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**Obutev in sestavni deli obutve - Kakovostna preskusna metoda za oceno protiglivične aktivnosti (preskus rasti) (ISO/DIS 19574:2020)**

Footwear and footwear components - Qualitative test method to assess antifungal activity (growth test) (ISO/DIS 19574:2020)

Schuhe und Schuhbestandteile - Qualitatives Prüfverfahren zur Bestimmung der antimykotischen Wirksamkeit (Wachstumsprüfung) (ISO/DIS 19574:2020)

Chaussures et composants de chaussures - Méthode d'essai de croissance qualitative pour évaluer les propriétés antifongiques contre les microchampignons filamenteux (ISO/DIS 19574:2020)

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|        |         |          |
|--------|---------|----------|
| 61.060 | Obuvala | Footwear |
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## Footwear and footwear components — Qualitative test method to assess antifungal activity (growth test)

*Chaussures et composants de chaussures — Méthode d'essai de croissance qualitative pour évaluer les propriétés antifongiques contre les microchampignons filamenteux*

ICS: 61.060

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## ISO/DIS 19574:2020(E)

### 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.

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](http://www.iso.org/directives)).

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

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# Footwear and footwear components — Qualitative test method to assess antifungal activity (growth test)

**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 International Standard specifies a test method (growth test) for qualitative evaluation of the antifungal activity of footwear and footwear components due to the action of filamentous micro-fungi.

This International Standard is applicable only to footwear and components that claim to have antifungal (antimycotic) or antimicrobial treatment effects.

## 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 11133, *Microbiology of food, animal feed and water — Preparation, production, storage and performance testing of culture media*

ISO 16187, *Footwear and footwear components — Test method to assess antibacterial activity*

ISO 19952, *Footwear — Vocabulary*

## 3 Terms and definitions

For the purposes 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 <http://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 specimens**

material identical to the test material but without antifungal treatment

Note 1 to entry: If no control specimens are available, sterilized filter paper can be used as control specimens.

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### 4 Principle

The test and control specimens of footwear are inoculated with a mixed spore suspension of selected mould test strains or with a single test strain according to the specific claim.

Antifungal performance is qualitatively determined by visual assessment of fungal growth after specified or agreed incubation period.

### 5 Safety

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 workers should consult IEC 60068-2-10, appendix A "Danger to personnel" and ISO 7218 "Microbiology — General guidance for microbiological examinations".

### 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

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#### 6.3 Microbiological incubator

Capable of maintaining a temperature of  $(28 \pm 2)$  °C and a relative humidity of  $(85 \pm 5)$  %.

#### 6.4 Autoclave

Capable of maintaining a temperature of  $(121 \pm 2)$  °C and a pressure of  $(103 \pm 5)$  kPa, for wet sterilization, used in accordance with ISO 7218.

#### 6.5 Vortex mixer

6.6 **pH-meter**, having an accuracy of  $\pm 0,1$  pH-units.

6.7 **Laboratory centrifugal**,  $2\ 000 \times g^1$ .

6.8 **Microscope**, magnification  $\times 50$ .

6.9 **Glass beads**, 2 mm to 3 mm in diameter, for preparation of fungal spore solutions.

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

6.11 **Wide mouth jars, with cap**, 500 ml, capable of being used with an autoclave (6.4).

1)  $2\ 000 \times g \approx 4\ 000$  r/min



**6.12 Oven**, for dry sterilization.

**6.13 Balance**, capable of weighing to  $\pm 0,01$  g.

**6.14 Spectrophotometer**, capable of measuring at a 500 nm to 700 nm wavelength, or McFarland's nephelometer.

**6.15 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.

**6.16 Pipette**, having the most suitable volume for each use.

## 7 Reagents and culture medium

### 7.1 General

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

Dehydrated products available on the commercial market are recommended for use in preparing the culture media. The manufacture user's instructions for the preparation of these products should be strictly followed.

Follow ISO 11133 for the preparation, production and performance testing of culture media.

### 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 RO (reverse osmosis).

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

### 7.3 Malt extract agar (MEA) medium

#### 7.3.1 Composition

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

#### 7.3.2 Preparation

After mixing, stir and adjust pH to  $(5,5 \pm 0,2)$  at room temperature. Heat with stirring on a hotplate or in a boiling-water bath until the components are completely dissolved, sterilize at  $(121 \pm 2)$  °C for 15 min in an autoclave (5.4) with saturated water vapour. Cool and shake solution well, then pour 25 ml into each sterile Petri dish. Leave to cool and solidify.

NOTE 1 The Potato Dextrose Agar (PDA) can also provide a complete medium for the growth of micro-fungi. The commercial PDA medium with standard composition can be used.

NOTE 2 Malt extract agar (MEA) medium can be obtained from commercial source.

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### 7.4 Physiological saline (sodium chloride solution)

#### 7.4.1 Composition

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

#### 7.4.2 Preparation

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

### 7.5 Wetting agent (nonionic surfactant)

To be used to harvest the spores and in the test spore suspension, it should not react with other reagents and not cause a reduction or increase in micro-fungi number, such as polysorbate 80 (TWEEN 80), N-methyltauride, Triton™ X-100<sup>2)</sup> or polyglycol ether and so on. Use final concentrations of 0,01 %.

NOTE Wetting agent (nonionic surfactant) can be used when the specimens have coating.

### 7.6 Buffer solution

#### 7.6.1 Buffer stock

|  |          |
|--|----------|
| Potassium dihydrogen phosphate, $\text{KH}_2\text{PO}_4$ | 34,0 g   |
| Water  | 1 000 ml |

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#### 7.6.2 Preparation of buffer stock

Weigh potassium dihydrogen phosphate into 1 000 ml flask, add 500 ml of distilled water, adjust pH to  $(7,2 \pm 0,2)$  (at room temperature) with diluted solution of 0,01 mol/L NaOH. Dilute to 1 000 ml with distilled water and store at 4 °C for maximum 6 month.

#### 7.6.3 Preparation of buffer solution

Transfer 1 ml of buffer stock solution and 0,08 g of wetting agent (7.5), corresponds to 0,01 % and dilute to 800 ml with distilled water. After mixing well, sterilize at  $(121 \pm 2)$  °C for 15 min.

NOTE If wetting agent (nonionic surfactant) (7.5) is not required, it might be omitted.

## 8 Test microorganisms

The strain used shall be stated in the test report.

The species to be used are listed in [Table 1](#).

If antimycotic properties are claimed against mould, tests can be carried out against one of *Aspergillus sp.* and *Penicillium funiculosum*, either as a mixed suspension or separately. If antimycotic properties are claimed against dermatophyte athlete's foot, *Trichophyton mentagrophytes* shall be tested.

Do not test a mixed suspension of mould strains and dermatophyte strains.

NOTE it's recommended to test each organism independently.

2) Triton™ 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.