INTERNATIONAL STANDARD

ISO 20645

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Textile fabrics — Determination of antibacterial activity — Agar diffusion plate test

Étoffes — Contrôle de l'activité antibactérienne — Essai de diffusion sur plaques de gélose

Reference number
ISO 20645:2004(E)

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Foreword

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ISO 20645 was prepared by the European Committee for Standardization (CEN) in collaboration with Technical Committee ISO/TC 38, *Textiles*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Throughout the text of this document, read “...this European Standard...” to mean “...this International Standard...”.

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Foreword

This document (EN ISO 20645:2004) has been prepared by Technical Committee CEN/TC 248 “Textiles and textile products”, the secretariat of which is held by BSI, in collaboration with Technical Committee ISO/TC 38 “Textiles”.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by June 2005, and conflicting national standards shall be withdrawn at the latest by June 2005.

This document includes a Bibliography.

CAUTION — This method involves the use of processes that could lead to a hazardous situation. Attention is drawn to the safety precautions in Clause 3.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

Introduction

The application of an antimicrobial finish to a textile can prevent bacterial growth and might reduce the effects of microbial pathway products, biodeterioration and microbiogenous odours.

This method determines the activity of such treatments qualitatively when different products are compared. Semi-quantitative information on the effect of treatments can be obtained when different concentrations of the same product are compared.

1 Scope

This document specifies a method for the determination of the effect of antibacterial treatments applied to woven, knitted and other flat textiles.

This method is applicable to testing hygienic finishes of hydrophilic, air-permeable materials or antibacterial products incorporated in the fibre. A minimum diffusion of the antibacterial treatment into the test agar is necessary with this procedure.

NOTE Other materials may be tested using this method, provided that it is adapted accordingly.

This method is not suitable for testing textiles treated with antibacterial treatments that react with the agar.

2 Terms and definitions

For the purposes of this document, the following term and definition applies.

antibacterial effect
inhibition of bacterial growth in favourable growing conditions

3 Safety precautions

This method requires the use of bacteria and conditions that promote bacterial growth. Since the bacteria might be pathogenic the tests should be carried out by trained personnel.

Appropriate safety precautions should be observed.

4 Principle

Specimens of the material to be tested are placed on two-layer agar plates. The lower layer consists of a culture medium free from bacteria and the upper layer is inoculated with the selected bacteria. The textiles are tested on both sides. The level of antibacterial activity is assessed by examining the extent of bacterial growth in the contact zone between the agar and the specimen and, if present, the extent of the inhibition zone around the specimen.

5 Apparatus, reagents and culture media

5.1 Apparatus

5.1.1 *Incubator* , capable of maintaining a temperature of (37 ± 1)°C.

5.1.2 *Autoclav*e, capable of operating at 121°C and 205 kPa (2,05 bar).

5.1.3 *Water bath*, capable of maintaining a temperature of (45 ± 2)°C.

5.1.4 *Shaker,* for test tubes

5.1.5 *Microscope*, 20 × magnification, lighting from beneath (lens 20x, stereomicroscope 20x).

5.1.6 *Petri dishes*, of glass or polystyrene construction and 9 cm inner diameter

5.2 Reagents and culture media

5.2.1 Reagents

Use only reagents of recognized analytical grade and distilled water or water of equivalent purity.

5.2.2 Culture media

5.2.2.1 Dry agar, available commercially with the following composition[1].

Should commercially available agars be unsuitable for the bacteria to be tested, the culture media shall be adapted or replaced accordingly. Such changes shall be mentioned in the test report

5.2.2.2 Composition of the nutrient broth for test strains:

trypton peptone	17 g
phyton peptone	3 g
sodium chloride	5 g
di-potassium hydrogen phosphate	2,5 g
dextrose	2,5 g
distilled water	1 000 ml

Preparation

Prepare the nutrient broth by heating the above solids in water until they are completely dissolved. Sterilize the broth at 121 °C (205 kPa) in the autoclave for 15 min. After sterilization, the pH of the broth shall be 7,3 ± 0,1 at 20 °C.

5.2.2.3 Composition of the medium for tests:

trypton peptone	15 g
phyton peptone	5 g
sodium chloride	5 g
agar-agar	15 g
distilled water	1 000 ml

1) Trypticase Soy Agar / Broth (BBL); Tryptic Soy Agar / Broth (Difco); CASO Agar / Broth (Merck); Trypton Soya Agar / Broth (Oxoid) are examples of suitable products available commercially This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN or ISO of these products.

6 Test bacteria

The following gram positive strain and one of the two gram negative strains shall be used.

