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Textiles — Determination of antifungal activity of textile products —

Part 1:
Luminescence method

Textiles — Détermination de l'activité antifongique des produits textiles —

Partie 1: Méthode par luminescence

Reference number
ISO 13629-1:2012(E)

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 13629-1 was prepared by Technical Committee ISO/TC 38, *Textiles*.

ISO 13629 consists of the following parts, under the general title *Textiles — Determination of antifungal activity of textile products*:

— *Part 1: Luminescence method*

— *Part 2: Plate count method* (under preparation)

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Introduction

Speciality products of antimicrobial-treated textiles have been increasing year by year in various applications and they certainly contribute to the prevention of a material's deterioration and to the improvement of the environment and quality of life.

For these reasons, ISO/TC 38/WG 23 developed ISO 20743 in 2007, and is continuing to study a test method on the antifungal activity of textile products for a series of International Standards.

This part of ISO 13629 adopts an ATP luminescence method as a basis for the quantitative determination of antifungal activity.

Characteristics of the luminescence method are as follows:

— extremely small margin of error compared to the colony count method;

— elimination of the culturing time for colony formation, enabling a shorter testing time;

— simplification of testing operation.

The other parts will be developed relating to:

— Part 2: Plate count method.

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Textiles — Determination of antifungal activity of textile products —

Part 1: Luminescence method

1 Scope

This part of ISO 13629 specifies a test method for the quantitative determination of the antifungal activity by measuring the intensity of luminescence produced by an enzymatic reaction [adenosine triphosphate (ATP) method].

The part of ISO 13629 is applicable to various kinds of textile products, such as fibres, yarns, fabrics, clothing, bedclothes, home furnishings and other miscellaneous goods.

Based on the intended application and on the environment in which the textile product is to be used, the user can select the most suitable evaluation method from the following methods before enumeration by the ATP method:

a) absorption method (an evaluation method in which test fungi suspension is inoculated directly onto the specimens);

b) transfer method (an evaluation method in which test fungi are placed on an agar plate and printed onto the specimens).

2 Normative reference

The following referenced documents are indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 105-F02, *Textiles — Tests for colour fastness —Part F02: Specification for cotton and viscose adjacent fabrics*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
control specimen
specimen used to validate the growth condition of test fungus

NOTE The control specimen may be taken from the same textile products as the textile products to be tested, but without antifungal treatment. If this is not available, a 100 % cotton specimen without fluorescent brighteners or other finish, complying with the requirements of ISO 105-F02, is used as the control specimen, after 10 cycles of washing for 10 min at a temperature of 60 °C without detergents or any brighteners and rinsing twice for 5 min in accordance with ISO 6330.

3.2
antifungal agent
agent to prevent or mitigate the growth of fungus or to reduce the number of fungus

3.3
antifungal treatment
treatment to prevent or mitigate the growth of fungus or to reduce the number of fungus

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3.4
spore suspension
liquid with evenly dispersed fungal spores in sterilized water containing an anionic surfactant (8.3)

3.5
ATP
adenosine triphosphate, a multifunctional nucleotide present in living fungi

3.6
antifungal activity
activity to prevent or mitigate the growth of fungus, expressed as the difference of growth value in logarithm of ATP between the control and test specimen

3.7
luminescence method
method in which the amount of ATP contained in fungal cells is measured in moles of ATP

4 Principle

A test specimen and control specimen are inoculated with a spore suspension of a reference fungus and incubated at 25 °C for 42 h.

In this part of ISO 13629, fungal growth or antifungal activity is determined quantitatively, by comparison with the result of a control specimen, by measuring the luminescence intensity of intracellular ATP.

5 Safety precaution

The test methods specified herein require the use of fungus.

This test shall be performed only by personnel with training and experience in microbiological techniques.

All regulations, rules and recommendations regarding appropriate safety precautions in the country concerned shall be consulted and followed.

6 Reference fungi

The fungi to be used shall be selected from Table A.1.

Equivalent fungi types obtained from other agencies of the World Federation for Culture Collection (WFCC) may be used as agreed upon between the interested parties.

The preservation number and supply source of the fungus used shall be stated in the test report.

7 Apparatus

Usual laboratory apparatus and, in particular, the following.

7.1 **Gauze**, for biochemical testing or glass wool (FR specification).

7.2 **Petri dishes**, with internal diameters of approximately 90 mm and 55 mm to 60 mm.

7.3 **Dry sterilizer**, capable of maintaining the temperature at 160 °C to 180 °C.

