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Footwear and footwear components — Test method to assess antibacterial activity

Chaussure et composants de chaussure — Méthode d'essai pour évaluer l'activité antibactérienne

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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. www.iso.org/directives

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The committee responsible for this document is ISO/TC 216, *Footwear*.

Footwear and footwear components — Test method to assess antibacterial activity

CAUTION — Test methods specified herein require the use of bacteria. 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. Appropriate safety precautions are to be observed with due consideration given to country-specific regulations.

1 Scope

This International Standard specifies quantitative test methods to evaluate the antibacterial activity of footwear and components.

This International Standard is applicable to all types of footwear and components employing non-diffusing antibacterial treatments.

2 Normative references

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 3696, *Water for analytical laboratory use — Specification and test methods*

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.

3.1
antibacterial activity
efficacy of a material or finish used to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.2
control sample
material identical to the test material but without antibacterial treatment

4 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. Codes of practice for disinfection, sterilization and personal hygiene shall be strictly observed.

NOTE It is recommended that workers consult IEC 60068–2-10, appendix A "Danger to personnel", and ISO 7218.

5 Apparatus and materials

5.1 General

Standard laboratory equipment and the following.

5.2 Biological safety cabinet.

5.3 Incubator, capable of maintaining a temperature of (37 ± 2) °C.

5.4 Autoclave.

5.5 Humidity chamber, capable of maintaining a temperature of (37 ± 2) °C and a relative humidity not less than 90 %.

5.6 Ultraviolet lamp.

5.7 Wide mouth jars, with cap, 100 ml, capable of being used with an autoclave (5.4).

5.8 Cover film that does not affect bacterial growth or absorb water, which can be made of either polyethylene, polypropylene or polyester [poly (ethylene terephthalate)]. Film that is 0,05 mm to 0,10 mm thick is recommended. For example, disposal bag suitable for use with an autoclave (5.4).

5.9 Vortex mixer.

5.10 Dimensional shaker, two dimensional or three dimensional, capable of adjusting to 50 rpm.

5.11 Shaking incubator, capable of maintaining a temperature of (37 ± 2) °C and a rotational frequency of (120 ± 10) rpm.

6 Reagents and culture medium

6.1 Principle

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

NOTE This can be done according to ISO/TS 11133-1, ISO/TS 11133-2, or according to national standards or regulations.

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

Use only water Grade 3 according to ISO 3696.

6.2 Nutrient broth (NB)

6.2.1 Composition

Beef extract, 3,0 g.

Peptone, 5,0 g.

Sodium chloride (NaCl), 5,0 g.

Water, 1 000 ml.

6.2.2 Preparation

Stir and adjust pH to (7,2 ± 0,2) (at room temperature). Heat with stirring on a hotplate or in a boiling-water bath until the components are completely dissolved. Sterilize with autoclave (5.4) at (121 ± 2) °C for 15 min.

6.3 Nutrient agar (NA)

6.3.1 Composition

Beef extract, 5,0 g.

Peptone, 10,0 g.

Sodium chloride (NaCl), 5,0 g.

Agar, 15,0 g.

Water, 1 000 ml.

NOTE If solidification is insufficient, 15 g to 18 g of agar can be used.

6.3.2 Preparation

Stir and adjust pH to (7,2 ± 0,2) (at room temperature). Heat with stirring on a hotplate or in a boiling-water bath until the components are completely dissolved. Sterilize with autoclave (5.4) at (121 ± 2) °C for 15 min. Cool and shake solution well, then pour into the Petri dishes.

6.4 Soybean casein digest broth with lecithin and polyoxyethylene medium (SCDLP)

6.4.1 Composition

Peptone, digest of casein, 17,0 g.

Peptone, digest of soybean, 3,0 g.

Sodium chloride (NaCl), 5,0 g.

Potassium dihydrogen phosphate, 2,5 g.

Glucose, 2,5 g.

Lecithin, 1,0 g.

Polysorbate 80, 7,0 g.

