Specified Requirements for Antibacterial Activity of Textiles

Doc. No. ：FTTS-FA-027

Version: 1.0

1.Scope

This requirement specifies the antibacterial test method and quality requirements for textiles.

Remark: applicant must include skin irritation (PII value[(1)]＜2) or allergic (no allergic reaction) report of the antibacterial processing agent, and the original acute oral toxicity (no death or anomaly in mice＞1000 mg/kg) report from approved labs, or the third party test report copy and warranty from the supplier.

Note[(1)]: primary irritation index.

2.Definition

2.1 Antibacterial agent: agent that can prevent or inhibit bacterial proliferation, and can reduce the number of bacteria or sterilize.

2.2 Antibacterial finish: activity that can prevent or inhibit bacterial proliferation, and can reduce the number of bacteria or sterilize.

2.3 Antibacterial activity: indicates a property that can prevent or inhibit bacterial proliferation, and can reduce the number of bacteria or sterilize.

2.4 Neutralizer: chemical agent that is capable of inactivating, neutralizing or inhibiting the antibacterial properties of antibacterial agents.

3.Quality

Status and antibacterial activity value after washing must comply with Table 1 requirements.

Table 1 Quality requirements of antibacterial textiles

Product	Type	Laundry resistance frequency	Grade	Antibacterial activity value
Medical textiles	I	100 times	A	Antibacterial activity value≧3.0
			B	2.0≦Antibacterial activity value＜3.0
	II	50 times	A	Antibacterial activity value≧3.0
			B	2.0≦Antibacterial activity value＜3.0
	III	No need to wash	A	Antibacterial activity value≧3.0
			B	2.0≦Antibacterial activity value＜3.0
General textiles	I	50 times	A	Antibacterial activity value≧3.0
			B	2.0≦Antibacterial activity value＜3.0
	II	20 times	A	Antibacterial activity value≧3.0
			B	2.0≦Antibacterial activity value＜3.0
	III	10 times	A	Antibacterial activity value≧3.0
			B	2.0≦Antibacterial activity value＜3.0
	IV	No need to wash	A	Antibacterial activity value≧3.0
			B	2.0≦Antibacterial activity value＜3.0

4. Method

4.1 Test strains

4.1.1 Medical textiles

(1) Methicillin resistant *Staphylococcus aureus* BCRC 15211[(2)] (ATCC 33591)

(2) Pseudomonas aeruginosa BCRC 10944[(2)] (ATCC 1014)

4.1.2 General textiles

(1) *Staphylococcus aureus* BCRC 12154[(2)] (ATCC 6538)

(2) *Klebsiella pneumoniae* BCRC 16082[(2)] (ATCC 4352)

Note[(2)] BCRC: Bioresource Collection and Research Center

4.2 Equipment, materials and reagents

4.2.1 Equipment

(1) Autoclave: sterilization at high temperature (121±2)℃ and pressure (103±5) kPa.

(2) Biological Safety Cabinet.

(3) Constant temperature incubator: maintain temperature at (37±2)℃.

(4) Micropipette: accuracy at 0.5%.

(5) Constant temperature refrigerator: maintain temperature at (2~8)℃

(6) Electronic balance: accuracy at 0.01 g.

(7) Circulating water bath: maintain temperature at (46±2)℃.

(8) Vortex mixer.

(9) Spectrophotometer: able to measure at (620~660) nm wavelength, or McFarland nephelometer.

(10) Reciprocal shaking incubator: maintain temperature at (37±2)℃.

(11) Washer: compliant with ISO 6330 requirements.

(12) Dryer: compliant with ISO 6330 requirements.

(13) Blender mixer: at least (6~8) strokes/second, use disposable blender bag.

4.2.2 Materials and reagents

4.2.2.1 Materials

(1) Petri dish: sterile, diameter about (90~100) mm, made of plastic or glass.

(2) Inoculating loop: use platinum loop with loop diameter about 2 mm or disposable loop.

(3) Glass stirring rod: diameter about 18 mm.

(4) Glass bottle: 30 mL, screw capped with Teflon or silica gaskets, cap can be made with polypropylene, polycarbonate or other suitable materials.

(5) Cotton: 100 % cotton, without fluorescent agent or other processing.
Must confirm that no antibacterial effect first.

4.2.2.2 Reagents

All reagents must be reagent grade or microbiological test grade.

(1) Beef extract.

(2) Peptone.

(3) Dehydrated yeast extract.

(4) Tryptone.

(5) Casein peptone.

(6) Soya peptone.

(7) Lecithin.

(8) Glucose.

(9) Agar

(10) Nonionic surfactants: polyoxyethylene sorbitan monooleate (Tween 80)

(11) NaCl.

(12) K2HPO4.

(13) KH2PO4.

(14) Na2HPO4 • 2H2O.

(15) Histidine hydrochloride.

(16) NaOH.

(17) HCl.

(18) Water for analysis: **for example deionized water,** ion exchange water, reverse osmosis filtered water, etc. Nontoxic and not containing antibacterial substances.