7.4 **Autoclave**, capable of maintaining the temperature at (121 ± 2) °C (equivalent to 103 kPa).

7.5 **Safety cabinet**, for biochemical testing, or one that offers equivalent performance.

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7.6 Platinum colony loop, with a loop of 2 mm to 4 mm in diameter.

7.7 L-shaped platinum colony hook.

7.8 Incubator, capable of maintaining the target temperature range of 20 °C to 37 °C with a margin of ± 2 °C.

7.9 Vial, 30 ml screw-top glass vial with polytetrafluoroethylene or silicone gasket and polypropylene cap.

7.10 Glass rod, between 5 mm and 18 mm in diameter and 1 g to 50 g in mass.

7.11 Glass funnel.

7.12 Pipettes, of capacity 0,05 ml, 0,1 ml, 0,2 ml,1 ml, 5 ml and 10 ml with a tolerance of 5,0 %.

7.13 Pasteur pipette, for microbiological testing.

7.14 Conical flask, of capacity 100 ml to 500 ml.

7.15 Tweezers.

7.16 Plastic test tube, especially for the luminometer.

7.17 Test-tube agitator.

7.18 Centrifugal separator, with a centrifugal acceleration of approximately 2 000g.

7.19 Centrifuge tube, used in a centrifugal separator.

7.20 Hemocytometer, capable of measuring 1×10^6/ml to 3×10^6/ml.

7.21 Microscope, magnification 200×.

7.22 Ultrasonic cleaner, compact, for experimental tools, with a frequency of approximately 30 kHz to 50 kHz.

7.23 Luminometer, measuring at a wavelength of 300 nm to 650 nm, and capable of ATP measurement at 1×10^{-8} mol/l to 1×10^{-5} mol/l, under the assay conditions defined in 8.4 and Clause 11.

7.24 pH-meter, with an accuracy of ± 0,1 at 25 °C.

7.25 Refrigerator, capable of maintaining a temperature of between 2 °C and 10 °C.

7.26 Freezers, one adjustable to a temperature below – 80 °C and another to a temperature below – 20 °C.

Test tubes, vials, flasks, pipettes and tweezers shall be carefully washed in alkaline or neutral detergent, rinsed, dried, and processed by dry sterilization or high-pressure steam sterilization before use.

8 Reagents and culture media

8.1 General

Reagents used in tests shall be of analytical quality and/or suitable for microbiological purposes.

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Dehydrated products available on the commercial market are recommended for use in preparing the culture media, strictly in accordance with the manufacture's instructions.

8.2 Pure water

Analytical-grade water for microbiological media and reagent preparation, which is freshly distilled and/or ion-exchanged and/or ultra-filtered and/or filtered with reverse osmosis (RO). It shall be free from all toxic or fungus-inhibitory substances.

8.3 Anionic surfactant

Dioctyl sodium sulfosuccinate to prepare the spore suspension.

8.4 Luminescent reagents, reagents and buffer solutions

8.4.1 General

Use reagents and buffer solutions prepared as shown in 8.4.2 to 8.4.8. Commercially prepared items may be used after appropriate validation.

8.4.2 ATP standard stock solution (1×10^{-3} mol/l) referred to as ATP standard hereafter

Adenosine-disodium 5'-triphosphate trihydrate ($C_{10}H_{14}O_{13}P_3Na_2 \cdot 3H_2O$)	60,5 mg
Pure water (8.2)	100 ml (final volume)

Place the prepared solution in a tightly sealed container and freeze at a temperature of – 20 °C or lower for storage of up to 6 months.

NOTE It is not recommended to refreeze and/or reuse a melted solution.

8.4.3 Buffer solution for ATP luminescent reagent

N-[Tris(hydroxymethyl)methyl] glycine	1 117 mg
Disodium salt of ethylenediaminetetraacetic acid dihydrate	183 mg
Magnesium acetate tetrahydrate	808 mg
DL-dithiothreitol	6,7 mg
Dextrin	25 000 mg
Sucrose	925 mg
Pure water	250 ml (final volume)
pH	7,5 ± 0,2

8.4.4 ATP luminescent reagent

ATP luminescent reagents shall enable a luminometer (7.23) to measure the ATP of 1×10^{-8} mol/l to 1×10^{-5} mol/l, under the assay conditions defined in 8.4 and Clause 11.

