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ISO 22196

Second edition
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Measurement of antibacterial activity on plastics and other non-porous surfaces

Mesurage de l'action antibactérienne sur les surfaces en plastique et autres surfaces non poreuses

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ISO copyright office
Case postale 56 • CH-1211 Geneva 20
Tel. + 41 22 749 01 11
Fax + 41 22 749 09 47
E-mail copyright@iso.org
Web www.iso.org

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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 22196 was prepared by Technical Committee ISO/TC 61, *Plastics*, Subcommittee SC 6, *Ageing, chemical and environmental resistance*.

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This second edition cancels and replaces the first edition (ISO 22196:2007). The main change is the extension of the scope of the standard to include non-porous surfaces other than plastics (for details, see the Introduction).

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Introduction

Antibacterial materials and products have been widely and rapidly accepted by general consumers as fulfilling a relatively new function, which is distinguishable from the more traditional function of material protection.

Antibacterial products created by incorporating an antibacterial agent (biocide) can suppress the growth of bacteria on the surfaces of products when conditions exist where growth can occur. They can keep surfaces clean and sanitary and can also have an advantage in minimizing the impact on the environment by minimizing diffusion of the agent. This technology is significant for the quality of life, not only in developed countries but also in developing countries.

Antibacterial products have been widely used in plastics, coating materials, ceramics, natural and artificial leather, stainless steel, rubber, etc. The products involved cover a variety of categories, such as electrical appliances, personal items, household goods, nursing-care articles, pet accessories and aircraft-interior fittings.

The scope of the first edition of ISO 22196 was limited to plastics surfaces. In this second edition, the scope has been extended to include surfaces made of other non-porous materials, thus making the second edition applicable to products of the kinds listed above. The test method, which is based on JIS Z 2801[11], has remained unchanged.

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Measurement of antibacterial activity on plastics and other non-porous surfaces

1 Scope

WARNING — Handling and manipulation of microorganisms which are potentially hazardous requires a high degree of technical competence and may be subject to current national legislation and regulations. Only personnel trained in microbiological techniques should carry out such tests. Appropriate practices for disinfection, sterilization and personal hygiene must be strictly observed.

This International Standard specifies a method of evaluating the antibacterial activity of antibacterial-treated plastics, and other non-porous, surfaces of products (including intermediate products).

It is not intended to be used to evaluate the effects and propagation of bacteria on non-porous surfaces without antibacterial treatments. ISO 846[1] describes tests to evaluate the effects and propagation of bacteria on non-porous surfaces, which are different from those covered by this International Standard (see e.g. ISO 846:1997, method C).

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Secondary effects of antibacterial treatments, such as the prevention of biodeterioration and odour, are not covered by this International Standard, which is not intended to be used or referenced as a method to document or claim biodegradability of, for instance, plastics materials. In the case of plastics, biodegradation is covered in ISO 14851[2], ISO 14852[3] and ISO 14855[4] and related standards.

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Building materials are excluded, except where they are used in the same manner as treated articles.

Antibacterial-treated textile products are excluded, even if the surfaces are coated or laminated (such products are covered by ISO 20743[5]).

Photocatalytic materials and products are excluded (such materials and products are covered by ISO 27447[6]).

The results obtained should include a reference to this International Standard and the conditions used. Results obtained with this International Standard indicate antibacterial activity under the specified experimental conditions used, and do not reflect activity under other circumstances where a variety of factors, such as temperature, humidity, different bacterial species, nutrient conditions, etc., have to be considered. A minimum diffusion of the antibacterial agents/chemicals into the test inoculum is necessary with this procedure.

It is recommended that workers consult ISO 7218.