(19) Detergent: composed by polyoxyethylene alkyl ether and α-olefin sulfonate (AOS). Reference: JAFET (Japan Textile Evaluation Technology Council, JTECT) standard detergent.

4.2.3 Culture medium and drugs[3]

(1) Tryptone soya broth, TSB

Put tryptone 17.0 g, soy peptone 3.0 g, NaCl 5.0 g, glucose 2.5 and potassium hydrogen phosphate 2.5g in 1000 mL of water for analysis. After mixing thoroughly, adjust pH value until 7.2±0.2, then sterilize in the autoclave.

(2) Tryptone soya agar, TSA

Put tryptone 15.0 g, soy peptone 5.0 g, NaCl 5.0 g and agar 15 g in 1000 mL of water for analysis. After mixing thoroughly, adjust pH value until 7.2±0.2, then sterilize in the autoclave.

(3) Nutrient broth, NB

Put meat extract 3.0 g, peptone 5.0 g, glucose 1.0 g and 15 of agar in 1000 mL of water for analysis. After mixing thoroughly, adjust pH value until 7.2±0.2, then sterilize in the autoclave.

(4) Plate count agar, PCA

Put yeast extract 2.5 g, tryptone 5.0 g, glucose 1.0 g and 15 g of agar in 1000 mL of water for analysis. After mixing thoroughly, adjust pH value until 7.2±0.2, then sterilize in the autoclave.

(5) Peptone salt solution

Put tryptone 1.0 g and NaCl 8.5 g in 1000 mL of water for analysis. After mixing thoroughly, adjust pH value until 7.2±0.2, then sterilize in the autoclave.

(6) SCDLP medium

Put casein peptone 17.0 g, soy peptone 3.0 g, NaCl 5.0 g, potassium hydrogen phosphate 2.5 g, glucose 2.5 g, lecithin 1.0 g and nonionic surfactant 7.0 g in 1000 mL of water for analysis. After mixing thoroughly, adjust pH value until 7.2±0.2, then sterilize in the autoclave.

(7) Shake-out physiological saline

Put NaCl 8.5 g and nonionic surfactant 2.0 g in 1000 mL of water for analysis. After mixing thoroughly, adjust pH value until 7.2±0.2, then sterilize in the autoclave.

(8) Neutralizing solution

Put nonionic surfactant 30.0 g, lecithin 3.0 g, histidine hydrochlorate 1.0 g, peptone 1.0 g, NaCl 4.3 g, potassium hydrogen phosphate 3.5 g, sodium dihydrogen phosphate dehydrate 7.2 g n 1000 mL of water for analysis. After mixing thoroughly, adjust pH value until 7.2±0.2, then sterilize in the autoclave.

Note(3) it is recommended to use commercial dry powdered products for preparing culture media, according to the manufacturers' instructions.

4.3 Washable test

4.3.1 Refer to Table 1 to choose washable type.

4.3.2 Test according to ISO 6330: 2012 regulations, 1 complete washable test include 1 washing process and 1 drying process.

(1) Medical textiles: choose 7N washing process, and use general drying process (highest exhaust gas temperature 80℃).

(2) General textiles: choose washing and drying process according to product's washing instructions.

4.4 Antibacterial test

4.4.1 Sample preparation

(1) Choose (0.40±0.05) g as 1 sample and cut into the appropriate dimensions.

(2) Must prepare control group (unprocessed cloth or cotton fabric) with 6 samples and 6 samples from the sample group.

(3) Place each sample in a 30 mL glass bottle (4), screw the cap (not tightly) and after sterilizing in the autoclave (5), dry, cool and wait for use.

Note(4) Preparation method of other samples:

(a) For samples that crimp easily, fillings, feathers or fibers, use the glass rod to press.
(b) For yarns, order and tie into a bundle, and use the glass rod to press.
(c) For carpet types, cut the fine down and use the glass rod to press.

Note(5) if samples cannot be autoclaved, other suitable sterilization methods may be used, but need to be noted in the report.

4.4.2 Microbe preservation: microbial strains are activated after purchase as per instructions, transplant the activated strains to PCA plate media, cultivate at (37±2)℃ for (18~24) hours. Afterwards store at (5~10)℃ for a maximum of 1 week.

4.4.3 Microbial pre-culture

(1) Use sterilized inoculating loop to acquire a suitable amount of microbes from the PCA plate, add 20 mL of NB or TSB into the serum bottle, shake incubate at (37±2)℃ for (18~24) hours. Microbial concentration should be (1~3)x10^8 CFU/mL.

(2) Acquire 0.4 mL of pre-culture bacteria solution [4.4.3(1)], add in a serum bottle with 20 mL of NB or TSB, shake incubate at (37±2)℃ for (3±1) hours. Microbial concentration should be 10^7 CFU/Ml (this solution can be stored at 0℃, for a maximum of 8 hours).

4.4.4 Test steps

(1) Prepare test bacteria solution

Use room temp. sterile water to dilute the NB or TSB into 1/20 NB or 1/20 TSB, then use the 1/20 NB or 1/20 TSB(6) to adjust the bacteria solution concentration to (1~3)x10^5 CFU/mL (this test bacteria solution can be stored at 0℃, for a maximum of 8 hours).

