

---

---

**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*

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

[ISO 20150:2019](https://standards.iteh.ai/catalog/standards/sist/f0e47c9a-4680-4bca-a0d7-52ad0f5cfe35/iso-20150-2019)

[https://standards.iteh.ai/catalog/standards/sist/f0e47c9a-4680-4bca-a0d7-  
52ad0f5cfe35/iso-20150-2019](https://standards.iteh.ai/catalog/standards/sist/f0e47c9a-4680-4bca-a0d7-52ad0f5cfe35/iso-20150-2019)



## iTeh STANDARD PREVIEW (standards.iteh.ai)

ISO 20150:2019

<https://standards.iteh.ai/catalog/standards/sist/f0e47c9a-4680-4bca-a0d7-52ad0f5cfe35/iso-20150-2019>



### **COPYRIGHT PROTECTED DOCUMENT**

© ISO 2019

All rights reserved. Unless otherwise specified, or required in the context of its implementation, no part of this publication may be reproduced or utilized otherwise in any form or by any means, electronic or mechanical, including photocopying, or posting on the internet or an intranet, without prior written permission. Permission can be requested from either ISO at the address below or ISO's member body in the country of the requester.

ISO copyright office  
CP 401 • Ch. de Blandonnet 8  
CH-1214 Vernier, Geneva  
Phone: +41 22 749 01 11  
Fax: +41 22 749 09 47  
Email: [copyright@iso.org](mailto:copyright@iso.org)  
Website: [www.iso.org](http://www.iso.org)

Published in Switzerland

# Contents

	Page
Foreword .....	v
<b>1 Scope</b> .....	<b>1</b>
<b>2 Normative references</b> .....	<b>1</b>
<b>3 Terms and definitions</b> .....	<b>1</b>
<b>4 Principle</b> .....	<b>2</b>
<b>5 Safety</b> .....	<b>2</b>
<b>6 Apparatus</b> .....	<b>2</b>
<b>7 Reagents and culture medium</b> .....	<b>3</b>
7.1 General .....	3
7.2 Water .....	3
7.3 Malt medium .....	3
7.3.1 Composition .....	3
7.3.2 Preparation .....	3
7.4 Malt extract agar (MEA) medium .....	4
7.4.1 Composition .....	4
7.4.2 Preparation .....	4
7.5 Physiological saline (sodium chloride solution) .....	4
7.5.1 Composition .....	4
7.5.2 Preparation .....	4
7.6 Wetting agent (nonionic surfactant) .....	4
7.7 Buffer solution .....	4
7.7.1 Buffer stock .....	4
7.7.2 Preparation of buffer stock .....	5
7.7.3 Preparation of buffer solution .....	5
<b>8 Test microorganisms</b> .....	<b>5</b>
<b>9 Preparation of test inoculums</b> .....	<b>5</b>
9.1 Indications for use of strains .....	5
9.2 Preparation of inoculums of <i>Candida albicans</i> .....	6
9.3 Preparation of test spore suspension of filamentous micro-fungi .....	6
<b>10 Preparation of test specimens</b> .....	<b>6</b>
10.1 General .....	6
10.2 Test specimen .....	7
10.3 Pre-treatment of test specimen .....	7
<b>11 Test procedure</b> .....	<b>7</b>
11.1 Summary of test methods .....	7
11.2 Dynamic challenge tests .....	7
11.2.1 Inoculation .....	7
11.2.2 Neutralization and elution after inoculation (time 0 h) .....	8
11.2.3 Incubation .....	8
11.2.4 Neutralization and elution after incubation (time 24 h) .....	8
11.2.5 Determination of the number of viable micro-fungi .....	8
11.3 Static challenge test .....	8
11.3.1 Inoculation .....	8
11.3.2 Neutralization and elution after inoculation (time 0 h) .....	9
11.3.3 Incubation .....	9
11.3.4 Neutralization and elution after incubation (time 24 h) .....	9
11.3.5 Determination of the number of viable micro-fungi .....	9
<b>12 Expression of results</b> .....	<b>9</b>
12.1 Calculation of the number of viable micro-fungi .....	9
12.2 Judgement of test effectiveness .....	9

12.3	Calculation of antifungal activity ratio .....	10
<b>13</b>	<b>Test report</b> .....	<b>10</b>
	<b>Bibliography</b> .....	<b>12</b>

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

ISO 20150:2019

<https://standards.iteh.ai/catalog/standards/sist/f0e47c9a-4680-4bca-a0d7-52ad0f5cfe35/iso-20150-2019>

## 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)).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see [www.iso.org/patents](http://www.iso.org/patents)).

