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Textiles — Determination of antibacterial activity of antibacterial finished products

Textiles — Détermination de l'activité antibactérienne des produits finis antibactériens

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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

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 20743 was prepared by Technical Committee ISO/TC 38, *Textiles*.

Introduction

These test methods were established in order to address the substantial need for an International Standard to determine antibacterial activity for antibacterial finished textile products.

Textiles — Determination of antibacterial activity of antibacterial finished products

1 Scope

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

This International Standard is applicable to all textile products, including cloth, wadding, thread and material for clothing, 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, the user can select the most suitable of the following three methods on determination of antibacterial activity:

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

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

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

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
textile
fabric
general term employed for designating textile surfaces, woven fabrics, knitted fabrics, etc., formed by the interlocking of textile materials having a certain cohesion and which are generally intended for clothing or furniture applications

NOTE Often includes certain types of nonwovens.

3.2
control fabric
fabric used to validate the growth condition of test bacteria

NOTE The same fabric as the fabric to be tested but without antibacterial treatment. If this is not possible, then 100 % cotton fabric without fluorescent brighteners or other finish.

3.3
antibacterial agent
product designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.4
antibacterial finish
treatment designed to prevent or mitigate the growth of bacteria, to reduce the number of bacteria or to kill bacteria

3.5
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

3.6
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 The results are expressed in “CFU (Colony Forming Unit)”.

3.7
luminescence method
method in which the amount of ATP contained in bacterial cells is measured

NOTE The results are expressed in “mol of ATP”.

3.8
neutralizer
chemical agents used to inactivate, neutralize, or quench the antibacterial properties of antibacterial agents

4 Safety precaution

Test methods specified herein 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/s to 8 blows/s, 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 atmospheric condition.

5.9 Luminescence photometer, capable of measuring ATP of 10^{-13} 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 piece and rotating the piece 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 other suitable material.

5.17 Petri dishes, that have been sterilized, made of glass or plastic, in diameter sizes of 90 mm to 100 mm and 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, 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, suitable for containing food products, to be used for storage of samples.

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.

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).

This solution shall be used for the resuscitation of the freeze-dried bacterial strains.

Tryptone, pancreatic digest of casein	15 g
Soya peptone, papain digest of soya	5 g
Sodium chloride (NaCl)	5 g
Water	1 000 ml (final volume)
pH after sterilization	7,2 ± 0,2

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 (final volume)
pH after sterilization	7,2 ± 0,2

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 (final volume)
pH after sterilization	7,2 ± 0,2

6.5 Nutrient broth (NB).

Beef extract	3 g
Peptone	5 g
Water	1 000 ml (final volume)
pH after sterilization	6,9 ± 0,2

6.6 Peptone-salt solution.

Tryptone, pancreatic digest of casein	1 g
Sodium chloride (NaCl)	8,5 g
Water	1 000 ml (final volume)
pH after sterilization	6,9 ± 0,2

6.7 Physiological saline.

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

6.8 SCDLP medium.

Peptone, digest of casein	17 g
Peptone, digest of soybean	3 g
Sodium chloride (NaCl)	5 g
Potassium dihydrogenphosphate	2,5 g
Glucose	2,5 g
Lecithin	1 g
Polysorbate 80	7 g
Water	1 000 ml (final volume)
pH after sterilization	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 (final volume)

When sufficient neutralizing power cannot be achieved, 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 (final volume)
pH after sterilization	7,2 ± 0,2

6.12 Agar for printing.

Agar	20 g
Water	1 000 ml (final volume)

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.

For solutions containing glycerol, prepare the nutritive solution and add 150 g of glycerol per litre prior to sterilizing.

For solutions containing dimethylsulfoxide, sterilize the dimethylsulfoxide by means of a filtering system equipped with a 0,22 µm membrane filter. Prepare the nutritive solution and, after sterilization, add 100 g of dimethylsulfoxide per litre.