Note(6) if sample is hydrophobic, add 0.05% Tween 80 into the test bacteria solution, and specify name and concentration in the report.

(2) Inoculate the test bacteria solution

Use the micropipette to aspire 0.2 mL of test bacteria solution, inoculate evenly and carefully on all sterilized samples (do not touch the bottle's internal walls and cap with the solution).

(3) Incubate

Shake incubate the inoculated bottles (3 test control groups and 3 test sample groups) at (37±2)℃ for (18~24) hours.

(4) Scrub the test bacteria

(a) Immediately scrub after the test bacteria inoculation

Add 20 mL scrub solution (SCDLP broth, neutralizing solution or saline scrub solution) into the 3 test control groups and 3 test sample groups, screw the caps (tightly), shake(7) with the vortex mixer (5sec/time, 5 times), so that the inoculated bacteria is evenly distributed in the scrub liquid, use the micropipette to aspire 1 mL of this solution into a tube with (9.0±0.1) mL of diluting solution (NB or peptone saline), mix thoroughly. Use a new micropipette to aspire 1 mL of this solution into another tube with (9.0±0.1) mL of diluting solution (NB or peptone saline), mix

thoroughly. Repeat this step to prepare a serial 10-fold dilution, place 1 mL each in a sterile petri dish, each solution has to be repeated twice (2 petri dishes). Add (15~20) mL of PCA or TSA medium at (45~46)℃ and shake horizontally to mix evenly, stand the containers for cooling and solidification, reverse the containers and incubate at (37±2)℃ for (24~48) hours.

Note(7) Another method for scrubbing the inoculated bacteria is to shake by hand for 30 seconds (with an arc of 30 cm), or put the sample in the blender and process each side for 1 minute.

(b) Scrub after incubation

Process the 3 control groups and 3 test groups from 4.4.3(3) after being incubated for (**18~24**) as per above procedures.

(5) Total plate count

(a) After incubation, take a container with (30~300) colonies for plate counting, if colony number is less than 30 on a plate with 1 mL of scrub solution, then its colony average value is 30. If colony number is less than 1 on a plate with 1 mL of scrub solution, its average value in the calculation formula is 1.

(b) According to formula (1) calculate bacteria concentration in the scrub solution

$C_B = Z \times R$……(1)

Wherein, C_B: bacteria concentration, unit is CFU/mL

Z: average colony number in 2 petri dishes

R: dilution rate

(c) According to formula (2) calculate number of bacteria

$M = C_B \times 20$……(2)

Wherein, M: number of bacteria from each sample

C_B: bacteria concentration

20: quantity of scrub solution (mL)

4.5 Results

4.5.1 Test's condition of establishment

When test conditions are compliant with the following 3, the test is determined to be established. Otherwise the test is determined to be not established, and must be re-done.

(1) Test bacteria solution should be $(1\sim3)\times10^5$ CFU/mL

(2) The maximum difference value of each 3 bacteria logarithms of the control group after immediate scrubbing and incubation is < 1.

(3) According to formula (3) calculate the control group proliferation value, which should be≧1.0.

$$F=\log C_t - \log C_0 \quad \ldots\ldots(3)$$

Wherein, F: control group proliferation value

$\log C_t$: average value of control group bacteria number after incubation

$\log C_0$: average value of control group bacteria number after immediate scrubbing

4.5.2 Formula (4) to calculate antibacterial activity value.

$$A=(\log C_t - \log C_0) - (\log T_t - \log T_0) = F - G \quad \ldots\ldots(4)$$

Wherein, A: antimicrobial activity value

F: control group proliferation value

G: sample group proliferation value

$\log C_t$: average value of control group bacteria number after incubation

$\log C_0$: average value of control group bacteria number after immediate scrubbing

$\log T_t$: average value of sample group bacteria number after incubation[8]

$\log T_0$: average value of sample group bacteria number after immediate scrubbing[8][9]

Note[8] If the maximum difference value of each 3 bacteria logarithms of the sample group after immediate scrubbing and incubation is≧2, **repeat the test to confirm whether the neutralizer has failed**.

Note[9] When $C_0 > T_0$, use C_0 to substitute T_0.

5.Report

(1) Test method

(2) Sample name and type

(3) Test bacteria strain name and number

(4) Test bacteria concentration

(5) Antibacterial activity value

(6) All differences with this requirement needs to the noted in the report.

6.Reference

6.1	ISO 20743:2013	Textiles-Determination of antibacterial activity of textile products
6.2	ISO 6330:2012	Textiles-Domestic washing and drying procedures for textile testing
6.3	CNS 14945	Performance evaluation of general antibacterial textiles
6.4	CNS 14946	Performance evaluation of medical antibacterial textiles

7.Annex:

This standard has been verified by the Specified Requirements Execution Team convenor, and was issued after approval from the chairman of the Assessment Committee, same for all revisions.

The Committee for Conformity Assessment of Accreditation and Certification on Functional and Technical Textiles

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Specified Requirements Execution Team	Convener Hsing Wen-Hao	Chairman Lin Neng-Chung

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