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Footwear and footwear components — Quantitative challenge test method to assess antifungal activity

Chaussures et composants de chaussure — Méthode de test d'épreuve quantitatif pour évaluer l'activité antifongique

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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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This document was prepared by Technical Committee ISO/TC 216, *Footwear*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Footwear and footwear components — Quantitative challenge test method to assess antifungal activity

CAUTION — Test methods specified herein require the use of micro-fungi. These tests are only to be carried out in facilities with containment techniques for handling microorganisms and by persons with training and experience in the use of microbiological techniques.

1 Scope

This document specifies quantitative challenge test methods for evaluating the antifungal activity of footwear and footwear components.

This document is applicable only to footwear and components that claim to have antifungal (antimycotic) properties or antimicrobial properties.

Two methods can be applied. The choice of method depends on the material properties and test microorganisms. Dynamic challenge test method can be applied to all types of materials. For single absorbent materials, static challenge test method is recommended. Brief descriptions of each method are given in 11.2 and 11.3.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

ISO 19952, *Footwear — Vocabulary*

3 Terms and definitions

For the purpose of this document, the terms and definitions given in ISO 19952 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
antifungal activity
antimycotic activity
efficacy of a material or finish used to prevent or mitigate the growth of micro-fungi, to reduce the number of micro-fungi or to kill micro-fungi

3.2
control specimen
material identical to the test material but without antifungal treatment

Note 1 to entry: If no control specimen is available, sterilized conical flask can be used as control specimen.

3.3
neutralizer
chemical agents used to inactivate, neutralize, or quench the antifungal properties of antifungal agents

[SOURCE: ISO 20743:2013, 3.7, modified — "antibacterial" has been replaced with "antifungal".]

4 Principle

The test specimens and control specimens are inoculated with a spore suspension of a selected test strain of micro-fungi specified or claimed. Two test methods are available to assess antifungal activity.

Antifungal performance is quantitatively determined by counting the number of viable micro-fungi and calculating the antifungal activity ratio.

5 Safety

The handling of microorganisms which are potentially hazardous requires a high degree of technical competence and can be subject to current national legislation and regulations. Only personnel trained in microbiological techniques should carry out such tests.

NOTE: Refer to country-specific codes of practice for personal hygiene, disinfection and sterilization.

It is recommended that the person who perform the test should consult IEC 60068-2-10:2005, Appendix A, and ISO 7218.

6 Apparatus

6.1 General

Disposable apparatus is an acceptable alternative to re-usable glassware and plastic if it has suitable specifications.

Usual microbiological laboratory equipment in accordance with ISO 7218 and in particular the following.

6.2 Biological safety cabinet.

6.3 Incubator, capable of maintaining a temperature of (28 ± 2) °C.

6.4 Autoclave, capable of maintaining a temperature of (121 ± 2) °C and a pressure of (103 ± 5) kPa, for wet sterilization, used in accordance with ISO 7218.

6.5 Humidity chamber, capable of maintaining a temperature of (28 ± 2) °C and a relative humidity of (85 ± 5) %.

6.6 Ultraviolet lamp.

6.7 Wide mouth jars, with cap, 100 ml, capable of being used with an autoclave (6.4).

6.8 Vortex mixer.

6.9 Centrifugal machine, 2 000 × *g*.

6.10 Dimensional shaker, two dimensional or three dimensional, capable of adjusting to 50 r/min.

6.11 Shaking incubator, capable of maintaining a temperature of (28 ± 2) °C and a rotational frequency of (120 ± 10) r/min.

6.12 Glass beads, 2 mm to 3 mm in diameter, 10 beads to 15 beads per conical flask, for preparation of fungal spore solutions.

6.13 Glass wool or medical gauze (double layers), for preparation of fungal spore solutions.

6.14 Oven, for dry sterilization.

6.15 pH-meter, capable of measuring to ±0,2 units.

6.16 Balance, capable of weighing to ±0,01 g.

6.17 Spectrophotometer, capable of measuring at 500 nm to 660 nm wavelength, or McFarland's nephelometer.

6.18 Petri dishes, that have been sterilized, made of glass or plastic, in diameter sizes of 90 mm to 100 mm or 55 mm to 60 mm.

6.19 Pipette, having the most suitable volume for each use.

7 Reagents and culture medium

7.1 General

The preparation and test shall be freshly prepared in order to ensure the culture quality.

NOTE This could be done according to ISO 11133, or according to national standards or regulations.

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

7.2 Water

Water used in tests shall be analytical-grade water for microbiological media preparation, which is freshly distilled and/or ion-exchanged and/or ultra-filtered and/or filtered with reverse osmosis.

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

7.3 Malt medium

7.3.1 Composition

Malt extract	30,0 g
Soya peptone	3,0 g
Water	1 000 ml

7.3.2 Preparation

Dissolve the designated amounts of components in distilled water, stir and adjust pH to (5,5 ± 0,2) at room temperature, sterilize at (121 ± 2) °C for 15 min in an autoclave (6.4) with saturated water vapour.

7.4 Malt extract agar (MEA) medium

7.4.1 Composition

Malt extract	30,0 g
Soya peptone	3,0 g
Agar	15,0 g
Water	1 000 ml

7.4.2 Preparation

After mixing, stir and adjust pH to (5,5 ± 0,2) at room temperature. Heat with stirring on a hotplate or in a boiling-water bath until the components are completely dissolved, sterilize at (121 ± 2) °C for 15 min in an autoclave (6.4) with saturated water vapour. Cool and shake solution well, then pour into the Petri dishes.

NOTE The potato dextrose agar (PDA) can also provide a complete medium for the growth of micro-fungi. The standard PDA medium can be obtained as a commercial product thereby avoiding the preparation steps of cooking and the variation in the composition of potato species. The commercial PDA medium with standard composition can be used to avoid the influence of composition of potato and operation when boiling it, and Malt Extract Agar (MEA) medium can be obtained from commercial source.

7.5 Physiological saline (sodium chloride solution)

7.5.1 Composition

Sodium chloride, NaCl	8,5 g
Water	1 000 ml

7.5.2 Preparation

After well mixing, adjust pH to (6,9 ± 0,2) at room temperature and sterilize at (121 ± 2) °C for 15 min.

7.6 Wetting agent (nonionic surfactant)

To be used for harvesting the spores, does not react with other reagents and does not cause a reduction or increase in micro-fungi number, such as polysorbate 80 (TWEEN 80), N-methyltauride, Triton™ X-100[1)] or polyglycol ether and so on.

NOTE Wetting agent (nonionic surfactant) can be used when the specimen has coating.

7.7 Buffer solution

7.7.1 Buffer stock

Potassium dihydrogen phosphate, KH_2PO_4	34,0 g
Water	1 000 ml

1) TritonTM X-100 is the trade name of a product supplied by SIGMA-ALDRICH. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.