Staphylococcus aureus gram positive ATCC[2)] 6538 or NCCB[3)] 46064

Escherichia coli gram negative ATCC 11229 or NCCB 1500

Klebsiella pneumoniae gram negative ATCC 4352 or NCCB 89160

In order to guarantee comparable, reproducible results, only strains supplied by a recognised culture collection shall be used.

NOTE Depending on the range of application and composition of the textile under test, the spectrum of test bacteria may be enlarged. Should bacteria other than those specified be used, the method of culture, the culture media and the incubation temperature may need to be adjusted. Such changes should be indicated in the report.

7 Preparation of the bacteria cultures

7.1 General

The procedure described refers to the culturing of stock and test strains of bacteria specifically for the tests. Laboratories should apply the procedure EN 12353 for the preservation of microbial strains.

7.2 Culturing with lyophilized bacteria

Suspend the lyophilized bacteria in an adequate quantity of nutrient broth.

Prepare liquid sub-cultures from the suspension and prepare an agar plate culture. Check the purity of the culture by streak plates, and confirm the identity by microscopic examination and gram stain. Effect "liquid to liquid" transfers for up to three days to avoid the opportunity for contamination. Incubate for 24 h in each case at (37 ± 1) °C.

Verify the purity of the colonies again by spreading on agars.

Should the series of three to four "liquid to liquid" transfers be interrupted by a weekend, a 16 h to 24 h old culture shall be placed in the refrigerator (3 °C to 4 °C) on Friday and reinoculated at the latest on Tuesday for a "liquid" transfer over a minimum of 24 h.

7.3 Culturing from the agar

Prepare a first liquid sub-culture from the agar and prepare an agar plate culture. The culture should be no older than 4 weeks. Check the purity of the culture by streak plates and confirm the identity by microscopic examination and gram stain. Effect "liquid to liquid" transfers for up to three days to avoid the opportunity for contamination. Incubate for 24 h in each case at (37± 1) °C.

Verify the purity of the colonies again by spreading on agar.

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2) American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209,USA,. Tel: +703.365.2700

3) The Netherlands Culture Collection of Bacteria, Utrecht Univ., Uppsalalaan 8, P.O. Box 85167, 3508 AD Utrecht, The Netherlands,Tel: +31 (30) 2122634

Should the series of three to four "liquid to liquid" transfers be interrupted by a weekend, a 16 h to 24 h old culture shall be placed in the refrigerator (3 °C to 4 °C) on Friday and reinoculated on Tuesday at the latest for a "liquid" transfer over a minimum of 24 h.

At the end of the week, destroy all the working cultures and replace with new ones prepared from stock strains cultivated on agar or taken from lyophilized bacteria.

After a maximum of 6 months testing, all new cultures shall be based on lyophilized bacteria.

8 Preparation of test specimens

8.1 General

The test specimens shall be circular, with a diameter of (25 ± 5) mm.

Tests on four specimens are necessary and shall be carried out on both sides of the specimens.

NOTE In special cases, the specimens should be prepared in accordance with Annex A.

8.2 Preparation of the test material

Unsterilized specimens only shall be used. Any deviations shall be included in the test report.

NOTE The test materials or their antibacterial treatment might be altered if the specimens are sterilized.

8.3 Material for blind tests

Wherever possible, prepare blind test specimens of identical quality and finish but without antibacterial treatment. If such material is not available, use a cotton fabric without antibacterial treatment for the bacterial growth control.

8.4 Conditioning of specimens

Store the specimens between 12 h and 24 h in sterilized petri dishes at room temperature.

9 Test procedure

9.1 Prepare, for the lower layer free from bacteria, the required agar volume. Pour (10 ± 0,1) ml into each of the sterilized petri dishes and let the agar congeal.

9.2 For the upper layer, prepare the required agar quantity and cool to (45 ± 1) °C in the water bath (5.1.3). Inoculate 150 ml agar with (1 ± 0,1) ml of bacterial working culture (1-5 x10^8 cfu/ml). Shake the vessel vigorously to distribute the bacteria evenly. Pour (5 ± 0,1) ml into each petri dish and let the agar congeal. Use the inoculated agar plates within 1 h.

9.3 Since agar plates tend to dry on the surface after casting and varying degrees of dryness from one zone to the other may lead to an irregular growth of the bacteria, not more than 50 plates, i.e. 500 ml of agar (250 ml per layer) shall be used at a time.

9.4 Press the test specimens taken from woven, knitted and other flat materials with a pair of sterilized tweezers or with a bent glass rod evenly on the nutrient medium, until the texture of the fabric is uniformly imprinted and there is good contact between specimen and agar. If necessary, place a sterilized glass ring or inox ring on the specimens to guarantee this contact.

