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Textiles — Determination of antibacterial activity of textile products

Textiles — Détermination de l'activité antibactérienne des produits textiles

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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 38, *Textiles*.

This second edition cancels and replaces the first edition (ISO 20743:2007), which has been technically revised.

Introduction

Speciality products of antibacterial-treated textiles have been introduced in the market and are expanding year by year in various applications. Those textiles certainly meet the consumer's requirement to seek prevention and protection from the negative effects caused by bacteria and to secure the quality of life.

In this situation, the test methods to determine the antibacterial activity for antibacterial textile products were expected to be established in order to address the substantial need for an International Standard.

The test method for antibacterial activity was developed as ISO 20645 which was a qualitative test method. There are no testing standards for the quantitative method which gives more objective information for the antibacterial activity of the textile products.

There are several practical test methods to determine the quantitative antibacterial activity specified in this International Standard. The test methods are composed of 2 major steps, such as inoculation of bacteria and quantitative measurement of bacteria.

The methods for the inoculation of bacteria specified in this International Standard are the absorption method, transfer method and printing method.

The methods of the quantitative measurement of bacteria specified in this International Standard are colony plate count method and ATP luminescence methods.

Although there are 6 ways for the combination of inoculation methods and quantitative measurements to execute this test, the choice of the ways depends on the user's availability and consensus between the concerned parties.

Textiles — Determination of antibacterial activity of textile products

1 Scope

This International Standard specifies quantitative test methods to determine the antibacterial activity of all antibacterial textile products including nonwovens.

This International Standard is applicable to all textile products, including cloth, wadding, thread and material for clothing, bedclothes, home furnishings and miscellaneous goods, regardless of the type of antibacterial agent used (organic, inorganic, natural or man-made) or the method of application (built-in, after-treatment or grafting).

Based on the intended application and on the environment in which the textile product is to be used and also on the surface properties of the textile properties, the user can select the most suitable of the following three inoculation methods on determination of antibacterial activity:

a) absorption method (an evaluation method in which the test bacterial suspension is inoculated directly onto specimens);

b) transfer method (an evaluation method in which test bacteria are placed on an agar plate and transferred onto specimens);

c) printing method (an evaluation method in which test bacteria are placed on a filter and printed onto specimens).

The colony plate count method and the ATP (ATP = Adenosine Tri-phosphate) luminescence method are also specified for measuring the enumeration of bacteria.

2 Normative references

The following referenced documents are indispensable for the application 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 6330, *Textiles — Domestic washing and drying procedures for textile testing*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
control fabric
fabric used to validate the growth condition of test bacteria and validate the test

Note 1 to entry: The same fabric as the fabric to be tested but without antibacterial treatment or a 100 % cotton fabric without fluorescent brighteners or other finish can be used.

3.2
antibacterial agent
product designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.3
antibacterial finish
treatment designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.4
antibacterial activity
activity of an antibacterial finish used to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.5
plate count method
method in which the number of bacteria present after incubation is calculated by counting the number of colonies according to a ten-time dilution method

Note 1 to entry: The results are expressed in "CFU (Colony Forming Unit)".

3.6
luminescence method
method in which the amount of ATP contained in bacterial cells is measured

Note 1 to entry: The results are expressed in "moles of ATP".

3.7
neutralizer
chemical agents used to inactivate, neutralize or quench the antibacterial properties of antibacterial agents

4 Safety precaution

The test methods specified in this International Standard require the use of bacteria.

These tests should be carried out by persons with training and experience in the use of microbiological techniques.

Appropriate safety precautions should be observed with due consideration given to country-specific regulations.

5 Apparatus

Usual laboratory apparatus and, in particular, the following.

5.1 Spectrophotometer, capable of measuring at a 620 nm to 660 nm wavelength, or McFarland's nephelometer.

5.2 Incubator, capable of maintaining a constant temperature of 37 °C ± 2 °C.

5.3 Water baths, one capable of maintaining a constant temperature of 46 °C ± 2 °C and another capable of maintaining a temperature of 70 °C to 90 °C.

5.4 Mixer, producing a vortex shaking action.

5.5 Stomacher, capable of speeds of 6 blows per second to 8 blows per second, with the corresponding disposable containers.

5.6 Clean bench, for microbial test.

5.7 Washing machine, in accordance with the specifications of ISO 6330.

5.8 Humidity chamber, tropical chamber or other container capable of maintaining a high-humidity more than 70 %RH atmospheric condition.

5.9 Luminescence photometer, capable of measuring ATP of 10^{-12} mol/l to 10^{-7} mol/l at 300 nm to 650 nm with a luminescence-measuring reagent.

5.10 Printing apparatus, capable of applying a 4 N load to a test specimen and rotating the specimen 180° in one direction for a period of 3,0 s.

5.11 Refrigerator, capable of maintaining a temperature of between 2 °C and 8 °C.

5.12 Freezers, one adjustable to a temperature below −70 °C and another to a temperature below −20 °C.

5.13 Balance, which can be read to the nearest 0,01 g.

5.14 Filtering apparatus, consisting of an upper container equipped with a membrane filter and a lower container equipped with a suction opening.

5.15 Pipette, having the most suitable volume for each use, with a tip made of glass or plastic, and with a tolerance of 0,5 % or less.

5.16 Vials, 30 ml glass bottles, with screw openings, polytetrafluoroethylene or silicone packing and caps made of polypropylene, polycarbonate or another suitable material.

5.17 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.

