1. Purpose and Scope

This criterion is applicable to the evaluation and testing of antifungal activity of textiles.

2. Terminology

Antifungal treatment: A finishing treatment on aiming at inhibiting the growth of fungi on textile.

Area of fungi growth (%): Percentage of fungi growth area on the surface of tested sample.

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3. Performance Specification

3.1 Classification

To meet the requirement, textile must achieve antifungal activity expressed by area of fungi growth (%) by an approved lab to grade C or above on 4 specified test organisms or on *Trichophyton metagrophytes* (ATCC 9533), after being washed for 50 , 20, 10, or 5 cycles or no washing required for disposable product.

3.2 Washing requirement

Table 1. Washing requirement.

Type	Washing cycles
I	Antifungal activity after 50 washing
II	Antifungal activity after 20 washing
III	Antifungal activity after 10 washing
IV	Antifungal activity after 5 washing
V	Antifungal activity without washing (for disposable products)

3.3 Classification of antifungal activity

Table 2. Calssification of antifungal activity.

Area of fungi growth on specimens (%)	Grade	Classification
No growth, None	A	Excellent
Growth area $\leq$ 10	B	Good
10< growth area $\leq$ 30	C	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.

4. Test Method

4.1 Test organisms: fungus to be used in tests

4.1.1 *Aspergillus niger* (BCRC 31512, ATCC 9642)(1)

4.1.2 *Penicillium pinophilum* (BCRC 30438, ATCC 11797)

4.1.3 *Chaetomium globosum* (BCRC 31605, ATCC 6205)

4.1.4 *Myrothecium verrucaria* (BCRC 31545, ATCC 9095)

4.1.5 *Trichophyton mentagrophytes* (BCRC 32066, ATCC 9533)

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

ATCC: American Type Culture Collection

4.2 Preparation for tests

4.2.1 Chemicals, materials and implements

(1) Ethanol (C_2H_5OH): Reagent grade.

(2) Agar: For microbial test, Sabouraud Dextrose Agar (SDA) or Potato Dextrose Agar (PDA).

(3) Wetting agent: Sodium Dioctyl Sulfosuccinate (SDS) or others.

(4) Purified water: Distilled water or deioned water.

(5) Ammonium nitrate (NH_4NO_3): Reagent grade.

(6) Potassium dihydrogen phosphate(KH_2PO_4): Reagent grade.

(7) Magnesium sulfate ($MgSO_4 \cdot 7H_2O$): Reagent grade.

(8) Potassium chloride (KCl): Reagent grade.

(9) Ferrous sulfate ($FeSO_4 \cdot 7H_2O$): Reagent grade.

(10) 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.

(11) Autoclave: Capable of keeping at 121℃, 103 kPa (1.05 kg/cm^2), for over 15 minutes.

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

(13) Incubator: Capable of keeping at 28±2 ℃, humidity ≥ 85 %RH.

(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) Detergent: Polyoxyethylene Alkyl Ether.

(18) Oven: Adjustable temperature setting, capable of keeping temperature from room temperature

to 100℃ or above, ±2 ℃ in accuracy.

(19) Water bath shaker: Adjustable temperature setting, ±2℃ in accuracy.

(20) Washing machine: Agitator speed 179 ± 2 spm, Spin speed 645 ± 15 rpm, Total liquor ≥ 64 L .

4.2.2 Preparation of specimen

The specimen can be 5 × 5 cm or 3.8±0.5 cm in diameter. Randomly select 3 to 5 specimens and for test.

4.2.3 Sterilization

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

4.2.4 Culture medium

Appropriate amount of SDA or PDA dissolved in 1L water. Heat in water bath to disperse ingredient. Sterilize by autoclave. Cool down to 50-60 ℃ before pouring to Petri dishes. Pour about 20 mL agar to Petri dish and solidified. If it is not used immediately after preparation, pour to Petri dish to make the plate and preserve it at 4-10℃. Never use the agar kept for one month or longer after preparation.

4.2.5 Mineral Salt Agar (MSA)

$MgSO_4 \cdot 7H_2O$	0.5 g
KH_2PO_4	1 g
NH_4NO_3	3 g
KCl	0.25 g
$FeSO_4 \cdot 7H_2O$	0.002 g
Agar	25 g
distilled water	to 1000 mL

Heat in water bath to disperse ingredients. Adjust to pH 6.4±0.4. Sterilize by autoclave. Cool down to 50-60℃ before pouring to Petri dishes. Pour about 20 ml agar to Petri dish and let it solidified. When it is not used immediately after preparation, pour to Petri dish to make the plate and preserve it at 4-10℃. Never use the nutrient agar kept for one month or longer after preparation.

