

1. Purpose and Scope

This criterion is applicable to the evaluation and testing of antibacterial activity of textile for medical use. The quantitative evaluation of antibacterial activity is judged by the percentage of bacteria reduction.

2. Terminology

Antibacterial treatment: A finishing treatment on aiming at inhibiting the growth of bacteria on textile.

Reduction rate: Percentage of bacteria reduction (%)

3. Performance Specification

3.1 Classification

To meet the requirement, textile must achieve antibacterial activity expressed by percentage reduction of bacteria (%) by an approved lab to grade 2 or above on two specified test organisms, after being washed for 100 cycles or no washing required for disposable product.

3.2 Washing requirement

Table 1. Type of Washing Cycles

Type	Washing cycles
I	Antibacterial activity after 100 washing
II	Antibacterial activity without washing (for disposable products)

3.3 Classification of antibacterial activity

Table 2. Classification of antibacterial activity

Percent reduction of bacteria (%)	Grade	Classification
$R \geq 99.9$	3	Excellent
$99 \leq R < 99.9$	2	Good
$0 < R < 99$	1	Fair

3.4 Toxicity test

The applicant must provide animal test reports from a third party with dermal irritation test (PII primary irritation index <2) or allergenic test (negative, positive 0%), and acute oral toxicity report (LD50 in mice >1000 mg/kg, no mortality nor abnormal symptom) for the antibacterial finishing reagent used for the treated textile. This can also be provided by test report copy or guarantee letter from a third party by antibacterial finishing reagent supplier.

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4. Test Method

4.1 Bacteria to be used in tests

4.1.1 *Methicilin resistant Staphylococcus aureus* (BCRC 15211, ATCC 33591)⁽¹⁾

4.1.2 *Pseudomonas aeruginosa* (BCRC 10944, ATCC 10145)

Remark⁽¹⁾: BCRC : Bioresource Collection and Research Center of Food Industry Research Development Institute.

ATCC: American Type Culture Collection.

4.2 Test Preparation

4.2.1 Chemicals, materials and implements.

- (1) Ethanol (C₂H₅OH): Reagent grade.
- (2) Agar: For microbial test.
- (3) Beef Extract: For microbial test.
- (4) Peptone: For microbial test.
- (5) Sodium Chloride: Reagent grade.
- (6) Wetting agent: Sodium Dioctyl Sulfosuccinate or others.
- (7) Purified water: Distilled water or deionized water.
- (8) Petri dish: Conforming to CNS 7320 90A or 90B with about 9 cm inside diameter, 1.5-1.8 cm depth. The surface of the Petri dish should be smooth, no bubbles, scratches or other damages.
- (9) Autoclave: Capable of keeping at 121 °C, 103 kPa (1.05 kg/cm²), for over 15 minutes.
- (10) Spectrophotometer: Capable of measuring at 660 nm.
- (11) Inoculating loop: 4mm loop at its point, platinum or disposable.
- (12) Incubator: Capable of keeping at 37±2°C.
- (13) Water bath shaker: Temperature setting ±2°C in accuracy, rate of shaking 110 ± 10 rpm and 3 cm in width.
- (14) Flask: Can be autoclaved (121 °C, 103 kPa (1.05 kg/cm²)).
- (15) Test tube: Can be autoclaved (121 °C, 103 kPa (1.05 kg/cm²)).
- (16) Laminar flow: Class II.
- (17) Standard untreated fabric: 100% cotton cloth, which has no antimicrobial effectiveness and the test bacteria can grow on it normally.
- (18) Vortex mixer: Adjustable to 1800-2500 rpm.
- (19) Detergent: Polyoxyethylene Alkyl Ether.
- (20) Oven: Adjustable temperature setting, capable of keeping temperature from room temperature to 100 °C or above, ±2°C in accuracy.
- (21) Colony counter: 4-digit.

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4.2.2 Preparation of test specimen: Cut circular swatches 4.8 ± 0.1 cm in diameter from sample.

4.2.3 Culture medium: Nutrient, Trypticase Soy and Brain-Heart Infusion are suitable broth / agar media.

(1) Example 1 - Nutrient agar (NA)

peptone	5 g
beef extract	3 g
agar	15 g
distilled water	to 1000 mL

Heat to a boil to disperse ingredients. Adjust to pH 6.8 ± 0.1 with 1 N sodium hydroxide (NaOH) solution. (This is not necessary if prepared, dehydrated medium is used.) Sterilize by autoclave. Cool in water bath with temperature set at $45-50^{\circ}\text{C}$ before pouring to Petri dishes. If it is not used immediately after preparation, preserve it at $5-10^{\circ}\text{C}$. Never use the nutrient agar kept for one month or longer after preparation.

(2) Example 2 - Nutrient broth (NB)

peptone	5 g
beef extract	3 g
distilled water	to 1000 mL

Heat to a boil to disperse ingredients. Adjust to pH 6.8 ± 0.1 with 1 N sodium hydroxide (NaOH) solution. (This is not necessary if prepared, dehydrated medium is used.) Sterilize by autoclave. When it is not used immediately after preparation, preserve it at $5-10^{\circ}\text{C}$. Never use the nutrient broth kept for one month or longer after preparation.

4.2.4 Incubation of test bacteria

The incubation of bacteria stock strain shall be carried out according to the buying instruction. Transfer the stock strain to NA slant (or other suitable medium). Incubate the bacteria transferred slant culture medium at $37 \pm 2^{\circ}\text{C}$ for 24 h, and then transfer to NB (or other suitable medium broth) at $37 \pm 2^{\circ}\text{C}$ for 24 h.

4.2.5 Sterilization

The test specimens, flask, test tube, pipette, Petri dish, culture medium, water should be autoclaved before use.

