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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

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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Contents

Page

Foreword ... iv

Introduction ... v

1 Scope ... 1

2 Normative reference ... 1

3 Terms and definitions ... 1

4 Principle ... 2

5 Safety precaution ... 2

6 Reference fungi ... 2

7 Apparatus ... 2

8 Reagents and culture media ... 3
8.1 General ... 3
8.2 Pure water ... 4
8.3 Anionic surfactant ... 4
8.4 Luminescent reagents, reagents and buffer solutions ... 4
8.5 Culture medium ... 5

9 Fungus preservation and use ... 6
9.1 Fungus preservation ... 6
9.2 Use of fungus ... 7

10 Spore suspension ... 7
10.1 Suspending spores in culture media ... 8
10.2 Collection and dispersion of spore suspension from a culture medium ... 8
10.3 Filtering to remove hyphae and spore threads ... 8
10.4 Using centrifugal separation and resuspension to remove supernatant ... 8
10.5 Confirming the concentration of spore suspension ... 9
10.6 Adjusting spore suspension for testing ... 9

11 Preparing the ATP calibration curve ... 9

12 Testing method ... 10
12.1 Preparation of test specimens and inoculation ... 10
12.2 Incubation ... 12

13 Measurement of luminescence intensity ... 12
13.1 Absorption method ... 12
13.2 Transfer method ... 13

14 Calculation ... 14
14.1 Judgment of test effectiveness ... 14
14.2 Calculation of antifungal activity value ... 14

15 Test report ... 15

Annex A (normative) **The fungi used in this part of ISO 13629** ... 16

Annex B (informative) **Antifungal efficacy** ... 17

Bibliography ... 18

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https://standards.iteh.ai/catalog/standards/sist/198645c4-c10b-467e-89dd-beae866af984/iso-13629-1-2012

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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).

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2 Normative reference

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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.

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5 Safety precaution

The test methods specified herein require the use of fungus.

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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.

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7.17 Test-tube agitator.

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

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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)

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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.

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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.

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The composition is as follows:

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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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