5.18 Glass rod, with a diameter of approximately 18 mm.

5.19 Anti-bumping granules (glass beads), with a diameter of 3 mm to 4 mm.

5.20 Erlenmeyer flask, of capacity 100 ml.

5.21 Cutting template, made of a sterilizable material (stainless steel or glass) with a diameter of 3,8 cm ± 0,1 cm.

5.22 Disposable plastic bags, sterile bags suitable for containing food products, to be used for one of the shaking methods of the specimens.

5.23 Tweezers, made of a material which can be sterilized.

5.24 Stainless-steel cylinder, with a mass of 200 g ± 10 g and a diameter of 3,5 cm ± 0,1 cm.

5.25 Metal wire basket, for autoclaving.

5.26 Aluminium foil.

5.27 Reciprocal incubation shaker.

5.28 Autoclave, capable of sterilizing at 121 °C ± 2 °C and 103 kPa ± 5 kPa.

6 Reagents and culture media

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 manufacturer's instructions for the preparation of these products should be strictly followed.

6.1 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 bacteria inhibitory substances.

6.2 Tryptone soya broth (TSB)

Tryptone, pancreatic digest of casein	17 g
Soya peptone, papain digest of soya	3 g
Sodium chloride (NaCl)	5 g
Glucose	2,5 g
Dipotassium hydrogen phosphate	2,5 g
Water	1 000 ml
Mix well and adjust pH,	7,2 ± 0,2
then sterilize by autoclave (5.28).	

6.3 Tryptone soya agar (TSA)

Tryptone, pancreatic digest of casein	15 g
Soya peptone, papain digest of soya	5 g
Sodium chloride (NaCl)	5 g
Agar	15 g
Water	1 000 ml
Mix well and adjust pH,	7,2 ± 0,2
then sterilize by autoclave (5.28).	

6.4 Agar for transfer

Tryptone, pancreatic digest of casein	0,75 g
Soya peptone, papain digest of soya	0,25 g
Sodium chloride (NaCl)	5 g
Agar	15 g
Water	1 000 ml
Mix well and adjust pH,	7,2 ± 0,2
then sterilize by autoclave (5.28).	

6.5 Nutrient broth (NB)

Beef extract	3 g
Peptone	5 g
Water	1 000 ml
Mix well and adjust pH, then sterilize by autoclave (5.28).	
pH	6,9 ± 0,2

6.6 Peptone salt solution

Peptone, pancreatic digest of casein	1 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml
Mix well and adjust pH,	6,9 ± 0,2
then sterilize by autoclave (5.28).	

6.7 Physiological saline

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

Mix well, then sterilize by autoclave (5.28).

6.8 SCDLP medium

Peptone, digest of casein	17 g
Peptone, digest of soybean	3 g
Sodium chloride (NaCl)	5 g
Dipotassium hydrogenphosphate	2,5 g
Glucose	2,5 g
Lecithin	1 g
Polysorbate 80	7 g
Water	1 000 ml
Mix well and adjust pH, then sterilize by autoclave (5.28).	7,2 ± 0,2

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.

6.9 Dilution buffer for shake-out bacterial suspension

This buffer solution consists of 0,005 mol/l sodium dihydrogenphosphate containing 0,037 % sucrose.

pH	7,2 ± 0,2

6.10 Neutralizing solution

The composition of the standard neutralizing solution shall be as follows.

Polysorbate 80	30 g
Egg-yolk lecithin	3 g
Histidine hydrochloride	1 g
Meat or casein peptone	1 g
Sodium chloride (NaCl)	4,3 g
Monopotassium phosphate	3,6 g
Disodium phosphate dihydrate	7,2 g
Water	1 000 ml
Mix well and sterilize by autoclave (5.28).	

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.

6.11 Enumeration agar (EA)

Dehydrated yeast extract	2,5 g
Casein tryptone	5,0 g
Glucose	1,0 g
Agar	12 g to 18 g (depending on the gel strength of the product)
Water	1 000 ml
Mix well and adjust pH,	7,2 ± 0,2

then sterilize by autoclave (5.28).

6.12 Agar for printing

Agar	20 g
Water	1 000 ml

Mix well and sterilize by autoclave (5.28).

6.13 Cryoprotective solution for bacterial species

For freezing, a cryoprotective solution containing 150 g/l of glycerol or 100 g/l of dimethylsulfoxide shall be used and prepared as follows.

TSB (6.2) or NB (6.5): 1 000 ml

Add,

Glycerol: 150 g

or

dimethylsulfoxide: 100 g

Mix well and sterilize by autoclave (5.28).

For solutions containing glycerol, sterilize the mixed solution by autoclave (5.28). For solutions containing dimethylsulfoxide, sterilize the mixed solution by using 0,22 µm membrane filter.

NOTE Any commercially available product may be used as long as it is a cryoprotective solution or preserving system that contains glycerol or dimethylsulfoxide and allows preservation of the strains in the same manner as the specified solutions.

6.14 Stock solution of ATP standard reagent

The concentration of ATP standard reagent is 1 X 10^{-4} mol/l which is obtained by the following mixing.

Adenosine-disodium 5'-triphosphate trihydrate	60,5 mg
Water	1 000 ml (final volume)

After preparation, the solution shall be placed in a tightly sealed container and cryopreserved at a temperature of –20 °C or lower. The solution shall be used no later than 6 months from the date of preparation.

NOTE The suitable amount of adenosine-disodium 5'-triphosphate trihydrate may be calculated from the ATP content of each commercial product.