Test Method of Specified Requirements of Antibacterial Textiles for Medical Use

FTTS-FA-002

FTTS-FA-002 Antibacterial Textiles for Medical Use

Antibacterial Textiles suppress and even kill harmful bacteria that tend to grow on fabrics. To achieve the level of antibacterial textile for medical use, the antibacterial textiles have to pass severe qualification tests, and the antibacterial activity can be effective on various bacteria. Antibacterial Textiles for medical use should be either bacteriocidal or bacteriostatic. These textiles are used in hospitals to prevent or decrease the spread of infections. All medical institutions need these textiles for general use in hospital bedding, sheets, pillowcases, quarantine curtains, and in operating rooms for robes, hats, aprons, covers, lining cloths, gauze, bandages, masks, shelter wear, air filters and more.

As to current antibacterial technology in Taiwan, the effectiveness of antibacterial textile for medical use generally achieve bacteria reduction rate to over 99% (The best grade for such textiles is 99.9% (test method applied is AATCC 100)). Performance on the Bacteriostasis Activity Value is not less than 2.0. Bacteria used for testing are Staphylococcus aureus and the test method applied is JIS L1902.

1. Scope

This criterion is applicable for the evaluation and testing of antibacterial activity of textile for medical 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.

2.2Red: 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 100 cycles by an approved lab, or no washing required for disposible product so to meet the requirement set for FTTS-FA-002.

3.2 Washing requirement

Type	Washing cycles
I	Antibacterial Activity after 100 washing
II	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 ≦ 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 leter from antibacterial finishing reagent supplier.

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: 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: 4mm loop at its point, platinum or disposable.
(12) Incubator: Capable of keeping at 37+2℃.
(13) Water bath shaker: temperature setting ±2℃ in accuracy, rate of shaking: 110 ± 10 rpm and 3 cm width

(14) Flask: can be autoclaved (121℃, 103kPa (1.05kg/cm2))

(15) Test tube: can be autoclaved (121℃, 103kPa (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℃ and above, ±2℃ in accuracy.

(21) Colony counter: 4-digit.

4.2.2 *Preparation* of specimen: Cut circular swatches 4.8 ± 0.1cm 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 1000 ml

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 45-50℃ before pouring to Petri dishes. When 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	5g
beef extract	3g
distilled water	to 1000 ml

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 5-10℃. 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℃± 2℃ for 24 hr., and then transfer to NB (or other suitable medium broth) at 37℃± 2℃ 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℃± 2℃ for 24-48 hr.. Maintain at 5-10℃, 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℃± 2℃, 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 X 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℃± 2℃, 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 X 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 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±1℃ 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±2℃ 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 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±2℃ 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 operation:

(1)Washing condition:

Washing according to the below condition. 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.

Specimen size L x W (cm)	Temperature (℃)	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. Remover excess water by hand squeezing or centrifuge. Dry the specimen in an air circulating oven in which the temperature does not exceed 71℃.

(3) Repeat the washing procedure according to the above washing condition, rinsing, removing excess water, and drying to the required washing cycle.

Calculate percentage of bacteria reduction by the formula as:

R %= [(A-C)/A] x 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: Log (B)-Log (A)> 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.