated per test piece shall be constant at 1.0 to 5.0 x 10^5 cells as with standard test pieces, even if the area decreases or the volume of liquid inoculum decreases. Note that even when the volume of liquid inoculum meets these requirements, highly hydrophilic samples are likely to encounter accidents such as unwanted migration of the cover film with slight inclination and leakage of liquid inoculum from the margin of the cover film. To overcome this drawback, liquid inoculum volume may be reduced to a quarter of the specified volume.

[3] Use a low-temperature constant-temperature chamber, or air-condition the laboratory to a room temperature of 20 to 25°C. Measure the room temperature and humidity during the testing and indicate this in the Test Results section. The test may be performed at storage temperatures befitting the service conditions for the product. In this case, the test temperature shall be indicated in the Test Results section.

[4] Store under the same conditions, except for light exposure, as the bright conditions whenever possible.

[5] The type and model of the fluorescent lamp, distance from the test piece, illuminance and ultraviolet intensity at the position of the test piece (whatever the light source, black light fluorescent lamp or white fluorescent lamp, both illuminance and ultraviolet intensity shall be indicated), the illuminometer, ultraviolet intensity tester and the like used shall be indicated in the Test Results section. Ultraviolet intensity and illuminance shall be measured with the substrate between the light source and the sample on the light receiving port of the illuminometer or ultraviolet intensity tester.

[6] Under bright conditions, bacteria can die due to dehydration of the liquid inoculum caused by the heat from the fluorescent lamp. To prevent this, the Petri dishes shall be kept in a moisture retention container containing a saturated aqueous solution of ammonium dihydrogen phosphate at not less than 5% of its capacity, covered with glass of high ultraviolet permeability* (quartz or borosilicate glass such as Pyrex or hard glass) on the upper surface, and shall be exposed to light from above. Although it has been confirmed that clouding of the glass of the moisture retention container causes almost no change in the light intensity, appropriate measures shall be taken to minimize said clouding. Provided that the cell count for the "bright conditions control plot" does not decrease, the test may be performed with the light source in the moisture retention container. In this case, however, anti-moisture measures for the light source shall be taken to prevent short circuits and other accidents. (A moisture retention container based on another method confirmed to produce a relative humidity of 90% or more may be used.) The specifications for the moisture retention container shall be indicated in the Test Results section. According to the intended use, a substrate may be inserted between the sample and the light source to change the light intensity and light wavelength spectrum. In this case, the fact shall be stated in the Test Results section.

*: Place all substrates, including the cover film, between the light source and the sample, on the light receiving port of the ultraviolet intensity tester and determine their ultraviolet transmittance: the ultraviolet transmittance shall not be less than 70%.

$$\text{Ultraviolet transmittance (\%)} = \frac{\text{(measured value obtained with substrates on the light receiving port of ultraviolet intensity tester)}}{\text{(measured value obtained with nothing on the light receiving port of ultraviolet intensity tester)}} \times 100$$

(3) Dark conditions test plot
Place each of four photocatalytic antibacterial test pieces (2 pieces × 2 bacterial species) in a sterile Petri dish, inoculate 0.4 ml of the liquid inoculum (including 1.0 to 5.0 × 10^5 cells) to the test surface, place a cover film[1], and store under shade at 20 to 25°C (dark conditions).

(4) Bright conditions test plot
Place each of four photocatalytic antibacterial test pieces (2 pieces × 2 bacterial species) in a sterile Petri dish, inoculate 0.4 ml of the liquid inoculum to the test surface, place a cover film, then expose to light, and store at 20 to 25°C (bright conditions).
When a hybrid antibacterial product is evaluated, the following test shall also be performed (supplementary test).

(5) Dark conditions no-treatment test plot
Place each of four untreated test pieces (2 pieces × 2 bacterial species) in a sterile Petri dish, inoculate 0.4 ml of the liquid inoculum (including 1.0 to 5.0 × 10^5 cells) to the test surface, place a cover film, and store under shade at 20 to 25°C (dark conditions).