4.2.6 Fungus transference from the freeze-dried stock

The transference of fungus stock strain shall be carried out according to the instruction. Transfer the stock strain to SDA or PDA slant. Incubate the fungus transferred slant culture medium at 28±2 ℃ , Humidity >85 %RH for 14 days.

4.2.7 Incubation and preparation of test inoculum

Flame the platinum inoculum loop or use disposable inoculum loop, scrap out a platinum loop of spores from a breeding surface of fungus on slant SDA or PDA slant agar. Streak on the center of agar surface. Incubate the fungus transferred slant culture medium at 28±2 ℃, Humidity >85 %RH. Indicate the date of inoculation, fungus strain and other items. Observe the fungus growth.

4.2.8 Preparation of spore suspension

(1) Water containing wetting agent

Take 50 mg SDS and dissolved into 1 L water (0.005%). Take 10 mL into 50 mL Erlenmeyer flask and autoclaved.

(2) Prepare spores suspension

Operate the following procedure in laminar flow. Add scrapings of fungus spores to the water containing wetting agent (0.005% SDS). Adjust the spores concentration to $10^6 \pm 2 \times 10^5$ CFU/mL, shake thoroughly to bring the spores into suspension. Use this suspension in 24 h.

4.3 Test Procedure

4.3.1 Washing operation

(1) Washing condition

Washing according to the below condition. Each specimen is washed with a standard cloth to make up the washing load to 4 lb (include the test specimen) to the washing machine. Different treatment of test specimen should be washed individually. It is not allowed to place test specimen with different treatments into the same load to avoid cross contamination.

Table 3. Washing condition.

Specimen size L x W (cm)	Temperature (℃)	Total liquor volume (L)	Detergent (g)	Agitator speed (spm)	Time (min)	Spin Speed (rpm)	Spin time (min)
15 x 15 3-5 specimens	49±3	68±4	66±1	179±2	12	645±15	6

(2) Remove each specimen from the washing machine. Dry the specimen in an air circulating oven in which the temperature does not exceed 71℃.

(3) Repeat the selected washing and drying cycle to an agreed number of cycles.

4.3.2 Antifungal activity test

The test specimen is sterilized by autoclave. Place the sterilized specimen in contact with the hardened MSA surface. Distribute 1±0.1 mL of the spore suspension evenly over the specimen by micropipette. Set up a control in a similar way distributing 1±0.1 mL of the spore suspension

on MSA without the test specimen. Incubate all plates in a condition of temperature of 28+1 ℃, humidity >85 %RH for 14 days.

4.4 Evaluation

Report the percentage of surface area of the specimen covered with the growth of tested fungus using a microscope (50 times) in accordance with the below scheme.

5. Mark

Table 4. Classification of antifungal textiles.

Type	Grade	Classification	Washing cycle	Growth of fungus (%)
I	AAAAA	Excellent	50 washing	No growth
	AAA	Good	50 washing	Percentage of growth area ≤10
	A	Fair	50 washing	10 < percentage of growth area ≤30
II	AAAAA	Excellent	20 washing	No growth
	AAA	Good	20 washing	Percentage of growth area ≤10
	A	Fair	20 washing	10 < percentage of growth area ≤30
III	AAAAA	Excellent	10 washing	No growth
	AAA	Good	10 washing	Percentage of growth area ≤10
	A	Fair	10 washing	10 < percentage of growth area ≤30
IV	AAAAA	Excellent	5 washing	No growth
	AAA	Good	5 washing	Percentage of growth area ≤10
	A	Fair	5 washing	10 < percentage of growth area ≤30
V	AAAAA	Excellent	No washing required (disposable)	No growth
	AAA	Good	No washing required (disposable)	Percentage of growth area ≤10
	A	Fair	No washing required (disposable)	10 < percentage of growth area ≤30

6. Reference

AATCC 30-1999 Antifungal Activity, Assessment on Textile Materials: Mildew and Rot Resistance of Textile Materials

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

ASTM G21-1990 Determining Resistance of Synthetic Polymeric Materials to Fungi

JIS Z 2911-2000 Method of Test Fungus Resistance

CNS 2690 L3063-1975 Method of Test for Mold-Proof of Textile Products