Luciferase	0,7 mg
D-luciferin	12,6 mg

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Bovine serum albumin	56 mg
Buffer solution (see 8.4.3)	30 ml

Once fully dissolved, stand at room temperature for 15 min before use. Use within 3 h of preparation.

8.4.5 ATP extraction reagent

ATP extraction reagents must be able to extract intracellular ATP from the incubated fungus with an efficiency of 80 % or higher.

N-[Tris(hydroxymethyl)methyl] glycine	45 mg
Benzalkonium chloride, 10 % aqueous solution	0,2 ml
Pure water	9,8 ml
pH (use sodium hydroxide to adjust pH)	12,0 ± 0,5

The use of any unspecified extracting agent in the composition shall be recorded.

8.4.6 ATP eliminating reagent

ATP eliminating reagents shall reduce the culture medium ATP content to less than 10^{-11} mol/l within 15 min when one-tenth of the reagent in quantity is added to the culture medium (defined in 8.5.1).

Use within 8 h of preparation.

The composition is as follows:

Apyrase (EC: 3.6.1.5)	4,6 international units/ml
Adenosine phosphate deaminase (EC: 3.5.4.6 or 3.5.4.17)	46 international units/ml
Sucrose	37 mg
Bovine serum albumin	20 mg
0,05 mol/l buffer solution of 2-morpholinoethanesulfonic acid, monohydrate	10 ml
pH	6,0 ± 0,5

When a different eliminating reagent is used, its composition shall be recorded.

8.4.7 Physiological saline solution

Place 8,5 g of sodium chloride in 1 000 ml of pure water in a flask. Thoroughly dissolve and pour it into test tubes as needed for steam pressure sterilization.

8.4.8 Sterilized water containing anionic surfactant (8.3)

Dissolve 50 mg of anionic surfactant in pure water and make up to 1 000 ml, then pour it into test tubes as needed for high-pressure steam sterilization.

8.5 Culture medium

Use a culture medium prepared as described in 8.5.1 to 8.5.4. Commercially prepared items may be used after appropriate validation.

For culture media that will not be used immediately after preparation, it is recommended that they be stored at 5 °C to 10 °C and discarded after one month.

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8.5.1 Sabouraud dextrose broth (SDB)

Peptone	10 g
Dextrose	20 g to 40 g
Pure water	1 000 ml
pH after sterilization	5,6 ± 0,2

8.5.2 Potato dextrose agar (PDA)

Wash and peel a large potato with minimal blemishes, removing approximately 10 mm around each bud, and dice it into 10 mm cubes. Boil 200 g of these cubes in 1 000 ml of pure water for 1 h. Use several layers of gauze (7.1) to drain, then add distilled water so that the volume of the mixture equals 1 000 ml. Add 20 g of glucose and 15 g to 20 g of agar, fully dissolving the solids before placing in the autoclave (7.4) for sterilization.

8.5.3 Slant culture

Pour approximately 10 ml of preheated and fully dissolved PDA (described in 8.5.2) into a test tube. Stopper with a cotton plug and sterilize with steam. After sterilization, place the test tube at an approximately 15° angle against a level surface on a clean laboratory table, and leave the contents to solidify. When there is no bleed water on the solidified agar, dissolve and solidify it again for use.

8.5.4 Sabouraud dextrose agar (SDA)

Pepsic meat pepton	10 g
Dextrose	35 g
Agar	15 g
Pure water	1 000 ml (final volume)

Follow the indications of the supplier.

pH after sterilization	5,6 ± 0,2

NOTE This medium will be used for the transfer method.

9 Fungus preservation and use

9.1 Fungus preservation

Reference fungi and spores are subcultured and handled inside a safety cabinet (7.5) or other equivalent system.

- Conduct either flame sterilization or the chemical sterilization on the cotton plugs and necks of the test tubes before and after subculture.
- Scrape off a small amount of fungus from the original fungus, spread the spores over the bleed water at the bottom of the slant culture and smear onto the top end of the slant culture, in either a straight or wavy line (see Figure 1).
- Use the flame-sterilized platinum colony loop (7.6) and hook (7.7) each time when different types of fungus are subcultured.
- Place the subcultured slant cultures in an incubator (7.8) at 25 °C ± 2 °C for at least 8 days, and confirm that sufficient spores have been generated before preserving them at 5 °C to 10 °C.
- Within 3 months, transplant the subcultured fungus to new slant cultures for further incubation and preservation.

