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**Antibacterial products — Test for
antibacterial activity and efficacy**

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Foreword

This translation has been made based on the original Japanese Industrial Standard revised by the Minister of Economy, Trade and Industry through deliberations at the Japanese Industrial Standards Committee as the result of proposal for revision of Japanese Industrial Standard submitted by Society of Industrial technology for Antimicrobial Articles (SIAA)/Japanese Standards Association (JSA) with the draft being attached, based on the provision of Article 12 Clause 1 of the Industrial Standardization Law applicable to the case of revision by the provision of Article 14.

Consequently JIS Z 2801 : 2006 is replaced with this Standard.

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Attention is drawn to the possibility that some parts of this Standard may conflict with a patent right, application for a patent after opening to the public or utility model right which have technical properties. The relevant Minister and the Japanese Industrial Standards Committee are not responsible for identifying the patent right, application for a patent after opening to the public or utility model right which have the said technical properties.

Antibacterial products — Test for antibacterial activity and efficacy

Introduction

This Japanese Industrial Standard has been prepared based on the first edition of ISO 22196 published in 2007 with some modifications of the technical contents to meet technology trend, actual situation, etc. in Japan.

The portions given sidelines or dotted underlines are the matters in which the contents of the original International Standard have been modified. A list of modifications with the explanations is given in Annex JA.

1 Scope

This Standard specifies the test for antibacterial activity and efficacy to bacteria on the surface of antibacterial products (including intermediate products) of plastic products, metal products, ceramics products, etc. excluding the textile products and the photocatalyst products.

Secondary effects of antibacterial efficacy such as fungal resistance, odour prevention and biodeterioration are not covered by this Standard.

NOTE 1 For the products so determined that the test method for textile products should be applied from their usage, shape, etc., clause 10 (Quantitative test) of JIS L 1902 may be applied.

NOTE 2 The International Standard corresponding to this Standard and the symbol of degree of correspondence are as follows.

ISO 22196 : 2007 *Plastics — Measurement of antibacterial activity on plastics surfaces* (MOD)

In addition, symbols which denote the degree of correspondence in the contents between the relevant International Standards and JIS are IDT (identical), MOD (modified), and NEQ (not equivalent) according to ISO/IEC Guide 21-1.

2 Normative references

The following standards contain provisions which, through reference in this text, constitute provisions of this Standard. The most recent editions of the standards (including amendments) indicated below shall be applied.

JIS K 0050 *General rules for chemical analysis*

JIS K 0950 *Sterilized plastic petri dishes*

JIS K 0970	<i>Piston operated micro-volumetric apparatus</i>
JIS K 3800	<i>Class II biological safety cabinets</i>
JIS K 8101	<i>Ethanol (99.5) (Reagent)</i>
JIS K 8150	<i>Sodium chloride (Reagent)</i>
JIS K 8180	<i>Hydrochloric acid (Reagent)</i>
JIS K 8263	<i>Agar (Reagent)</i>
JIS K 8576	<i>Sodium hydroxide (Reagent)</i>
JIS K 9007	<i>Potassium dihydrogen phosphate (Reagent)</i>
JIS K 9017	<i>Dipotassium hydrogenphosphate (Reagent)</i>
JIS R 3505	<i>Volumetric glassware</i>
JIS Z 8802	<i>Methods for determination of pH of aqueous solutions</i>

3 Terms and definitions

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

3.1 antibacterial

a state where the growth of bacteria on the surfaces of products is inhibited

3.2 antibacterial agent

agent that inhibits the growth of bacteria on the surfaces of products by using it directly or as a compounded agent

3.3 antibacterial treatment

treatment for antibacterial purpose

3.4 antibacterial product

product on which antibacterial treatment has been performed

3.5 antibacterial activity

value which shows the difference in logarithmic values of number of viable bacteria between the antibacterial product and the untreated product after inoculation followed by incubation of bacteria

3.6 antibacterial effectiveness

effectiveness of antibacterial product determined from the antibacterial activity

4 Antibacterial effectiveness

The antibacterial product is determined that it has the antibacterial effectiveness, when the antibacterial activity thereof obtained according to the testing method of this

Standard is 2.0 or more.

