

### **3. Antibacterial Power Sustainability Test Methods for Antibacterial Products (2012 Version)**

#### **(1) Waterproofing Test**

##### **1. Outline**

An antibacterial product conferred with an antibacterial function (hereinafter simply referred to as a product) can lose antibacterial power due to a loss of its antibacterial ingredients from its surface when in contact with water (or warm water). With this in mind, an accelerated test is performed under test conditions (water temperature and immersion time) specified for each product category to prepare test pieces<sup>1</sup>. Using these test pieces, an antibacterial power test is performed to evaluate the sustainability of the antibacterial power (waterproofing performance) of the product.

##### **2. Procurement of Supplies**

The following instrumentation and equipment shall be provided. 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.

In addition to the items shown below, instruments etc. shall be provided as required.

- (1) Desiccator
- (2) Measuring cylinders
- (3) Immersion containers (shall be made of glass, polyethylene or polypropylene, and may have an optionally chosen shape and capacity; shall be thoroughly cleaned before use<sup>2</sup>.)
- (4) Dryer (air bath)
- (5) Deionized water or distilled water
- (6) Heater
- (7) Others

##### **3. Preparation of Test Pieces**

The test piece subjected to this 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,<sup>3</sup> it may be used as the test piece.

The test piece may be cut to desired size after a waterproofing test is performed or prior to a waterproofing test. Described below is how to prepare a test piece previously cut to a size suitable for the antibacterial power test.

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<sup>1</sup> The test piece subjected to the antibacterial power test of an antibacterial product must, as a rule, be a test piece obtained by this method of preparation.

<sup>2</sup> As clean containers as possible shall be used. It is desirable that dedicated immersion containers be used for untreated test pieces and antibacterial test pieces, respectively.

<sup>3</sup> Should this test method be difficult to apply to prepare a test piece due to a special shape of the product and the like, a sample prepared by another method of treatment deemed to produce nearly the same results for antibacterial power may be used as the test sample. If a non-actual supply of the product is used as the test sample, however, the fact shall be stated in the Test Results section.

- (1) Provide twelve test pieces cut into  $50\pm 2$  mm square (thickness not more than 10 mm) standard pieces and use these as the antibacterial test pieces<sup>1</sup>.
- (2) Provide six untreated samples, previously cut into the same size as the test pieces, and use these as the untreated test pieces<sup>2</sup>.

#### 4. Test Procedures

Proceed as directed below.

- (1) Place the specified amount ( $2\pm 0.4$  ml per  $\text{cm}^2$  of antibacterial treatment area of test piece) of deionized water or distilled water in an immersion container and maintain a temperature in the range specified for the product category of the test piece. The water temperature shall be determined with reference to the four categories by intended use of product shown in Table 1<sup>3</sup>.
- (2) Place the test piece in the container to completely immerse the entire test piece in the deionized water or distilled water and allow to stand for the specified time<sup>4</sup>. In the case of warming, wait until the specified water temperature is reached, and then place the test piece in the immersion container.
- (3) After 16 hours of immersion, remove the test piece, drain the water on the test surface, and immediately perform the antibacterial power test. If the antibacterial power test cannot immediately be performed, keep the test piece in a dry state in a desiccator or dryer (air bath)<sup>5</sup>. If impossible, the test piece may be kept in a container that does not influence its quality, such as a glass Petri dish.

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<sup>1</sup> The thickness of the test piece shall be up to 10 mm to allow easy entry into a Petri dish. If the thickness exceeds 10 mm, the test piece shall be sliced to obtain a thickness of less than 10 mm. In this case, be sure to reserve the original surface of the test piece, and to perform the test on the reserved surface. 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 original area. If a standard test piece area cannot be assured because of the size of the test piece and the like, the size of the test piece shall be indicated.

In the antibacterial power test after preparation of the test pieces, use three test pieces (6 in total) for each of the bacteria *Staphylococcus aureus* NBRC12732 (ATCC 6538P) and *Escherichia coli* NBRC3972 (ATCC 8739).

<sup>2</sup> An untreated sample refers to a product not undergoing antibacterial treatment, and must be prepared with the same material using the same method of treatment as those for the test pieces.

<sup>3</sup> The category applied in the waterproofing test shall be indicated. If the test is performed under test conditions (water temperature and immersion time) other than those categorized in Table 1, the fact and the test conditions used shall be indicated.

<sup>4</sup> The immersion need not always be continuous, as long as the immersion time reaches the specified level on a cumulative basis. If more than one test piece is placed in the same container, they shall be placed with their antibacterial power test surfaces without overlapping, while keeping a sufficient gap to avoid the contact of the antibacterial power test surfaces with the inside wall of the container and contact with the other test pieces. However, an antibacterial test piece and an untreated test piece must not be placed in the same container. The test pieces shall, as a rule, be allowed to stand, with no stirring, shaking and the like, but stirring, shaking and the like may be performed if judged to be necessary for the sake of test convenience.

<sup>5</sup> The temperature range shall be between 30 and 40°C.

### 5. Standard Test Conditions and Categories

The following two standard conditions for the waterproofing test shall be established.

- (1) Immersion temperature<sup>1</sup>
- (2) Immersion time

Regarding product categorization for the waterproofing test, intended uses of products are divided into four categories in view of the degree of contact of product and water. Relationships between the categories and immersion temperature and time are shown in Table 1.

Table 1 Product categories for waterproofing test and immersion temperature and time

Category	Immersion conditions		Applicability (range)
	Water temperature (°C)	Immersion time (hr)	
0	Not performed		Products that do not come into contact with water (products that do not come into contact with water in ordinary use settings, or come into contact with water only during cleaning etc.)
1	Normal temperature <sup>2</sup>	16	Products that are unlikely to come into contact with water (products that do not come into contact with water except for incidental splashing)
2	50±5	16	Products that often come into contact with water (products for containing water or for use in water)
3	90±5	16	Products that often come into contact with warm water (products for containing warm water or for use in warm water)

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<sup>1</sup> The test may be performed under severer conditions than those specified for the category of the product shown in Table 1. Specifically, the test may take place at a higher temperature and for a longer immersion time than those specified for the category of the product. In the case of a test piece to which the antibacterial power test is difficult due to deformation etc. under the temperature conditions specified for the category 3 of the product (thermoplastic resins that do not endure 90°C heat, etc.), the temperature conditions may be set at lower levels that do not cause such deformation. In this case, however, the immersion time shall be doubled per 10°C of temperature reduction.

<sup>2</sup> Normal temperature is 5 to 35°C (JIS Z 8703). Temperature shall be set in view of the intended use of the product. However, temperature controls are unessential.