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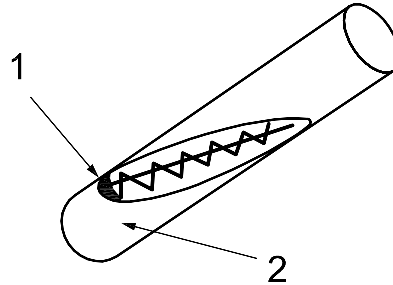
— Repeat the subculture at intervals of up to 3 months. The passage culture, however, shall not be subcultured more than five times. Do not use fungus over 3 months old for further subculture.

NOTE Long-term preservation can be possible by freeze-drying at −80 °C.



Key

1 bleed water

2 slant culture

Figure 1 — Subculture to a slant culture

9.2 Use of fungus

To prepare the fungus for spore suspension, as described in Clause 10, transplant the fungus preserved in 9.1 onto a plate culture, incubating at 25 °C ± 2 °C for at least 8 days. When the fungus is not used immediately after incubation, preserve it at 5 °C to 10 °C and use within 7 days.

10 Spore suspension

See Figure 2.

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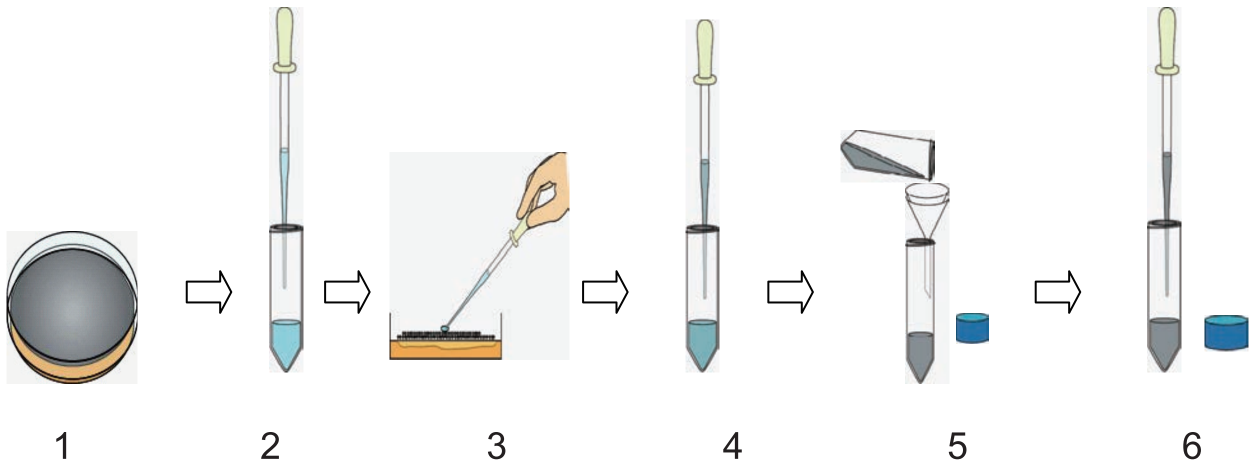

Key

1 pre-culture on PDA plate
2 Step 1
3 Step 2
4 Step 3
5 Step 4
6 Step 5

Figure 2 — Steps of procedure for adjusting spore suspension

10.1 Suspending spores in culture media

— Use a short Pasteur pipette (7.13) or similar apparatus to take 0,5 ml of sterilized water containing anionic surfactant (8.3) (Step 1).

— Slowly release it about 5 times on the spores in the centre of an agar plate to wash off the surface gently (Step 2).

NOTE A minor modification, such as increasing the amount of washing water, can be acceptable. Keep a record of all the conditions in such cases.

10.2 Collection and dispersion of spore suspension from a culture medium

— Take the spore suspension in 10.1 using a short pasteur pipette (7.13) or similar apparatus.

— Transfer it to approximately 5 ml of sterilized water containing anionic surfactant (8.3).

— Pipette about 100 times or agitate with a test tube agitator or apply light ultrasonic cleaning for about 5 min so that spores can be sufficiently dispersed.

— Visually check that the suspension looks slightly cloudy (Step 3).

10.3 Filtering to remove hyphae and spore threads

Use a funnel or other apparatus with gauze (7.1) or glass wool for filtration (Step 4).

NOTE The gauze (7.1) or glass wool can consist of a ply of 4 sheets ± 1 sheet of 5 cm × 5 cm square.

10.4 Using centrifugal separation and resuspension to remove supernatant

— After filtration, carry out centrifugal separation at approximately 2 000g for at least 5 min at 25 °C ± 2 °C, or at room temperature if the centrifugal separator (7.18) has no temperature-control device.