In addition, the value of over 2.0 may be applicable to the determination of antibacterial effectiveness upon the agreement between the parties concerned with delivery.

5 Testing method

5.1 Bacteria to be used for test

The species of bacteria to be used for the test shall be as follows, and the test shall be carried out on respective bacteria.

- a) *Staphylococcus aureus*
- b) *Escherichia coli*

Examples of bacterial strain to be used for the test are shown in table 1. If the bacterial strain is contributed by the agency of culture collection other than that shown in table 1, it shall be obtained from member agencies of World Federation for Culture Collections (WFCC) or Japan Society for Culture Collections (JSCC), and it shall be the bacterial strain of the same series as that shown in table 1.

Table 1 Bacterial strain used for test

Type of bacteria	Preservation number of bacterial strain	Agency of culture collection
<i>Staphylococcus aureus</i>	ATCC 6538P FDA 209P NBRC 12732 CIP 53.156 DSM 346 NCIB 8625	American Type Culture Collection Food and Drug Administration Bioresource Information Center, Department of Biotechnology of National Institute of Technology and Evaluation Collection des Bacteries de l'Institut Pasteur Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH National Collection of Industrial and Marine Bacteria Ltd.
<i>Escherichia coli</i>	ATCC 8739 NBRC 3972 CIP 53.126 DSM 1576 NCIB 8545	American Type Culture Collection Bioresource Information Center, Department of Biotechnology of National Institute of Technology and Evaluation Collection des Bacteries de l'Institut Pasteur Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH National Collection of Industrial and Marine Bacteria Ltd.

5.2 Chemicals, materials, instruments and apparatuses

The chemicals, materials, instruments and apparatuses used in this Standard shall be as follows, unless otherwise designated.

Ethanol (C ₂ H ₅ OH)	Guaranteed reagent specified in JIS K 8101 or superior
Beef extract	For microbial test
Peptone	For microbial test
Sodium chloride (NaCl)	Guaranteed reagent specified in JIS K 8150
Purified water	Conforming to the reference of the 15th revised Japanese Pharmacopoeia
Agar	Guaranteed reagent specified in JIS K 8263 or superior
Yeast extract	For microbial test
Tryptone	For microbial test
Glucose	For microbial test
Casein peptone	For microbial test
Soybean peptone	For microbial test
Lecithin	For microbial test
Nonionic surfactant	Polyoxyethylene sorbitan monooleate ..[Polysorbate 80 (Tween80)]..
Potassium dihydrogenphosphate (KH ₂ PO ₄)	Guaranteed reagent specified in JIS K 9007
Dipotassium hydrogenphosphate (K ₂ HPO ₄)	Guaranteed reagent specified in JIS K 9017
Sodium hydroxide (NaOH)	Guaranteed reagent specified in JIS K 8576
Hydrochloric acid (HCl)	Guaranteed reagent specified in JIS K 8180
Cotton stopper	OME cotton, or silicone stopper, metal stopper, morton stopper, etc.
Platinum loop	With a loop of about 4 mm at the point
Dry-heat sterilizer	Capable of keeping the temperature from 160 °C to 180 °C
Autoclave	Capable of keeping at 121 °C in temperature (corresponding to 103 kPa in pressure)
Safety cabinet	Having performance conforming to JIS K 3800 or equivalent
pH meter	Conforming to JIS Z 8802
Chemical balance	Having performance conforming to JIS K 0050 or equivalent
Clean bench	For microbial test
Measuring pipette	Having precision conforming to JIS K 0970 or Class A in

	<u>JIS R 3505, or equivalent</u>
Incubator	Capable of keeping the temperature $\pm 1\text{ }^{\circ}\text{C}$
Petri dish	Made of glass with about 90 mm of inside diameter, or conforming to No. 90A or No. 90B specified in JIS K 0950
Stomacher pouch	For microbial test
Stomacher	For microbial test
Film	Material such as polyethylene film not affecting microbial growth and that does not absorb water but with good adherence, the thickness of which is not specified

5.3 Method of sterilization

The glassware such as test tube and measuring pipette to be used shall be washed sufficiently with alkaline or neutral detergent, rinsed sufficiently with water, then dried and sterilized with dry-heat, or sterilized with high-pressure steam. The method of sterilization shall be in accordance with the following a) or b). The case where the platinum loop and the test tube are sterilized with flame shall be in accordance with the following c).