5.5 Standard test conditions and categories

The following three standard conditions of light exposure shall be established.

Table 1. Light exposure conditions

Category	Light source	Light intensity[2]	Examples
(I) Cases of aggressive ultraviolet exposure or outdoor use.	Black light	Not less than 20 μW/cm^2 [3]	Air cleaners, air-conditioners etc.
(II) Cases of use of light of relatively high intensity in daily living spaces. (Including visible light responding photocatalysts)	White fluorescent lamp	4,000 – 6,000 lx	Products for use in vicinity of light source or by windows (blinds, curtains, desk lamp shades, etc.)
(III, reference test) Cases of use of light of ordinary intensity in daily living spaces. (Including visible light responding photocatalysts)	White fluorescent lamp	1,000 – 2,000 lx	Products for use in ordinary indoor settings

[1] In the case of a test piece of non-standard size, the cover film shall be of a size such that there is a distance of 2.5 to 5.0 mm from the outer periphery.

[2] Prior to measuring the light intensity, wait at least 30 minutes after the light source is switched on.

[3] This figure is for an ultraviolet intensity tester with a flat light receiving port. In the case of an ultraviolet intensity tester with a dome-shaped light receiving port, the ultraviolet intensity shall not be less than 60 μW/cm^2. Although no provision is given here with respect to the upper limit of ultraviolet intensity, the ultraviolet intensity shall be up to a level fulfilling the requirements for test validity shown in section 6-(2). The ultraviolet intensity irradiated may be changed according to the kind of test microorganism. In this case, however, the ultraviolet intensity irradiated and the requirements for test validity shall be indicated along with the test results.

5.6 Viable cell counting

(1) Provide four sterile Petri dishes (2 dishes × 2 bacterial species), place an underlay film on each dish, place the liquid inoculum in an equal volume to that inoculated to each test piece, place a cover film, immediately thoroughly wash down the adhering cells into a Petri dish using SCDLP medium (10 ml) [1], determine the viable cell count[2] in 1 ml of the washings by the agar plate culture method using SA medium (incubation at 35±1°C for 40 to 48 hours) [3], calculate the mean[4] of the viable cell counts from the two dishes, and multiply by a factor of 10 (value A, "just-after-inoculation control plot") [5]. Sterile phosphate-buffered physiological saline shall be used as the diluent for viable cell counting.

(2) Calculate the mean of the viable cell counts from the two sterile Petri dishes for the dark conditions control plot after elapse of the specified time[6], measured in the same manner as with the sterile Petri dishes described in the previous term, and multiply by a factor of 10 (value B0, "dark conditions control plot").

(3) Calculate the mean of the viable cell counts from the two sterile Petri dishes for the bright conditions control plot after elapse of the specified time, measured in the same manner as with the sterile Petri dish described in the previous term, and multiply by a factor of 10 (value B1, "bright conditions control plot").

(4) Calculate the mean of the viable cell counts from the two antibacterial test pieces for the dark conditions photocatalytic plot after elapse of the specified time, measured in the same manner as with the sterile Petri dishes described in the previous term, and multiply by a factor of 10 (value C0, "dark conditions test plot").

(5) Calculate the mean of the viable cell counts from the two antibacterial test pieces for the bright conditions photocatalytic plot after elapse of the specified time, measured in the same manner as with the sterile Petri dishes described in the previous term, and multiply by a factor of 10 (value C1, "bright conditions test plot").

(6) Calculate the mean of the viable cell counts from the two dark conditions untreated test pieces after elapse of the specified time, measured in the same manner as with the sterile Petri dishes described in the previous term, and multiply by a factor of 10 (value D0, "dark conditions no-treatment test plot").

6. Requirements for Test Validity[7]

The test shall not be regarded as valid unless all the following four requirements for test validity are fulfilled.