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— Remove the supernatant.

— Add sterilized water containing anionic surfactant (8.3).

— Thoroughly pipette to disperse spores or agitate with a test tube agitator or apply light ultrasonic cleaning for about 5 min so that spores can be sufficiently dispersed (Step 5).

10.5 Confirming the concentration of spore suspension

Check the following items with a haemocytometer.

a) Spore count and spore status: confirm that spore count is 1×10^6/ml to 3×10^6/ml and that over 90 % of the spores are single spores free from hyphae.

b) In the case of too many spores: dilute the suspension with sterilized water containing anionic surfactant (8.3) to reduce the spore count to 1×10^6/ml to 3×10^6/ml, and check the spore count again.

c) In the case of not enough spores: repeat centrifugal separation to remove supernatant, and use sterilized water containing anionic surfactant (8.3) to adjust spore count to 1×10^6/ml to 3×10^6/ml, and check the spore count again.

d) For the transfer method described in 12.1.3.3, the spore count shall be adjusted to 1×10^8/ml to 3×10^8/ml and this spore suspension shall be used for testing.

10.6 Adjusting spore suspension for testing

— Use 1/20 SDB to adjust the spore suspension into 1×10^5/ml to 3×10^5/ml for testing.

NOTE To make 1/20 SDB, as described in 8.5.1, dilute with sterilized water containing anionic surfactant (8.3).

— Agitate the suspension well on dilution.

— Cool the suspension in ice and use within 4 h.

11 Preparing the ATP calibration curve

The procedure to prepare the ATP calibration curve is as follows. The curve shall be obtained on a daily basis.

a) Dilute the ATP standard stock solution prepared in 8.4.2 with pure water to prepare three dilutions having an accurate concentration of 1×10^{-8} mol/l, 1×10^{-7} mol/l, and 1×10^{-6} mol/l, respectively.

b) Prepare the first ATP dilution sample as follows: transfer 0,1 ml from each dilution into separate plastic test tubes, then add 0,05 ml of pure water and 0,35 ml of physiological saline solution to each plastic tube and agitate well.

c) Prepare the second ATP dilution sample as follows: transfer 0,1 ml again from the first ATP dilution sample into a test tube. Add 0,4 ml of physiological saline solution to the test tube and agitate well.

d) Prepare for ATP dilution sample measurement: transfer 0,1 ml of the second ATP sample into two plastic test tubes for ATP dilution sample measurement.

e) Prepare the first blank samples as follows: add 0,1 ml of sterilized water containing anionic surfactant (8.3), 0,35 ml of physiological saline solution and 0,05 ml of ATP eliminating reagent to a plastic test tube. Agitate well and leave it to rest for 10 min to 20 min.

f) Prepare the second blank sample as follows: transfer 0,1 ml of the first blank sample again into a test tube and add 0,4 ml of physiological saline solution to the test tube and agitate well.

g) Prepare for blank measurement: transfer 0,1 ml of the second blank sample into two plastic tubes and use for the blank measurement.

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h) Add 0,1 ml of ATP extraction reagent to each of the second blank samples in two plastic tubes prepared in g) and agitate well.

i) Add 0,1 ml of ATP luminescent reagent and agitate for 5 s with a test tube agitator.

j) Luminescence intensity of second blank samples: immediately measure the luminescence intensity of both blank samples with a luminometer (7.23).

k) Add 0,1 ml of ATP extraction reagent to the second ATP dilution samples prepared in d), starting from the sample with the lowest concentration to that with the highest concentration in sequential order, and agitate well.

l) Add 0,1 ml of ATP luminescent reagent, and agitate for 5 s with a test tube agitator.

m) Luminescence intensity of second ATP dilution samples: immediately measure the luminescence intensity of both second ATP dilution samples with a luminometer (7.23).

n) Using Equation (1), calculate coefficient A and coefficient B in order to set up a calibration curve:

- Coefficient A can be the average of three values obtained by dividing the corresponding ATP concentration (mol/l) by the average values of luminescence intensity of each of the second ATP dilution samples from the measurement described in m).
- Coefficient B is calculated by applying the coefficient A value and substituting the average value of the second blank sample for X and using the number zero for Y in the following calibration curve equation :

$$Y = AX + B \quad (1)$$

where

Y is the ATP concentration (mol/l);

X is the luminescence intensity (RLU = Relative Light Unit).

If the correlation coefficient between average luminescence intensities and ATP concentration is less than 0,99, the preparation of ATP calibration shall be done from the beginning.