- a) **Dry-heat sterilization** Objects to be sterilized shall be placed in a dry-heat sterilizer for 60 min or more in the case of the temperature of $170\text{ }^{\circ}\text{C}$ or 120 min or more in the case of the temperature of $160\text{ }^{\circ}\text{C}$. After the completion of dry-heat sterilization, if the cotton stopper or the wrapping paper of objects to be sterilized is wetted with water, that instrument shall not be used.
- b) **High pressure steam sterilization** Water is put in an autoclave, and the objects to be sterilized which are in a metal net basket shall be placed on a metal net shelf in the autoclave. The lid of autoclave is tightened, heated, and kept at temperature of $121\text{ }^{\circ}\text{C}$ (corresponding to 103 kPa in pressure) for 15 min to 20 min. After heating is suspended followed by naturally cooling to $100\text{ }^{\circ}\text{C}$ or under, the exhaust valve is opened to draw off the steam. The sterilized objects shall be taken out after opening the lid, and cooled on a clean bench or in a safety cabinet, if necessary. In order to keep the autoclave clean from contamination by culture medium or processing chemicals, it shall be washed with neutral detergent if necessary, and rinsed sufficiently with water.
- c) **Flame sterilization** The objects or parts to be sterilized shall be placed into a gas or alcohol flame. For a platinum loop, its shall be red heated sufficiently, and for a test tube, it shall be touched with the flame for 2 s or 3 s.

5.4 Culture medium, etc.

The culture medium, etc. of which the composition is as follows shall be used. A commercially available article may be used, if it is of the same composition.

- a) **Nutrient broth [1/500 nutrient broth (1/500 NB)]** Prepared so that 3.0 g of beef extract, 10.0 g of peptone and 5.0 g of sodium chloride which are weighed out by the chemical balance shall be added to 1 000 ml of purified water or ion-exchanged

water, then mixed and dissolved completely, and diluted 500 times with purified water. The pH thereof shall be adjusted to 6.8 to 7.2 (25 °C) with a sodium hydroxide solution or a hydrochloric acid solution by using the pH meter, and it shall be sterilized with high pressure steam. If it is not used immediately after preparation, it shall be preserved at a temperature of 5 °C to 10 °C. 1/500 NB that has been kept for one week or longer after preparation shall not be used.

- b) **Nutrient agar** Prepared so that 5.0 g of beef extract, 10.0 g of peptone and 5.0 g of sodium chloride shall be added to 1 000 ml of purified water or ion-exchanged water, and mixed. The pH thereof shall be adjusted to 7.0 to 7.2 (25 °C) with a sodium hydroxide solution or a hydrochloric acid solution. 15.0 g of powder agar shall be added and dissolved by heating, a cotton stopper shall be put, and it shall be sterilized with high pressure steam. If it is not used immediately after preparation, it shall be preserved at a temperature of 5 °C to 10 °C. The nutrient agar that has been kept for one month or longer after preparation shall not be used.
- c) **Plate count agar** Prepared so that 2.5 g of yeast extract, 5.0 g of tryptone and 1.0 g of glucose which are weighed out by the chemical balance shall be added to 1 000 ml of purified water or ion-exchanged water, and mixed. The pH thereof shall be adjusted to 7.0 to 7.2 (25 °C) with a sodium hydroxide solution or a hydrochloric acid solution by using the pH meter. 15.0 g of powder agar shall be added and dissolved by heating, and it shall be sterilized with high pressure steam. If it is not used immediately after preparation, it shall be preserved at a temperature of 5 °C to 10 °C. The plate count agar that has been kept for one month or longer after preparation shall not be used.
- d) **Slant culture medium** Prepared so that 6 ml to 10 ml of the nutrient agar of b) which has been preliminary warmed and dissolved shall be poured into a test tube, a cotton stopper shall be put, and it shall be sterilized with high pressure steam. After sterilization, the test tube shall be placed in a clean room at a slant of about 15° to the horizontal plane, and the content shall be solidified. If it is not used immediately after preparation, it shall be preserved at a temperature of 5 °C to 10 °C. If there is no condensed water, the content shall be dissolved and solidified again, then used. The slant culture medium that has been kept for one month or longer after preparation shall not be used.
- e) **SCDLP broth** Prepared so that 17.0 g of casein peptone, 3.0 g of soybean peptone, 5.0 g of sodium chloride, 2.5 g of disodium hydrogen phosphate, 2.5 g of glucose and 1.0 g of lecithin which are weighed out by the chemical balance shall be added to 1 000 ml of purified water or ion-exchanged water, then mixed and dissolved. Then, 7.0 g of nonionic surfactant shall be added and dissolved, The pH thereof shall be adjusted to 6.8 to 7.2 (25 °C) with a sodium hydroxide solution or a hydrochloric acid solution by using the pH meter, and it shall be sterilized with high pressure steam. If it is not used immediately after preparation, it shall be preserved at 5 °C to 10 °C. The SCDLP broth that has been kept for one month or longer after preparation shall not be used.
- f) **Phosphate buffer solution** Prepared so that 34.0 g of potassium dihydrogen phos-

