INTERNATIONAL STANDARD

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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
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Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

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of an identical text or by endorsement, at the latest by June 2005, and conflicting national standards shall be withdrawn at the latest by June 2005.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 *Autoclave*, 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