Water, 1 000 ml.

If the neutralizing power is insufficient, the content of polysorbate 80 or lecithin may be adjusted or another neutralizing agent may be added. The use of any unspecified neutralizer shall be recorded along with the name and concentration.

NOTE Information about selection and evaluation of alternative antibacterial neutralizing agents can be found in ASTM E 1054 and EN 1040.

6.4.2 Preparation

After mixing well, adjust pH to (7,2 ± 0,2) (at room temperature) and sterilize with autoclave (5.4) at (121 ± 2) °C for 15 min.

6.5 Sodium chloride solution (physiological saline)

6.5.1 Composition

Sodium chloride (NaCl), 8,5 g.

Water, 1 000 ml.

6.5.2 Preparation

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

7 Test microorganisms

7.1 Test strains

The following species shall be used in all antibacterial activity tests.

a) *Staphylococcus aureus* AS 1.89 or ATCC 6538.

b) *Klebsiella pneumoniae* AS 1.1736 or ATCC 4352.

NOTE 1 If required, other species or other strains can be used. However, the selected organisms should contain at least one gram-positive and one gram-negative organism as the antibacterial agents may have different activities.

Test strains shall be obtained from agencies of the World Federation of Culture Collection (WFCC).

The bacteria species and their supply sources shall be included in the test report.

NOTE 2 AS refers to the China General Microbiological Culture Collection Centre (CGMCC), ATCC is the American Type Culture Collection.

7.2 Storage of strains

Inoculate the strains to the nutrient agar (NA) (6.3), and incubate at (37 ± 2) °C for 24 h. Store at (5 ± 3) °C (maximum one month) and keep it as stock culture of the strains. Transfer and incubate one time each month.

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

8 Preparation of test inoculums

Using a sterile inoculating loop, transfer one colony (7.2) into 20 ml of nutrient broth (NB) (6.2) and incubate in the shaking incubator (5.11) at (37 ± 2) °C for about 16 h (overnight culture). Estimate the number of bacteria with microscopic observation or other methods. Prepare physiological saline (6.5) with 1 % nutrient broth (NB) (6.2). Use this media to prepare a suspension with a bacterial concentration of (2,5 approximately 10) × 10^5 CFU/ml as test inoculum.

If necessary, store the test inoculum on ice and use it within 4 h.

9 Preparation of test samples

9.1 General

Test only the components or material which are claimed to be antibacterial. If the whole footwear is claimed as antibacterial, major components, including upper, lining, insole, insock, outsole shall be tested separately.

In the case where only one material of a component is claimed to be antibacterial, it shall be tested separately, if possible. Otherwise, the whole component shall be tested.

Each test sample shall be at least 80 % of the surface area of the component or material. If single material accounts for less than 80 %, take two main materials used in the composition of the component.

The test samples can be obtained directly from the footwear raw materials.

9.2 Test specimen

The area of test specimen should be about 500 mm^2. For test method A (see Annex A), the area of test specimen shall have a thickness of less than 2,0 mm. The area and the weight shall be reported in the test report. If a larger test specimen is used then the volume of bacterial suspension should be increased proportionally.

If it is impossible to lower the thickness of the test specimen (for example, components are thicker and cannot be separated or cut without changing critical properties like surface morphology which may affect how the bacteria interact with the surface), the thickness shall be indicated in the test report.

At least six test specimens shall be taken for each material or component and for each test strain.

9.3 Pre-treatment of the test specimen

Pre-treatment of the test specimen is optional and should only be conducted if necessary due to high bioburden (contamination etc.).

If sterilization methods are applied, they shall be reported in detail, and shall not affect the antibacterial properties or the material itself.

NOTE The test and control sample can be sterilized with the autoclave (5.4) at (121 ± 2) °C and 103 kPa for 15 min, or with ultraviolet rays [ultraviolet lamp (5.6), 30 W, away from the sample 300 mm, each side for one hour respectively] or other suitable sterilizing methods.