phate which is weighed out by the chemical balance shall be added to 500 ml of purified water or ion-exchanged water, then mixed and dissolved. The pH thereof shall be adjusted to 6.8 to 7.2 (25 °C) with a sodium hydroxide solution or a hydrochloric acid solution by using the pH meter. Further, purified water or ion-exchanged water shall be added to make it 1 000 ml, and it shall be sterilized with high pressure steam. The phosphate buffer solution that has been kept for one month or longer after preparation shall not be used.

- g) **Phosphate-buffered physiological saline** The phosphate buffer solution of f) (0.85 % sodium chloride solution) shall be diluted 800 times with physiological saline. If necessary, it shall be dispensed into test tubes or Erlenmeyer flasks, cotton stoppers shall be put, and it shall be sterilized with high-pressure steam. If it is not used immediately after preparation, it shall be preserved at 5 °C to 10 °C. The phosphate-buffered physiological saline that has been kept for one month or longer after preparation shall not be used.

5.5 Preservation of bacteria

Bacteria shall be transferred aseptically. A safety cabinet shall be used, if necessary. Both the original strain and the slant culture medium (nutrient agar) of 5.4 d) to be transferred shall be held in one hand, the stem of the platinum loop shall be held in the other hand, and the cotton stopper shall be pulled out with this hand, then the mouth of the test tube shall be sterilized with flame. The platinum loop shall be sterilized with flame, the tip of the platinum loop shall be put in the condensed water part on the new slant culture medium to be cooled. The bacteria shall be scraped out from the part of original strain by using this platinum loop, and shall be streaked on a fresh slant culture medium.

The method thereof shall be such that, as shown in figure 1, the tip of the platinum loop shall be put in the condensed water to disperse the bacteria, and a straight line shall be drawn aslant to the upper part with the platinum loop, or the tip of the platinum loop shall be put in the condensed water again and a zigzag line shall be drawn aslant to the upper part with the platinum loop.

The mouth of the test tube shall be sterilized again with flame, and a cotton stopper shall be put as it was. The platinum loop used shall be sterilized with flame. The transferred slant culture medium shall be incubated at a temperature of 35 °C ± 1 °C for 24 h to 48 h in the incubator, then it shall be stored at 5 °C to 10 °C. Within one month of the transfer, the next transfer shall be carried out in a same way to be the passage culture. The number of passages shall be up to five times counted from the original strain obtained from the agency of culture collection. Furthermore, that kept for one month or more from the last transfer shall not be used for the following transfer.

For the bacterial strain obtained from the agency of culture collection which has been preserved by methods such as lyophilization and freezing for long life preservation, the number of passages cultured from the original strain to prepare the preserved bacterial strain shall be considered as the number of passages of the preserved bacter-

rial strain. If this preserved bacterial strain is used for the test, it shall be used up to times of the number which is obtained by subtracting the number of passages of the preserved bacterial strain from five.

