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Textiles — Determination of antifungal activity of textile products —

Part 2: Plate count method

Textiles — Détermination de l'activité antifongique des produits textiles —
Partie 2: Méthode par dénombrement sur plaque de gélose

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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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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information

The committee responsible for this document is 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*

Introduction

This part of ISO 13629 adopts the plate count method as a basis of quantitative determination of antifungal activity.

The following are characteristics of the plate count method:

— conventional method which is easy to operate in bacteriological laboratories;

— no need to use special apparatus such as a lumino photometer;

— long history and common procedure.

Textiles — Determination of antifungal activity of textile products —

Part 2:
Plate count method

1 Scope

This part of ISO 13629 specifies a test method for quantitative determination of antifungal activity by plate count method.

This 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.

2 Normative reference

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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*

ISO 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

3 Terms and definitions

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

3.1
control fabric
fabric used to validate the growth condition of test fungi

Note 1 to entry: Control specimens are sampled from the control fabric.

Note 2 to entry: The control fabric may be the same fabric as the fabric to be tested but without antifungal treatment. If this is not available, a 100 % cotton fabric without fluorescent brighteners or other finish, complying with the requirements of ISO 105-F02, is used as control fabric, after washing at the temperature of 60 °C without detergents or any brighteners, with mechanical agitation and rinsing.

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

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

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

3.5
plate count method
method in which the number of fungi present after incubation is calculated by counting the number of colonies according to a ten-time dilution method

Note 1 to entry: The results are expressed in CFU (Colony Forming Unit).

3.6
neutralizer
chemical agent used to inactivate, neutralize, or quench the antifungal properties of antifungal agents

4 Principle

A test specimen and a control specimen are inoculated with spore suspension of reference fungi and incubated at 30 °C for 48 h.

In this part of ISO 13629, fungal growth is quantitatively determined by the visual counting of colonies on the agar plate as CFU and the fungal activity is calculated by CFU.

In case the test specimen absorbs water, the absorption method is recommended. In case the test specimen does not absorb water, the transfer method is recommended.

5 Safety precaution

The test method specified herein requires use of fungi.

According to ISO 7218, 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 may be consulted and followed.

6 Reference fungi

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

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

The strain number and supply source of the fungi used shall be stated in the test report.

7 Apparatus

Usual laboratory apparatuses and, in particular, the following apparatuses are used. When relevant, the items have to be sterilized before using.

7.1 Gauze, sterilized.

7.2 Petri dish, made of glass or plastic, with a diameter of about 60 mm or 90 mm.

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

7.4 Platinum loop, with a loop of 2 mm to 4 mm in diameter (or plastic equivalent).

7.5 L-shaped platinum colony hook (or plastic equivalent).

7.6 Incubator, capable of maintaining a temperature in a range from 25 °C to 37 °C with a tolerance of ±2 °C.

7.7 Vial, capacity of 30 ml screw-top glass vial with polytetrafluoroethylene or silicone gasket and polypropylene cap. It shall be carefully washed in alkaline or neutral detergent, rinsed, and dried.

7.8 Glass funnel.

7.9 Pipettes, capacity of 0,2 ml, 1 ml, 5 ml, and 10 ml with a tolerance of 0,5 % or less and with a tip made of glass or plastic.

7.10 Pasteur pipette, for microbiological testing (or plastic equivalent).

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

7.12 Tweezers, made of material which can be sterilized.

7.13 Centrifuge, with centrifugal acceleration of approximate 2 000 × *g*.

7.14 Centrifuge tube, used for centrifuge.

7.15 Hemacytometer, capable of measuring 1 × 10⁶ cells/ml to 3 × 10⁶ cells/ml.

7.16 Microscope, capable of 200x magnification.

7.17 Ultrasonic cleaner, compact for experiment tools, with frequency of approximately 30 kHz to 50 kHz.

7.18 pH meter, with glass electrodes for biochemical testing or equivalent pH paper.

7.19 Erlenmeyer flask, capacity of 100 ml.

7.20 Cutting template, made of stainless steel with a diameter of (3,8 ± 0,1) cm.

7.21 Stainless steel cylinder, with a weight of (200 ± 10) g and a diameter of (3,5 ± 0,1) cm.

7.22 Shaker, capable of producing a Vortex shaking action.

7.23 Paddle blender [Stomacher-type], capable of speed 6 blows/s to 8 blows/s with the corresponding disposable containers.

7.24 Humidity chamber, a tropical chamber or other container capable of maintaining a high humidity atmospheric condition.

7.25 Refrigerator, capable of maintaining a temperature of between 2 °C and 8 °C with a tolerance of ±2 °C.

7.26 Freezers, adjustable to a temperature below −70 °C and below −20 °C with a tolerance of ±2 °C.

7.27 Balance, capable of measuring 0,01 g as the readability.

7.28 Disposable plastic bags, suitable for containing food products to be used for the shake-out of sample.

7.29 Microbiological safety cabinet (MSC Type II), designed for microbiological tests use, or other system with equivalent performances.

7.30 Water baths, one capable of maintaining a constant temperature of (46 ± 2) °C and another capable of maintaining a temperature of 70 °C to 90 °C.

8 Reagents and culture media

Reagents used in tests shall be of analytical grade and/or suited for microbiological purposes.

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

8.1 Pure water

Analytical-grade water for microbiological media preparation which is freshly distilled and/or ion-exchanged and/or ultra-filtered and/or filtered with RO (reverse osmosis).

It shall be free from all toxic or fungi inhibitory substances.

8.2 Anionic surfactant

Dioctyl sodium sulfosuccinate to prepare spore suspension. The concentration of the anionic surfactant in pure water (8.1) is 50 mg/l. Sterilize this solution by an autoclave (7.3) at 121 °C for 20 min.

8.3 Culture medium

Use a culture medium prepared as described below. Commercially prepared items may be used after appropriate validation.

Culture media which will not be used immediately after preparation shall be stored at 5 °C to 10 °C and discarded after one month.

8.3.1 Sabouraud dextrose broth (SDB)

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

8.3.2 Potato dextrose agar (PDA)

Potato infusion from	200 g
Dextrose	20 g
Agar	15 g
Pure water	1 000 ml
pH after sterilization	5,6 ± 0,2

This medium encourages mould sporulation.

8.3.3 Sabouraud dextrose agar (SDA)

Pepsic meat peptone	10 g
Dextrose	40 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.

8.3.4 Slant culture

8.3.4.1 Pour approximately 10 ml of pre-heated and fully dissolved PDA (described in 8.3.2) into a sterilized test tube.

8.3.4.2 Put a cotton plug on and sterilize it with steam after sterilization.

8.3.4.3 Place the test tube at an approximately 15° angle against a level surface on a clean laboratory table, and leave the contents to solidify.

8.3.4.4 When there is no bleed water on the solidified agar, dissolve, and solidify it again for use.

8.3.5 Neutralizing solution, SCDLP medium

Polysorbate 80	30 g
Egg yolk lecithin	3 g
Histidine hydrochloride	1 g
Meat or casein peptone	1 g
Sodium chloride (NaCl)	4,3 g
Monopotassium phosphate	3,6 g
Disodium phosphate dehydrate	7,2 g
Water	1 000 ml (final volume)
pH after sterilization	7,2 ± 0,2

When sufficient neutralizing power cannot be achieved, the content of polysorbate 80 or lecithin may be adjusted or another neutralizing agent may be added. Commercial solutions of neutralizer can be used after having tested their efficacy (see Annex B). The use of any unspecified neutralizer shall be recorded along with the name and concentration.