(1) Calculate the following value from the viable cell counts from two Petri dishes in each of the "just-after-inoculation control plot" and the "dark conditions control plot" using the formula shown below: the calculated value shall not be more than 0.2.

(Maximum logarithmic value - minimum logarithmic value) / (logarithmic mean) ≤ 0.2

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[1] Cells adhering to the test piece and cover film shall be washed down as described below. First, remove the cover film from the test piece using sterile tweezers and apply a washing liquid (10 ml of SCDLP medium) using a sterile pipette or sterile dropper. Next, aspirate the liquid in the Petri dish using a sterile pipette etc., and again apply the liquid. Repeat this operation three times to wash down the cells, then thoroughly mix the washings, and immediately use for viable cell counting.

[2] Use phosphate-buffered physiological saline as the diluent for viable cell counting.

[3] Viable cell counting shall be performed with reference to the "Methods of Counting Bacterial Cells (Viable Cells)" stipulated in the "Specifications and Standards for Foods, Additives etc. (Ministry of Health and Welfare Notification No. 370 dated December 28, 1959)" and the like.

[4] To obtain the mean of the viable cell counts from the two Petri dishes, calculate the arithmetic mean of the measured values rounded at the third decimal place, and indicate the value to the second decimal place by rounding at the third decimal place.

[5] If a viable cell count of less than 10 is obtained, the indication shall be "<10", and use "10" as a basis for calculating the mean viable cell count. However, provided that all measured values are "<10", the mean shall be indicated as "<10".

[6] For standard storage conditions, the sterile Petri dishes shall be stored for 24 hours. The storage time may be reduced to less than 24 hours according to the service conditions for the product, or in the case of an inorganic (silver etc.)-photocatalytic hybrid product. In this case, the storage time shall be indicted in the Test Results section.

[7] In addition to the four requirements for test validity described in the text, the following requirement is desirable. To fulfill this, it is necessary to establish experimental conditions that do not reduce the viable cell count for the "bright conditions control plot", i.e., conditions that do not reduce the cell count with light and heat only.
The following formula shall apply between B0 (viable cell count for dark conditions control plot after elapse of specified time) and B1 (viable cell count for bright conditions control plot after elapse of specified time):
$\{\log (B0/B1)\} \leq 1.0$

(2) The reduction rate of each of value B0 (mean for "dark conditions control plot") and value B1 (mean for "bright conditions control plot") compared to value A (mean for "just-after-inoculation control plot") shall not be more than 90%.

$\{(A - B0)/A\} \times 100 \leq 90$
$\{(A - B1)/A\} \times 100 \leq 90$

(3) The mean of the viable cell counts from the two Petri dishes for the "just-after-inoculation control plot" shall be between 1.0 and 5.0×10^5 cells/dish.

(4) (In evaluation of an ordinary photocatalytic product) Both of the viable cell counts from the two Petri dishes for the "dark conditions test plot" shall not be less than 1.0×10^3 cells/dish.
(In evaluation of a hybrid product) Both the viable cell counts from the two Petri dishes for the "dark conditions non-treatment test plot" shall not be less than 1.0×10^3 cells/dish[1].

7. Indication of Test Results

Calculate[2] the "antibacterial activity value" using the equation below and indicate the value to the first decimal place by rounding down the second decimal place. In addition to the test results, the test conditions shall also be indicated.

7.1 Antibacterial activity value

(1) Photocatalytic products

Antibacterial activity value (1)
$= \{\log (C0/A) - \log (C1/A)\} = \{\log (C0/C1)\}$

[1] If the calculated value from the "dark conditions non-treatment test plot" does not meet the requirement (not less than 1.0×10^3 cells/cup), the product per se may have antibacterial power. In this case, re-testing following the pretreatment etc. of the test piece described below may produce good results.

① Canceling antibacterial power by increasing the nutrient content of the liquid inoculum
In the case of a product of relatively weak antibacterial power deriving from paints, synthetic resin, etc., the antibacterial power of the untreated sample can be cancelled by increasing the nutrient content of the liquid inoculum from the standard level of 1/500 NB to, for example, 1/100 NB, 1/50 NB, 1/10 NB etc. In this case, the nutrients in the liquid inoculum shall be indicated in the Test Results section.