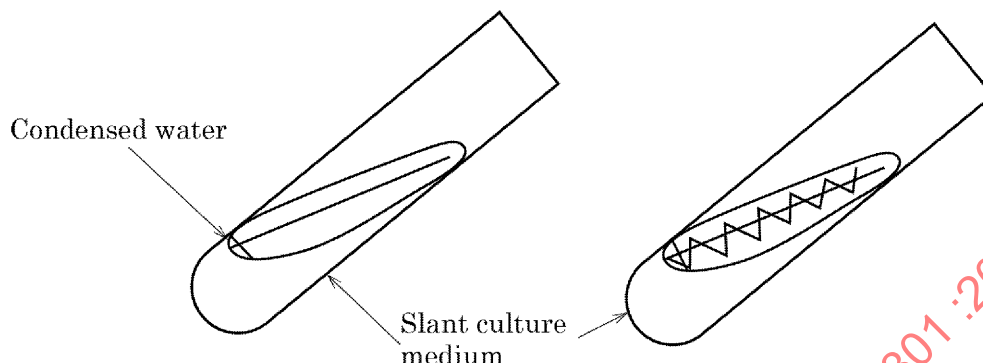


Figure 1 Transfer of bacteria

5.6 Test operation

Bacteria shall be handled aseptically, and attention shall be paid to the contamination of testing personnel, instruments and working environment with bacteria as follows. A safety cabinet shall be used, if necessary.

- a) **Pre-incubation of test bacteria** One platinum loop of bacteria shall be transferred from the preserved bacterial strain of 5.5 to the slant culture medium of 5.4 d), and incubated at a temperature of $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 h to 24 h in the incubator. Further, from this incubated bacteria, one platinum loop of bacteria shall be transferred to a fresh slant culture medium and incubated at a temperature of $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 16 h to 20 h.
- b) **Preparation of test piece** The preparation of test piece shall be as follows.
 - 1) The flat part of the product shall be cut into a square of $50\text{ mm} \pm 2\text{ mm}$ (within 10 mm in thickness) and made as the test piece of standard size. If it is difficult or impossible to cut the product into this square, a test piece of shape and size other than specified may be used if it can be covered with a film of the surface area of 400 mm^2 to $1\,600\text{ mm}^2$.
 - 2) Untreated test pieces shall be cut out from the antibacterial untreated product or the film. Among six untreated test pieces, three shall be used for the measurement of number of viable bacteria immediately after inoculation with test inoculum and another three shall be used for the measurement of number of viable bacteria after the incubation for 24 h.
 - 3) If untreated test pieces cannot be prepared, the film of 5.2 may be used. Attention shall be sufficiently paid to contamination with micro-organisms, mutual contamination between products and filth for the preparation of test pieces. It is desirable to sample the test pieces from the product itself, but if it is difficult to

prepare the test pieces because of the shape of the product, test pieces may be prepared from the product separately processed to a plate shape with the same raw material and the processing method.

- 4) If the prescribed number of untreated test pieces cannot be prepared and half the number (three pieces) can be prepared, the three untreated test pieces shall be used for the measurement of number of viable bacteria after incubation for 24 h and the film shall be alternatively used for the measurement of number of viable bacteria immediately after inoculation with test inoculum. When half the number (three pieces) cannot be prepared, the film shall be used for all.
- c) **Cleaning of test piece** The whole surface of test piece of b) shall be lightly wiped with pharmacopeia gauze or absorbent cotton immersed in ethanol two or three times, and dried completely.

If changes such as softening of test piece, dissolution of surface coating and elution of components occur after these treatments and it is considered that these treatments affect the test results, the test piece shall be cleaned by another appropriate method, or used as it is without cleaning.
- d) **Preparation of test inoculum** One platinum loop of bacteria of the test bacteria pre-incubated in a) shall be dispersed evenly in a small amount of 1/500 NB of 5.4 a), and the bacteria concentration shall be estimated with direct microscopic observation or other appropriate method. This inoculum shall be diluted with 1/500 NB appropriately and adjusted so that the bacteria concentration becomes 2.5×10^5 to 10×10^5 cells/ml, and this shall be used as the test inoculum. If the test inoculum is not used immediately, it shall be cooled on ice (0 °C) and shall be used within 2 h after storage.
- e) **Inoculation with test inoculum** The inoculation with test inoculum shall be as follows.
 - 1) Each test piece of c) shall be placed in a sterilized petri dish making the test surface up. The test surface shall be the surface of the product on which antibacterial treatment is performed. Even when the antibacterial treatment is processed to a depth, the cross section shall not be used as the test surface.
 - 2) Exactly 0.4 ml of test inoculum of d) shall be taken with a measuring pipette and instilled onto each test piece in the petri dish. The volume of inoculated inoculum on the test piece whose size is other than the standard size shall be proportionally divided by the ratio of the area of covering film. Even if the test piece is of the standard size, when the volume of inoculum based on the provision is inoculated on the test piece of very good wettability such as ceramics, tile, enamel and glass, the film may move at a small slant and the inoculum may escape from the edge of the film. In this case, the volume of inoculated inoculum may be reduced up to 1/4 of the specified volume. However, even when the volume of inoculated inoculum is reduced, the bacteria concentration inoculated on the test piece shall be 6.2×10^3 to 2.5×10^4 cells/cm².
 - 3) The instilled test inoculum shall be covered with a film, the film shall be gently