12 Testing method

12.1 Preparation of test specimens and inoculation

12.1.1 General

For the inoculation method, the absorption method and transfer method can be used. The transfer method is applicable to textile product samples which do not absorb water.

12.1.2 Absorption method

12.1.2.1 Mass and shape of test pieces

Obtain test pieces with a mass of 0,20 g ± 0,03 g and cut to a size suitable for the test. Obtain six control specimens and six test specimens.

NOTE Three of the control specimens and three of the test specimens are used for the time zero, immediately after inoculation. The remaining specimens are used for the contact time, after incubation.

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12.1.2.2 Setting the test specimens

Place each of the test specimens in separate vials (7.9) by selecting one of the following methods appropriate to the nature of the specimen

a) If the test specimen is a textile product that tends to curl easily, or if it contains wadding or down, place a glass rod onto the specimens in the vial (7.9). Alternatively, secure both ends of the test specimens with thread.

b) If the test specimen is yarn, arrange the yarn in a bundle and place a glass rod onto the specimen in the vial (7.9).

c) If the test specimens are carpets or of similar construction, cut off the pile and place a glass rod onto the pile specimen in the vial (7.9).

d) When necessary, test specimens may be washed in accordance with ISO 6330 or another suitable method, and after the final washing, the specimens are rinsed with water to eliminate the washing detergent. Use of an unspecified method shall be recorded.

e) When it is necessary, such as for dirty specimens, sterilize them by autoclave (7.4) according to the following procedure.

   1) Cover the upper portion of the vials (7.9) containing the test specimens with aluminium foil.

   2) Place the covered vials (7.9) in a metal wire basket for autoclaving.

   3) Wrap the vial caps with aluminium foil and place them in the wire basket.

   4) Sterilize the caps and the vials (7.9) containing the test specimens by autoclave (7.4) at 121 °C (103 kPa) for 15 min to 20 min.

   5) After sterilization, remove the aluminium foil and allow the test specimens in the vials (7.9) to dry for 60 min or more by placing them on a safety cabinet (7.5) or any other place where there is no risk of airborne contamination.

   6) Tighten the vial caps securely.

NOTE 1 If an autoclave (7.4) or alternative sterilization method, e.g. ethylene oxide gas or gamma-ray, is used, record this in the test report.

NOTE 2 A certain sterilization method can deactivate or increase the release of certain antimicrobial agents and hence give false results.

NOTE 3 The control specimen can be sterilized by the method described above for the test specimens.

12.1.2.3 Inoculation of test specimens

Accurately pipette 0,2 ml of the spore suspension, 1×10^5/ml to 3×10^5/ml, prepared in 10.6, on each specimen prepared in 12.1.2.1.

NOTE 1 Agitate the suspension well before pipetting it onto the specimen.

Make sure that the spore suspension is dropped at several locations to evenly distribute the suspension, and tamp with a glass rod to ensure that the suspension is fully absorbed. Follow the procedure described in Clause 13 to measure the luminescence intensity of the specimens immediately after inoculation of the suspension.

NOTE 2 For specimens that do not easily absorb the spore suspension, the transfer method can be used.

12.1.3 Transfer method

12.1.3.1 Preparation of test specimens

Using a template, cut six control specimens and six test specimens of approximately 3,8 cm in diameter. The test specimens shall not contain any seams, selvedges, embroidery, fasteners, etc. There should be a

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sufficient amount of sample to allow for repeat tests (a minimum of 0,5 m^2). Test specimens should be from the same batch and be free from selvedges or stemming.

Weigh each control specimen or test specimen, and record their mass (mass m_A).

When necessary, test specimens may be washed in accordance with ISO 6330 or another suitable method, and after the final washing, the specimens are rinsed with water to eliminate the washing detergent. Use of an unspecified method shall be recorded.

12.1.3.2 Inoculation to agar plates

Prepare 12 agar plates of the SDA for transfer to the 55 mm to 60 mm diameter Petri dish (7.2). Inoculate 1 ml of the initial spore suspension on the agar, inclining the plate in several directions so as to completely flood the surface of the plate. Suck up as much of the excess liquid as possible. Let stand for 300 s ± 30 s.

12.1.3.3 Transfer to specimens

Prepare three test specimens of the control textile product and three test specimens for use immediately after transfer and after incubation respectively. Set each specimen on the agar surface and weigh down with a 200 g stainless steel cylinder for 60 s ± 5 s. Place each specimen in a 55 mm to 60 mm diameter Petri dish (7.2) with the transferred surface face up.