10 Test procedure

Table 1 lists the circumstances under which each test method should be applied.

Test method A (see Annex A) should be used only for absorbent single material. Test method B (see Annex B) should be used only for non-absorbent single materials. Test method C (see Annex C) can be applied to both absorbent and non-absorbent materials or combinations.

NOTE For single material, method A and B are preferred.

Table 1 — List of test methods

No	Type of material	Test method	Remarks
1	Absorbent	Static challenge test in Annex A.	Textile and leather.
2	Non-absorbent	Film contact method in Annex B.	Micropore, i.e. coated or heavy leather, synthetic/ artificial materials, EVA foaming material, PU foaming material; and compacted material, i.e. plastic or coated material.
3	Absorbent and non-absorbent	Dynamic challenge test (shake flask test) in Annex C.	Components of different materials; shaped material; materials with fixed antibacterial agent.

11 Expression of results

Antibacterial performance of footwear or footwear components shall be reported separately based on the antibacterial activity ratio.

Calculate the antibacterial activity ratio (R) in accordance with Formula (1), or R^* in accordance with Formula (2). Record the result in percentage with three significant figures.

$$R = \frac{C_t - T_t}{C_t} \times 100\ \% \tag{1}$$

where

C_t is the average number of colonies of three control samples after 24 h or the specified incubation period, expressed as CFU/ml;

T_t is the average number of colonies of three test samples after 24 h or the specified incubation period, expressed as CFU/ml.

In cases where there is no control sample available, calculate R^* by replacing C_t on Formula (1) by T_0 using Formula 2.

$$R^* = \frac{T_0 - T_t}{T_0} \times 100\ \% \tag{2}$$

where

T_0 is the average number of colonies of three samples immediately after inoculation, expressed as CFU/ml.

12 Test report

The test report shall include at least the following information:

a) reference to this standard, i.e. ISO 16187;

b) the treated test components and their positions, areas and weights;

c) test methods for different materials;

d) preparation of the test specimen, including pre-treatment methods, if applied, for different samples (i.e. sterilization method);

e) species, serial number and number of viable cells of test strains for different materials;

f) surfactant and its concentration added into the test inoculums;

g) judgement of test effectiveness;

h) antibacterial activity ratio of different materials or components;

i) any deviations from this method.

Annex A
(normative)

Static challenge test

A.1 Test procedure

A.1.1 Inoculation

Place each of the six test samples and the six control samples into separate sterilized wide mouth jars (5.7). Pipette (1,0 ± 0,1) ml of the inoculum prepared in Clause 8 to each test sample and tightly close the screw cap. The number of swatches to be used is dependent on the sample type.

If no control samples are available, inoculate wide mouth jars without samples as control to determine the test effectiveness.

A.1.2 Elution after inoculation (time zero)

Add 20 ml SCDLP medium (6.4) to each of three inoculated test and control samples (if available). Tighten the caps and shake them in an arc of approximately 30 cm for 30 s, or mix for 5 s × 5 cycles using the vortex mixer (5.9) in order to elute out bacteria into the medium.

A.1.3 Incubating

Culture the three inoculated test and control samples (if available) at (37 ± 2) °C for (24 ± 1) h.

A.1.4 Elution after incubating (time 24 h)

Proceed as in A.1.2.

A.1.5 Determination of the number of viable bacteria — Surface culture

Take 1 ml elution from A.1.2 or elution from A.1.4 with a sterile pipette and add it into a test tube with (9,0 ± 0,1) ml physiological saline (6.5) and shake it well. Dilute the elution with physiological saline (6.5) and get 10-fold serial dilutions.

Inoculate 100 μL of each dilution onto nutrient agar (NA) (6.3) two times, turn the agar upside down and incubate it for 24 h to 48 h.

After incubation, count the number of colonies in the Petri dishes containing 30 to 300 colonies. If the minimum number of colonies is less than 30, then count and record the number of colonies in these plates. If there are no colonies recovered in the plate, record the number of colonies as < 1.