pressed so that the test inoculum spreads across the film while paying attention so that it does not spill over from the edge of film, and the lid of the petri dish shall be placed (see figure 2). The standard size of film shall be the square of $40 \text{ mm} \pm 2 \text{ mm}$. If the test piece is not that of the standard size, the size shall be adjusted so that the film can be placed within 2.5 mm to 5.0 mm from four sides of the test piece, but the size of film shall not be reduced to less than 400 mm^2 . Further, if it is difficult to adhere the film closely since the shape of test piece is not flat, if the test inoculum spreads over the test piece without covering the film since the test piece is hydrophilic or water absorbent or the like, the process of covering the film may be omitted. When the covering process of film is omitted, the standard size of test piece which is prepared in 5.6 b) 1) shall be the square of $40 \text{ mm} \pm 2 \text{ mm}$ (within 10 mm in thickness).

On the inoculation with test inoculum, if it is difficult to prevent the leakage beyond the edges of the film on the case where the surface of sample is very hydrophilic and the like, the volume of inoculum may be reduced up to 0.1 ml. In this case, the concentration of bacteria cells in inoculum shall be increased to provide the same number of bacterial cells as that of inoculated inoculum of normal volume.

Unit : mm

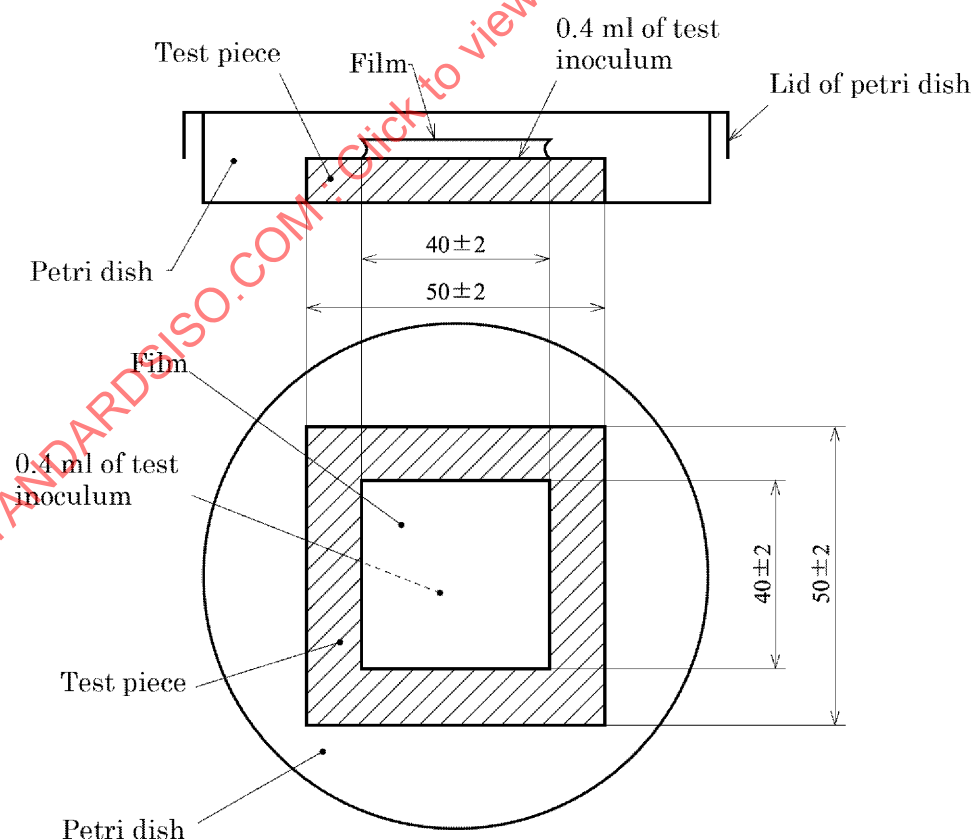


Figure 2 Instillation of inoculum on test piece and covering by film

- f) **Incubation of inoculated test piece with test inoculum** The petri dish containing the inoculated test piece with the test inoculum (three untreated test pieces and three antibacterial test pieces) shall be incubated at a temperature of $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ and a relative humidity of 90 % or more for $24\text{ h} \pm 1\text{ h}$.

NOTE : The antibacterial effectiveness of a product is evaluated from the antibacterial activity obtained from the test at the incubation temperature specified here; however the test at the temperature established considering the actual use of the antibacterial product (such as a room temperature) may be carried out together, if agreed upon all parties concerned with delivery.

- g) **Wash-out of test bacteria inoculated** The wash-out of test bacteria inoculated shall be as follows.

- 1) **Test piece immediately after inoculation with test inoculum** For three untreated test pieces immediately after inoculation with test inoculum, the covering film and the test piece shall be placed on another petri dish respectively with caution so that the inoculum does not spill. By adding 10 ml of SCDLP broth of 5.4 e), the inoculum on the untreated test piece shall be washed out with a measuring pipette at least four times, and this wash-out inoculum shall be recovered completely. The washings shall be immediately proceeded to the measurement of the number of viable bacteria.
