Specified Requirements of Antibacterial Textiles for General Use	Document No. FTTS-FA-001
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1. Purpose and Scope

This criterion is applicable to the evaluation and testing of antibacterial activity of textile for general 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 of bacteria reduction (%) by an approved lab to grade 2 or above on two specified test organisms, after being washed for 50, 20 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 50 washing
II	Antibacterial activity after 20 washing
III	Antibacterial activity without washing (for disposable products)

3.3 Classification of antibacterial activity

Table 2. Classification of antibacterial activity

Percentage of bacteria reduction(%)	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 test 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 for tests

4.1.1 *Staphylococcus aureus* (BCRC 10451, ATCC 6538P)[(1)]

4.1.2 *Klebsiella pneumoniae* (BCRC 16082, ATCC 4352)

Remark[(1)]: BCRC : Bioresouces 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 (C_2H_5OH): 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 deioned 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℃, 103 kPa (1.05kg/cm^2), for over 15 minutes.

(10) Spectrophotometer: Capable of measuring at 660 nm.

(11) Inoculating loop: 4 mm loop at its point, platinum or disposable.

(12) Incubator: Capable of keeping at 37 $\pm$ 2℃.

(13) Water bath shaker: Temperature setting $\pm$2℃ in accuracy, rate of shaking: 110 $\pm$ 10 rpm and 3 cm width.

(14) Flask: Can be autoclaved (121℃, 103 kPa (1.05 kg/cm^2)).

(15) Test tube: Can be autoclaved (121℃, 103 kPa (1.05 kg/cm^2)).

(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℃ or above, $\pm$2℃ in accuracy.

(21) Colony counter: 4-digit.

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℃ before pouring to Petri dishes. If it is not used immediately after preparation, preserve it at 5-10℃. 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 ℃. 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±2 ℃ for 24 h, and then transfer to NB (or other suitable medium broth) at 37±2 ℃ 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 a preserved bacteria and spread to slant NA agar (or other suitable medium). Incubate the transferred slant culture medium at 37±2 ℃ for 24-48 h, then maintain at 5-10℃. Never use it if it is kept for one week or longer after preparation. (Referred as “Preincubation a”)

(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 ℃, 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 × 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 ℃, 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 × 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 in 1000 mL distilled water) to the standard untreated fabtics. 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 and mix well. After agar solidified, place all plates in incubator at 37±2 ℃ 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 ℃ for 18-24 hours. 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 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℃ 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).

4.3.2 Washing operation

Refer to AATCC 135 (1)IV(A)i.

4.4 Result

4.4.1 Calculate percentage of bacteria reduction by the following formula

R %= [(A-C)/A] x 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 Log (B)-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	50 washing	R $\geq$ 99.9
	AAA	Good	50 washing	99 $\leq$ R $\leq$ 99.9
II	AAAAA	Excellent	20 washing	R $\geq$ 99.9
	AAA	Good	20 washing	99 $\leq$ R $\leq$ 99.9
III	AAAAA	Excellent	No washing required (disposable)	R $\geq$ 99.9
	AAA	Good	No washing required (disposable)	99 $\leq$ R $\leq$ 99.9

6. Reference

AATCC100-1999 Antibacterial Finishes on Textile Materials: Assessment of

AATCC 135-2001 Dimensional Changes in Automatic Home Laundering of Woven and Knit Fabrics

JIS L 1902-1998 Testing Method for Antibacterial of Textile