NOTE Other materials may need special tests (see Annex A).

9.5 Incubate the plates for 18 h to 24 h at (37 ± 1) °C immediately after placing the test specimens on the agar and then check for bacterial growth. Ensure that there is contact between specimen and agar for the whole incubation period. Deviations shall be mentioned in the test report.

10 Assessment of the tests

10.1 The assessment is based on the absence or presence of bacterial growth in the contact zone between agar and specimen and on the eventual appearance of an inhibition zone around the specimens.

10.2 Calculate the width of the inhibition zone, i.e. the zone free from bacteria near the specimen's edge, using the following formula:

$$H = \frac{D - d}{2}$$

where

H is the inhibition zone in mm

D is the total diameter of specimen and inhibition zone in mm

d is the diameter of specimen in mm

10.3 After measuring the inhibition zone, remove the specimens from the agar with a pair of tweezers. Examine the contact zones under the specimens for bacterial growth with a microscope at 20 times magnification and lighting from beneath.

10.4 Evaluate the antibacterial effect of the antibacterial treatment of the test specimen using Table 1.

Table 1 — Antibacterial effect of the antibacterial treatment

Inhibition zone (mm) Mean value	Growth [a]	Description	Assessment
> 1	none	inhibition zone exceeding 1 mm, no growth [b]	good effect
1 - 0	none	inhibition zone up to 1 mm, no growth [b]	
0	none	no inhibition zone, no growth [c]	
0	slight	no inhibition zone, only some restricted colonies, growth nearly totally suppressed [d]	limit of efficacy
0	moderate	no inhibition zone, compare to the control growth reduced to half [e]	insufficient effect
0	heavy	no inhibition zone, compare to the control no growth reduction or only slightly reduced growth	

[a] The growth of bacteria in the nutrient medium under the specimen.

[b] The extent of the inhibition shall only partly be taken into account. A large inhibition zone may indicate certain reserves of active substances or a weak fixation of a product on the substrate.

[c] The absence of growth, even without inhibition zone, may be regarded as a good effect, as the formation of such an inhibition zone may have been prevented by a low diffusibility of the active substance.

[d] "As good as no growth" indicates the limits of efficacy.

[e] Reduced density of bacterial growth means either the number of colonies or the colony diameter.

11 Evaluation

The antibacterial treatment requirements of 10.4 (good effect) are fulfilled by both the gram negative and gram positive bacteria prescribed for the tests.

Each side of the specimen shall be evaluated separately.

12 Test report

The following information shall be given in the test report,

a) reference to the method,

b) description of the type of material tested,

c) pretreatment of the test specimen (e.g. washing, exposition to light, weathering),

d) size, number and preparation of the specimens,

e) storage of the specimens prior to the test,

f) test bacteria,

g) derogations from the method,

h) results of the test according to the evaluation scheme (see Clause 10),

i) date, signature and name of the testing organization.

Annex A
(informative)

Special specimens and tests

A.1 Should the textile fabrics or materials for tests not be hydrophilic and not permeable to the air or the contact between the agar surface and the specimen insufficient, the specimens will have to be modified accordingly.

A.2 Staple fibres, cut flock, high-pile fabrics should be cut into small pieces and applied in the form of a thick layer on the agar. To facilitate the operation, a glass ring should be placed first on the agar, filled with the material and then removed.

If necessary, high pile materials of varying pile length should be cut evenly and placed on a good contact surface.

Unfinished parts should be removed from the specimen before testing.

NOTE 1 Should the yarn be composed of a mixture of finished and unfinished fibres, the test will still be feasible in many cases.

NOTE 2 Coated specimens might inhibit the growth of the test bacteria through lack of air. In such cases, the specimens should be cut in small strips and grouped on the agar, leaving small spaces between each strip.

Annex B
(informative)

European Suppliers of ATCC

Reference Materials at LGC
Queens Road
Teddington
Middlesex TW11 0LY
UK

Tel: +44(0)20 8943 7565
Fax: +44(0)20 8943 7554
Email: rmsales@lgc.co.uk
(UK, Ireland and all other countries not listed)

LGC Nordic AB
Brinellgatan 4
P O Box 1737
SE-501 17 Borås
Sweden

Tel: +46(0)33 165315/25
Fax: +46(0)33 165310
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Peru, 104 Nave 3
08018 Barcelona
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Tel: +34 (0)93 266 2731
Fax: +34 (0)93 307 3612
(Spain, Portugal)

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[1] EN 12353, *Chemical disinfectants and antiseptics — Preservation of microbial strains used for the determination of bactericidal and fungicidal activity.*

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