- 2) **Test piece after incubation** For the test piece after the incubation of f), the test bacteria shall be washed out in a similar way to 1). The washings shall be immediately proceeded to the measurement of the number of viable bacteria.
- 3) **For the wash-out of test bacteria**, the method such that the covering film and the test piece are placed in a sterilized stomacher pouch by using sterilized tweezers with caution so that the inoculum does not spill, 10 ml of SCDLP broth in 5.4 e) is added with a measuring pipette and the test piece and the covering film are kneaded sufficiently with hands or an extractor (such as stomacher) for the microbial test is applicable. Or if other methods show a recovery rate equivalent to or superior to the method above, such methods may be used. If it is difficult to wash out the test bacteria with 10 ml of SCDLP broth because of the size and characteristics of the test piece, the volume may be increased.

- h) **Measurement of number of viable bacteria by agar plate culture method** Exactly 1 ml of the washings of g) shall be taken with a measuring pipette and added in a test tube containing 9.0 ml of phosphate-buffered physiological saline of 5.4 g), and sufficiently mixed. Then, 1 ml shall be taken from this test tube with a new measuring pipette and add in another test tube containing 9.0 ml of phosphate-buffered physiological saline, and sufficiently mixed. These procedures shall be repeated to prepare 10-fold serial dilutions. 1 ml each of the washings and each dilution shall be dispensed into two sterilized petri dishes. To each petri dish, 15 ml to 20 ml of the plate count agar of 5.4 c) warmed at $46\text{ }^{\circ}\text{C}$ to $48\text{ }^{\circ}\text{C}$ shall be added and sufficiently mixed. By placing the lids, the petri dishes shall be left as they are at a room temperature. After solidifying the culture medium, the petri dishes shall be

turned over, and incubated in the incubator at a temperature of $35\text{ }^{\circ}\text{C} \pm 1\text{ }^{\circ}\text{C}$ for 40 h to 48 h. After incubation, the number of colonies in a serially diluted petri dish in which 30 to 300 colonies appear shall be measured, as a rule. If the number of colonies is less than 30 in the agar plate dispensed with 1 ml of the washings, the number of colonies shall be measured for this plate. If there are not any colony formations in any agar plate, then "< 1" shall be recorded. Further, if the number of colonies is not inversely proportional to the dilution ratio, since it is considered that the formation of colonies is inhibited by the effects of the antibacterial agent, the number of viable bacteria shall be determined using a method which forms colonies without being affected by the antibacterial agent with the use of an inactivating agent or dilution.