Weigh the Petri dish (7.2) and record the mass (mass m_B). Weigh the Petri dish and specimen after incubation and record the total mass (mass m_C).

The liquid mass, m_D, in the specimen is calculated as follows,

$$m_D = m_C - (m_A + m_B)$$

12.2 Incubation

12.2.1 Absorption method

Inoculate the spore suspension onto the specimen according to the procedure described in 12.1.2.3, incubating at 25 °C ± 2 °C for 42 h ± 2 h.

12.2.2 Transfer method

After transfer as described in 12.1.3.3, incubate in a humidity chamber at 25 °C ± 2 °C for 42 h ± 2 h.

13 Measurement of luminescence intensity

13.1 Absorption method

The preparation procedure for luminescence intensity measurement is as follows (see Figure 3).

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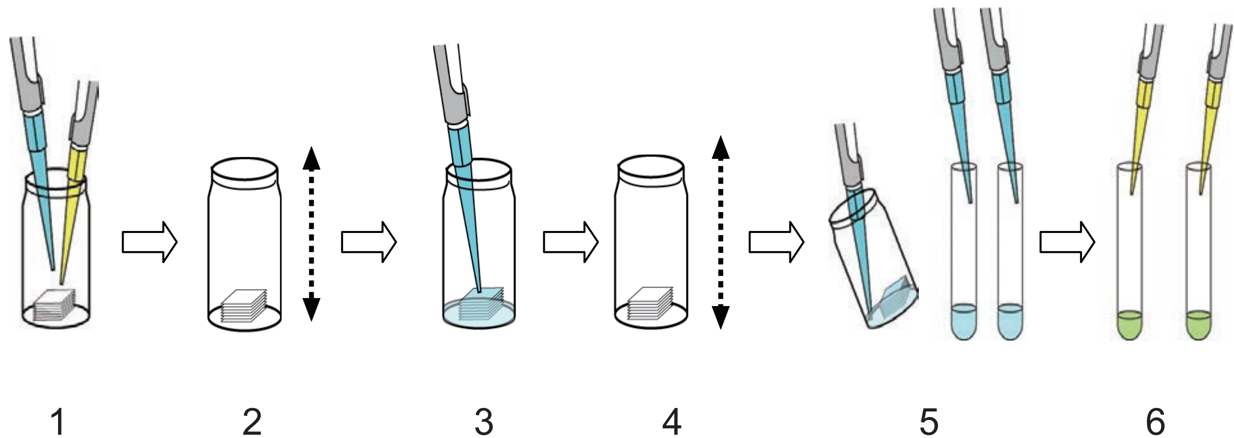

Key

1 Step 6
2 Step 7
3 Step 8
4 Step 9
5 Step 10
6 Step 11

Figure 3 — Steps of preparation procedure for luminescence intensity measurement

a) Add 0,1 ml of ATP-eliminating reagent to the specimen prepared in 12.2.1, and add 4,7 ml of physiological saline solution (Step 6).

b) Tighten the caps and agitate well. For agitation by hand, shake 30 times or, if using the test tube agitator, activate for 5 s and 5 times. Stand at room temperature for 20 min (Step 7).

NOTE If the specimen floats on the suspension liquid, use a glass rod to immerse the specimen completely.

c) Add 5,0 ml of ATP-extracting reagent (Step 8).

d) Tighten the caps and agitate well. For agitation by hand, shake 30 times or, if using the test tube agitater, activate for 5 s and 5 times. Stand at room temperature for 10 min (Step 9).

NOTE If the specimen floats on the suspension liquid, use a glass rod to immerse the specimen completely.

e) Agitate well, and transfer 0,2 ml of the solution prepared in d) into two plastic test tubes (Step 10).

f) Add 0,1 ml of ATP luminescent reagent to the samples prepared in e), use a test tube agitator to mix them for 5 s, and measure the luminescence intensity immediately using a luminometer (7.23) (Step 11).

g) Measure the luminescence intensity of both specimens.

13.2 Transfer method

a) Add 0,1 ml of ATP-eliminating reagent to the specimen prepared in 12.2.2, and add $(4,9 - m_D)$ ml of physiological saline solution (see 12.1.3.3).

b) Agitate well by pipetting about 30 times. Slant the Petri dish for easier pipetting. Stand at room temperature for 20 min.