② Volatilizing the antibacterial substances contained in the sample by drying
The majority of products containing free formalin, such as melamine resin, FRP resin, amino-series paints and melamine-series paints, exhibit strong antibacterial power. In this case, the antibacterial power can be cancelled by drying the sample to volatilize the formalin. Because optimal drying temperature and time vary depending on sample material and the like, judgment shall be made on the basis of the antibacterial power test results obtained by actually pre-treating the sample at different combinations of drying temperature and time. In this case, the pretreatment conditions shall be indicated in the Test Results section.

③ Preventing bacterial death due to dehydration in water-absorbing samples by impregnation with phosphate-buffered physiological saline
In the case of samples likely to absorb the liquid inoculum, such as floor coatings, the bacteria contained are often killed due to dehydration. In this case, it is recommended that a sterile test piece be tested after being allowed to stand in a Petri dish containing phosphate-buffered physiological saline for about 12 hours, and then wiped to remove water from the sample surface. In the case of flooring paints, however, free formalin is contained in the adhesive used in the substrate plywood; testing often fails to produce good results unless the above-described anti-formalin measures are taken. In this case, the pretreatment conditions shall be indicated in the Test Results section.

2 If the mean of value C0 (mean value for "dark conditions test plot"), value C1 (mean value for "bright conditions test plot") and value D0 (mean value for "dark conditions no-treatment test plot") is <10, it shall be regarded as "10" in calculating the antibacterial activity value.

(2) Hybrid products (supplementary test)

Antibacterial activity value (2a)
= {log (D0/A) - log (C0/A)} = {log (D0/C0)}

(a) If value C0 for the "dark conditions test plot" is not less than 1.0×10^2 cells/dish under the same test conditions as those used to obtain the antibacterial activity value (2a):

Antibacterial activity value (2b1)
= {log (C0/A) - log (C1/A)} = {log (C0/C1)}

(b) If value C0 for the "dark conditions test plot" is not more than 1.0×10^2 cells/dish under the same test conditions as those used to obtain the antibacterial activity value (2a), perform the test again at different sample storage times or liquid inoculum medium concentrations with reference to the footnote, and calculate the antibacterial activity value (2b2) [1].

Antibacterial activity value (2b2)
= {log (C0'/A')- log (C1'/A')} = {log (C0'/C1')}

7.2 Test conditions

- Type, model, and number of units of light source
- Presence or absence and kind of cover film
- Sample size
- Method of sample pretreatment
- Models and light receiving port shapes of ultraviolet intensity and illuminometer
- Method of preparing the liquid inoculum (choice of sterile water or phosphate-buffered solution)
- Amount of liquid inoculum
- NB concentration of liquid inoculum
- Sample storage temperature
- Sample storage time
- Requirements for test validity
- Specifications for moisture retention container
- Kinds of substrates between light source and sample

[1] If value C0 for the "dark conditions test plot" is not more than 1.0×10^2 cells/dish so that the difference from value C1 for the "bright conditions test plot" cannot be confirmed, the test conditions may be changed as described below to evaluate the difference between value C0 for the "dark conditions test plot" and value C1 for the "bright conditions test plot".

① Shortening storage time
Fix the medium concentration of the liquid inoculum at 1/500 NB and reduce the storage time to less than 24 hours to obtain a value C0 "dark conditions test plot" of not less than 1.0×10^2 cells/dish.

② Increasing the nutrient content of liquid inoculum
Fix the storage time at 24 hours and increase the nutrient content of the liquid inoculum from the standard level of 1/500 NB to, for example, 1/250 NB, 1/100 NB, 1/50 NB and the like, to obtain a value C0 "dark conditions test plot" of not less than 1.0×10^2 cells/dish.

③ Combination of ① and ② above