NOTE: For the methods of adoption of the number of colonies other than specified above, refer to 1.2 Microorganism tests, 3) Viable cell count of bacteria, (1) Pour plating method of Standard Method of Analysis for Hygienic Chemists (2005) edited by the Pharmaceutical Society of Japan, or Chapter 2 Bacteria 2. Spoilage indicator bacteria, 1. Total bacterial count of Standard Methods of Analysis in Food Safety Regulation (2004) supervised by the Environmental Health Bureau of the Ministry of Health and Welfare, Japan.

5.7 Calculation of number of viable bacteria

The number of viable bacteria shall be obtained by counts of colonies measured according to equation (1).

$$N = \frac{C \times D \times V}{A} \dots\dots\dots(1)$$

where, N : number of viable bacteria (per 1 cm² of test piece)
 C : count of colonies (average count of colonies of two petri dishes adopted)
 D : dilution factor (that of dilution dispensed into petri dishes adopted)
 V : volume of SCDLP broth used for wash-out (ml)
 A : surface area of covering film (cm²)

In the case where the covering film is omitted in 5.6 e) 3), A shall be the surface area (cm²) of the antibacterial test piece or the untreated test piece.

The number of viable bacteria shall be expressed with two significant figures by rounding off the third significant figure. When the count of colonies C is "<1", C is taken as "1", and the number of viable bacteria shall be calculated corresponding to V , A , D at that time. For example, when V is 10 ml, A is 16 cm² and D is 1, it shall be expressed as "<0.63".

5.8 Test results

The test results shall be as follows.

- a) **Determination of conditions of test validation** When the following three test conditions are all satisfied, the test shall be determined to be valid. Unless all the con-

ditions are satisfied, the test shall be determined to be not valid, and a retest shall be carried out.

- 1) The following equation (2) is established for the logarithmic value of the number of viable bacteria immediately after inoculation on untreated test piece.

$$\frac{L_{\max} - L_{\min}}{L_{\text{mean}}} \leq 0.2 \quad \dots\dots\dots(2)$$

where, $L_{\max}$: maximum logarithm number of viable bacteria
 $L_{\min}$: minimum logarithm number of viable bacteria
 L_{mean} : average of logarithm numbers of viable bacteria of three test pieces

- 2) The average of the number of viable bacteria immediately after inoculation on the untreated test piece shall be within the range of 6.2×10^3 to 2.5×10^4 cells/cm².
- 3) The number of viable bacteria on untreated test piece after 24 h shall be not less than 62 cells/cm² for all three test pieces. When a film is used for the untreated test piece; however, the number of viable cells of bacteria after 24 h shall be not less than 6.2×10^2 cells/cm² for all three test pieces.

- b) **Calculation of antibacterial activity** When the test has been determined to be valid, the antibacterial activity shall be obtained according to equation (3). The value shall be recorded to the first decimal place by rounding the second decimal place down. When the number of viable bacteria is "<0.63", it shall be taken as "0.63" and the average of logarithm numbers shall be calculated.

$$R = (U_t - U_o) - (A_t - U_o) = U_t - A_t \quad \dots\dots\dots(3)$$

where, R : antibacterial activity
 U_o : average of logarithm numbers of viable bacteria immediately after inoculation on untreated test pieces
 U_t : average of logarithm numbers of viable bacteria after inoculation on untreated test pieces after 24 h
 A_t : average of logarithm numbers of viable bacteria after inoculation on antibacterial test piece after 24 h

6 Record of test results

The following matters shall be listed in the test results of antibacterial products such as plastic products.

- a) Number or title of this Standard
- b) Commencement date of test
- c) Type, size, shape and thickness of antibacterial-treated test piece and untreated test piece
- d) Type, size, shape and thickness of film

- e) Type of test bacteria
- f) Preservation number of bacterial strain
- g) Volume of test inoculum inoculated
- h) Number of viable bacteria in test inoculum
- i) Cleaning method
- j) Respective value of U_0 , U_t , A_t and antibacterial activity of 5.8 b), identification of laboratory, name and signature of head, and date of test report
- k) Any deviation from this specification

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Bibliography

JIS L 1902 *Testing for antibacterial activity and efficacy on textile products*

