

**Test Method of Specified Requirements of
Antibacterial Textiles for General Use
FTTS-FA-001**

FTTS-FA-001 Antibacterial Textiles for General Use

Generally speaking, the application of anti-bacterial fibers is very extensive in the textile industry. There are very few textile manufacturers against the promotion claim of anti-bacterial functions on their products. The anti-bacterial effect could be applied in the areas of children's and infants' wears, underwear, sports shirts, shirts, working uniforms, medical supplies, patient wears, household textiles, beddings, furnishing textiles, non-woven fabrics, family and personal cleansing commodities, automobile textile products and many others.

1. 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 bacteria reduction.

2. Terminology

2.1 Anti-bacterial treatment: A finishing treatment on aiming at inhibiting the growth of bacteria on textile.

2.2 Red: Percentage reduction of bacteria (%)

3. Performance specification

3.1 Criteria

Textile must achieve antibacterial activity expressed by percentage reduction of bacteria (%) to grade 2 or above on two specified test organisms, after being washed for 50, 20 cycles by an approved lab, or no washing required for disposable product so to meet the requirement set for FTTS-FA-001.

3.2 Washing requirement

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

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

3.4 Toxicity test

The applicant must provide animal test reports 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 from a third party or guarantee letter from antibacterial finishing reagent supplier.**

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: BCRC : Bioresources 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 (1.05kg/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 width.
- (14) Flask: can be autoclaved (121°C, 103 kPa (1.05kg/cm²))
- (15) Test tube: can be autoclaved (121°C, 103 kPa (1.05kg/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 and above, $\pm 2^\circ\text{C}$ in accuracy.

(21) Colony counter: 4-digit.

4.2.2 Preparation of specimen: Cut circular swatches $4.8 \pm 0.1\text{cm}$ in diameter from the tested sample.

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

(1) Example 1 - Nutrient agar (NA)

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

Heat to a boil to disperse ingredients. Adjust to pH 6.8 ± 0.1 with 1N 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 $45\text{-}50^\circ\text{C}$ before pouring to Petri dishes. When it is not used immediately after preparation, preserve it at $5\text{-}10^\circ\text{C}$. Never use the nutrient agar kept for one month or longer after preparation.

(2) Example 2 - Nutrient broth (NB)

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

Heat to a boil to disperse ingredients. Adjust to pH 6.8 ± 0.1 with 1N 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 $5\text{-}10^\circ\text{C}$. Never use the nutrient broth kept for one month or longer after preparation.

4.2.4 Incubation of test bacteria

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

4.2.5 Sterilization

The test specimens, flask, test tube, pipette, Petri dish, culture medium, water

should be autoclaved before use.

4.2.6 Incubation 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 or use disposable inoculum loop, scrap out a platinum loop of bacteria from a preserved bacteria and spread to slant NA agar (or other suitable medium). Incubate the bacteria transferred slant culture medium at $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$ for 24-48 hr. Maintain at $5-10^{\circ}\text{C}$, and never use it if it is kept for one week or longer after preparation. (Referred as “Incubation a”)

(2) Place 20 ml of NB in a 100 ml Erlenmeyer flask, take one loop of bacteria from “Incubation a” with a platinum loop or disposable inoculum loop, inoculate it in the broth. Incubate it for 18-24 hr in the following condition : Temperature: $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$, rate of shaking: 110 ± 10 rpm and 3cm in width. (Referred as “Incubation b”)

(3) Use spectrophotometer or colony counter to determine the bacteria concentration of “Incubation b” to $1-2 \times 10^8$ CFU/ml, adjusting the concentration with sterilized NB. (CFU = Colony Forming Unit.)

(4) Place 20ml of NB (or other suitable broth) in a 100 ml Erlenmeyer flask, add 0.4ml of “Incubation b”, incubate it for 2 hr in the following condition : Temperature: $37^{\circ}\text{C} \pm 2^{\circ}\text{C}$, rate of shaking: 110 ± 10 rpm. The target number of bacteria is about 10^7 CFU/ml. (Referred as “Incubation c”)

4.3 Test Procedure

4.3.1 Antibacterial activity test

(1) Dilute 20 times the “Incubation c” using NB (or other suitable broth) to make the bacteria concentration to be $1-2 \times 10^5$ CFU/ml. Use the diluted nutrient broth as the test inoculum.

(2) Apply 1 ml of the diluted test inoculum onto 4 pieces of test specimen, 4 untreated control fabric swatches or untreated cotton fabric swatches respectively. Wetting agent can be added to the 20 times diluted test inoculum to enhance wetting of hydrophobic fabrics. See Remark.⁽³⁾

Remark.⁽³⁾ : The wetting agent must be shown not to cause a reduction in bacterial number, by prior testing at the intended use concentration. Report the use and concentration of wetting agent used.

(3) Shake-out immediately after inoculation

Add 100 ml of neutralizing solution (8.5g sodium chloride to 1000 ml

distilled water) to the tested specimen. Shake out bacteria from test specimen by a Vortex mixer. Make serial dilution of 10^1 , 10^2 , 10^3 , pipette 1 ml out to 9 cm petri dish. Pour 14-20 ml of Nutrient Agar and mix well. After agar solidified, place all plates in incubator at $37 \pm 1^\circ\text{C}$ for 24-48 hours and calculate the bacteria counts (A). (Test 4 specimens for one sample)

(4) Shake out after incubation

After inoculating 1 ml of the inoculum onto test specimens and untreated control fabrics, incubate at $37 \pm 2^\circ\text{C}$ for 24-48 hours. After incubation, add 100 ml of neutralizing solution to the test specimens and untreated control fabrics respectively. Shake out bacteria by a Vortex mixer. Make serial dilution of 10^1 , 10^2 , 10^3 and 10^4 , pipette 1ml diluted solution 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 $37 \pm 2^\circ\text{C}$ for 24-48 hours and calculate the bacteria counts of the untreated fabric (B) and the bacteria count of test specimen (C). (Test 4 specimens for one sample).

4.3.2 Washing requirement:

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

4.4 Result

Calculate percentage of bacteria reduction by the formula as:

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

R= Percent reduction of bacteria

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

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

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

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

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