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

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT) see [www.iso.org/iso/foreword.html](http://www.iso.org/iso/foreword.html).

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](http://www.iso.org/members.html).

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

ISO 20150:2019

<https://standards.iteh.ai/catalog/standards/sist/f0e47c9a-4680-4bca-a0d7-52ad0f5cfe35/iso-20150-2019>

# 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.

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

## 6 Apparatus

### 6.1 General

ISO 20150:2019

<https://standards.iteh.ai/catalog/standards/sist/f0e47c9a-4680-4bca-a0d7-71a1e1d54b-2019-02-14>

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  $(28 \pm 2)$  °C.

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 **Humidity chamber**, capable of maintaining a temperature of  $(28 \pm 2)$  °C and a relative humidity of  $(85 \pm 5)$  %.

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  $(28 \pm 2)$  °C and a rotational frequency of  $(120 \pm 10)$  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  $\pm 0,2$  units.

**6.16 Balance**, capable of weighing to  $\pm 0,01$  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  $(5,5 \pm 0,2)$  at room temperature, sterilize at  $(121 \pm 2)$  °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  $(5,5 \pm 0,2)$  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 (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)

**STANDARD PREVIEW**  
**(standards.iteh.ai)**

### 7.5.1 Composition

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

ISO 20150:2019

<https://standards.iteh.ai/catalog/standards/sist/f0e47c9a-4680-4bca-a0d7-52ad0f5cfe35/iso-20150-2019>

### 7.5.2 Preparation

After well mixing, adjust pH to  $(6,9 \pm 0,2)$  at room temperature and sterilize at  $(121 \pm 2)$  °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<sup>1)</sup> 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 <sub>2</sub> PO <sub>4</sub>	34,0 g
Water	1 000 ml

1) 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.

### 7.7.2 Preparation of buffer stock

Prepare phosphate buffer solution by placing 34,0 g of potassium dehydrogenate phosphate in a 1 000 ml volumetric flask. Add 500 ml of water and mix to dissolve. Adjust the pH to  $7,2 \pm 0,2$  at room temperature with sodium hydroxide. Add distilled water to make up to 1 000 ml.

### 7.7.3 Preparation of buffer solution

Transfer 1 ml of buffer stock solution and 0,08 g of wetting agent (nonionic surfactant) (7.6), 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) is not required, it might be omitted.

## 8 Test microorganisms

The strain used shall be stated in the test report.

The species that shall be used in antifungal activity tests are listed in Table 1.

Table 1 — Test strains

Micro-fungia <sup>a</sup>	Name	WDCM No.	CGMCC No.	ATCC® No.
Yeast	<i>Candida albicans</i>	00054	AS 1.2031	ATCC® 10231™ b
Filamentous micro-fungi/Mould	<i>Aspergillus niger</i>	00144	AS 3.4463	ATCC® 6275™ b
	<i>Aspergillus brasiliensis</i>	00053	AS 3.5487	ATCC® 16404™ b
Filamentous micro-fungi/Dermatophyte	<i>Trichophyton mentagrophytes</i>	00191	—	ATCC® 9533™ b

**Key** ISO 20150:2019  
WDCM: World Data Centre for Microorganisms  
CGMCC: China General Microbiological Culture Collection Centre  
ATCC®: American Type Culture Collection  
NOTE 1 Refer to WDCM and website: <http://refs.wdcm.org>.  
NOTE 2 Other micro-fungi (appropriate species or other appropriate strains) can be used after appropriate validation.  
<sup>a</sup> Test strains shall be obtained from agencies of the World Federation of Culture Collection (WFCC). The micro-fungal species and their supply sources shall be stated in the test reports.  
<sup>b</sup> ATCC® 10231™, ATCC® 6275™, ATCC® 16404™ and ATCC® 9533™ are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of these products.

Testing of yeast (*Candida albicans*) is mandatory.

If activity against mould is claimed, testing of one mould strain shall be performed.

If activity against dermatophyte is claimed, testing of *Trichophyton mentagrophytes* shall be performed.

Strains can be preserved in accordance with the supplier's instructions or EN 12353.

## 9 Preparation of test inoculums

### 9.1 Indications for use of strains

Apply strains within 4 generations.