NOTE If the specimen floats on the suspension liquid, use a glass rod to immerse the specimen completely.

c) Add 5,0 ml of ATP-extracting reagent.

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d) Agitate well by pipetting about 30 times. Slant the Petri dish for easier pipetting. Stand at room temperature for 10 min.

NOTE If the specimen floats on the suspension liquid, use a glass rod to make the specimen immerse completely.

e) Agitate well by pipetting, and transfer 0,2 ml of the solution prepared in d) into two plastic test tubes.

f) Add 0,1 ml of ATP luminescent reagent to the samples prepared in e), use a test tube agitator to mix them for 5 s, and measure the luminescence intensity immediately using a luminometer (7.23).

g) Measure the luminescence intensity of both specimens.

14 Calculation

14.1 Judgment of test effectiveness

When the growth value in b) is satisfied, the test is judged to be effective. When b) is not satisfied, the test is judged to be ineffective and a retest shall be carried out.

a) Calculate the growth value F of control specimens using Equation (2).

b) The growth value F obtained according to Equation (2) shall be $\geq$ 1,5.

Round the results to 2 significant digits.

When the growth value is less than 1,5 using a supplied control specimen, it is recommended to repeat the test using 100 % cotton-adjacent fabric as specified in ISO 105-F02.

$$F = \lg C_t - \lg C_o \quad (2)$$

where

F is the growth value on the control specimen;

$\lg C_o$ is the average common logarithm ATP amount from 3 control specimens immediately after inoculation;

$\lg C_t$ is the average common logarithm ATP amount from 3 control specimens after 42 h ± 2 h incubation.

14.2 Calculation of antifungal activity value

When the tests are confirmed valid, use Equation (3) to obtain the antifungal activity.

Round the results to 2 significant digits.

$$A_a = (\lg C_t - \lg C_o) - (\lg T_t - \lg T_o) \quad (3)$$

where

A_a is the antifungal activity value;

$\lg C_o$ is the average common logarithm ATP amount from three control specimens immediately after inoculation;

$\lg C_t$ is the average common logarithm ATP amount from three control specimens after 42 h ± 2 h incubation;

$\lg T_o$ is the average common logarithm ATP amount from three test specimens immediately after inoculation;

$\lg T_t$ is the average common logarithm ATP amount the three test specimens after 42 h ± 2 h incubation.

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15 Test report

The test report shall contain the following information:

a) a reference to this part of ISO 13629;

b) details of test textile products;

c) type of control specimen used;

d) type of reference fungus used;

e) details of the fungus strain: fungus preservation number and culture collection organization;

f) spore concentration;

g) inoculation method;

h) growth value F in Equation (2);

i) antifungal activity value A_a of each specimen;

j) evaluation and testing date;

k) name of laboratory, and name(s) and signature(s) of the test operator;

l) any deviation, by agreement or otherwise, from this part of ISO 13629.

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Annex A
(normative)

The fungi used in this part of ISO 13629

A.1 General

The fungi to be used in the test shall be identical to those listed in Table A.1, which are preserved by the members of the World Federation for Culture Collection (WFCC).

A.2 List of fungi

Table A.1 — Fungi for testing

Fungal type	WDCM code
Aspergillus niger	00144 http://refs.wdcm.org/getinfo.htm?sid=WDCM_00144
Penicillium citrinum	00189 http://refs.wdcm.org/getinfo.htm?sid=WDCM_00189
Cladosporium cladosporioides	00190 http://refs.wdcm.org/getinfo.htm?sid=WDCM_00190
Trichophyton mentagrophytes	00191 http://refs.wdcm.org/getinfo.htm?sid=WDCM_00191

NOTE 1 Other fungi can be used after appropriate validation.

NOTE 2 Refer to WDCM and its website: http://refs.wdcm.org/search.htm . (Note that WDCM stands for World Data Centre for Microorganisms.)

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Annex B
(informative)

Antifungal efficacy

The following criteria can be applied to show clear efficacy of the antifungal property.

NOTE The recommended criterion does not guarantee that there will be no growth of fungus. This means that fungus on the treated product shows slower growth or no growth as compared with a control textile product.

Table B.1 — Example of efficacy for antifungal property

Item	Criterion of antifungal efficacy by antifungal activity value A_a in Equation (3)	Explanation
Test specimen vs. control specimen	$1,0 > A_a$	No effect
	$2 > A_a \geq 1,0$	Small effect
	$3 > A_a \geq 2,0$	Medium effect
	$A_a \geq 3,0$	Full effect

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