4.2.6 Preincubation and preparation of test inoculum, and measurement of number of living bacteria

(1) Bacteria transference from the freeze-dried stock strain. Flame the platinum inoculum loop and cool it down or use disposable inoculum loop. Scrap out a loop of preserved bacteria and spread to slant NA agar (or other suitable medium). Incubate the transferred slant culture medium at $37 \pm 2^{\circ}\text{C}$ for 24-48 h, then maintain at $5-10^{\circ}\text{C}$. Never use it if it is kept for one week or longer after preparation. (Referred as "Preincubation a")

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- (2) Place 20 mL of NB in a 100 mL Erlenmeyer flask. Take one loop of bacteria from “Preincubation a” with a platinum loop or disposable inoculum loop, inoculate it in the broth. Incubate it for 18-24 h in 37 ± 2 °C, and shake with rate of 110 ± 10 rpm and 3 cm in width. (Referred as “Preincubation b”)
- (3) Use spectrophotometer or colony counter to measure the bacteria concentration of “Preincubation b”, adjusting to $1-2 \times 10^8$ CFU/mL, with sterilized NB. (CFU = Colony Forming Unit.)
- (4) Place 20mL of NB (or other suitable broth) in a 100 mL Erlenmeyer flask, add in 0.4 mL of “Preincubation b”. Incubate it for 2 h in 37 ± 2 °C, shaking in 110 ± 10 rpm. The target number of bacteria is about 10^7 CFU/mL. (Referred as “Preincubation c”)

4.3 Test Procedure

4.3.1 Antibacterial activity test

- (1) Bacteria Number of Incubation: Dilute 20 times the NB (or other suitable broth) and adjust “preincubation c” to make the bacteria concentration to be $1-2 \times 10^5$ CFU/mL. Use the diluted nutrient broth as the test inoculum.
- (2) Inoculation: Apply 1 mL of the diluted test inoculum onto 4 test specimens and 4 standard untreated fabric swatches respectively. Wetting agent can be added to the 20 times diluted test inoculum to enhance wetting of hydrophobic fabrics. See Remark.⁽²⁾
Remark⁽²⁾: By prior testing at the intending concentration, the wetting agent must not cause a reduction of bacterial number. Report the name and concentration of wetting agent used.
- (3) Shake-out immediately after inoculation: Place 100 mL of neutralizing solution (add 8.5 g sodium chloride to 1000 mL distilled water) to the standard untreated fabrics. Shake out bacteria by a Vortex mixer. Make serial dilution of 10^1 , 10^2 , 10^3 , pipette 1 mL out of each to 9 cm Petri dish. Pour 14-20 mL of Nutrient Agar (or other suitable agar medium) and mix well. After agar solidified, place all plates in incubator at 37 ± 2 °C for 24-48 h and calculate the bacteria counts (A). (Test 4 sets for each sample)
- (4) Shake out after incubation: After inoculating 1 mL of the inoculum onto test specimens and standard untreated fabrics, incubate at 37 ± 2 °C for 18-24 h. After incubation, add 100 mL of neutralizing solution to the test specimens and standard untreated fabrics respectively. Shake out bacteria by a Vortex mixer. Make serial dilution of 10^1 , 10^2 , 10^3 and 10^4 , pipette 1mL out of each to 9 cm petri dish. Pour 14-20 mL of Nutrient Agar and mix well. After agar solidified, place all plates in incubator at 37 ± 2 °C for 24-48 h and calculate the bacteria counts of the standard untreated fabric (B) and the bacteria counts of test specimen (C). (Test 4 sets for each sample).

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4.3.2 Washing operation

- (1) Washing condition: Washing according to the condition in Table 3. Each specimen is placed in the stainless steel canister individually. Different specimens are not allowed to put into the same canister to avoid cross contamination.

Table 3. Washing condition

Specimen size L x W (cm)	Temperature (°C)	Total liquor volume (mL)	Percent detergent of total volume (%)	No. steel balls	Time (min)
15X5	71	50	0.15	100	45

- (2) Remove each specimen from the canister, rinse thoroughly. Remove excess water by hand squeezing or centrifuge. Dry the specimen in an air circulating oven which temperature does not exceed 71 °C.
- (3) Repeat the washing procedure according to the above washing condition, rinsing, removing excess water, and drying to the required washing cycles.

4.4 Result

4.4.1 Calculate percentage of bacteria reduction by the following formula

$$R \% = [(A-C)/A] \times 100$$

R= Percent reduction of bacteria

A= The number of bacteria recovered from the inoculated standard untreated fabric immediately after inoculation

C= The number of bacteria recovered from the inoculated test specimen after incubation

4.4.2 Bacteria growth value: The test is judged as effective as $\text{Log}(B) - \text{Log}(A) \geq 1.5$

A= The number of bacteria recovered from inoculated standard untreated fabric immediately after inoculation

B= The number of bacteria recovered from inoculated standard untreated fabric after incubation for 18-24 h.

5. Mark

Type	Grade	Classification	Washing cycle	Reduction of bacteria (%)
I	AAAAA	Excellent	100 washing	$R \geq 99.9$
	AAA	Good	100 washing	$99 \leq R \leq 99.9$
II	AAAAA	Excellent	No washing required (disposable)	$R \geq 99.9$
	AAA	Good	No washing required (disposable)	$99 \leq R < 99.9$

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6. Reference

AATCC100-1999 Antibacterial Finishes on Textile Materials: Assessment of

AATCC 61 3A-2001 Colorfastness to Laundering, Home and Commercial Accelerated

JIS L 1902-1998 Testing Method for Antibacterial of Textile

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