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 7218, *Microbiology of food and animal feeding stuffs — General requirements and guidance for microbiological examinations*

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3 Terms and definitions

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

3.1
antibacterial
term describing a state where growth of bacteria on the surfaces of products is suppressed or describing the effect of an agent which suppresses the growth of bacteria on the surfaces of products

3.2
antibacterial agent
agent that inhibits the growth of bacteria on the surfaces of products, used either as a surface treatment or as a compounded ingredient

3.3
antibacterial activity
difference in the logarithm of the viable cell counts found on an antibacterial-treated product and an untreated product after inoculation with and incubation of bacteria

3.4
antibacterial effectiveness
ability of an antibacterial agent to inhibit the growth of bacteria on the surface of materials treated with an antibacterial agent, as determined by the value of the antibacterial activity

4 Materials

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4.1 Bacteria to be used for the tests

Both of the following species of bacteria shall be used:

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a) *Staphylococcus aureus*;

b) *Escherichia coli*.

The bacterial strains to be used are shown in Table 1. If bacterial strains obtained from culture collections other than those shown in Table 1 are used, they shall be obtained from a member agency of the World Federation for Culture Collections (WFCC) or of the Japan Society for Culture Collections (JSCC) and shall be the same strains as those shown in Table 1. Prepare stock cultures of these species in accordance with the supplier's directions.

Table 1 — Bacterial strains to be used

Name	Strain
Staphylococcus aureus	ATCC 6538P CIP 53.156 DSM 346 NBRC 12732 NCIB 8625
Escherichia coli	ATCC 8739 CIP 53.126 DSM 1576 NBRC 3972 NCIB 8545

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If required, other species can also be used, in which case the species and the reason for their use shall be included in the test report.

4.2 Reagents, culture media and solutions

Water shall be distilled or deionized and have a conductivity of < 1 µS/cm.

All reagents shall be of analytical grade and/or of a grade appropriate for microbiological purposes.

4.2.1 Nonionic surfactant

Polyoxyethylene sorbitan monooleate shall be used.

4.2.2 Biological materials

The following biological materials are required:

— lecithin;

— D-glucose;

— yeast extract;

— meat extract (see Annex A);

— peptone (see Annex A);

— casein peptone;

— soybean peptone;

— tryptone.

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4.2.3 Culture medium

4.2.3.1 General

The culture medium specified below shall be used. The medium may be obtained from commercial suppliers, in which case it shall be prepared for use in accordance with the manufacturer's instructions.

The quantity of the culture medium can be changed provided the same composition is retained.

4.2.3.2 Suspension medium — 1/500 nutrient broth (1/500 NB)

Prepare nutrient broth by dissolving 3,0 g of meat extract, 10,0 g of peptone and 5,0 g of sodium chloride in 1 000 ml of distilled or deionized water. Dilute the nutrient broth with distilled or deionized water to a 500-fold volume and adjust the pH to a value between 6,8 and 7,2 with sodium hydroxide or hydrochloric acid. Sterilize by autoclaving (see 6.2). If it is not used immediately after preparation, store it at 5 °C to 10 °C. A 1/500 NB that has been kept for one week or longer after preparation shall not be used.

4.2.3.3 Nutrient agar

Prepare nutrient agar by dissolving 5,0 g of meat extract, 10,0 g of peptone, 5,0 g of sodium chloride and 15,0 g of agar powder in 1 000 ml of distilled or deionized water. Heat, with stirring, on a hotplate or in a boiling-water bath until the agar has dissolved. Adjust the pH to a value between 7,0 and 7,2 (at 25 °C) with sodium hydroxide or hydrochloric acid. Sterilize by autoclaving (see 6.2). If it is not used immediately after preparation, store it at 5 °C to 10 °C. Nutrient agar that has been kept for one month or longer after preparation shall not be used.

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4.2.3.4 Plate count agar

Prepare plate count agar by dissolving 2,5 g of yeast extract, 5,0 g of tryptone, 1,0 g of glucose and 15,0 g of agar powder in 1 000 ml of distilled or deionized water. Heat, with stirring, on a hotplate or in a boiling-water bath until the agar has dissolved. Adjust the pH to a value between 7,0 and 7,2 (at 25 °C) with sodium hydroxide or hydrochloric acid. Sterilize by autoclaving (see 6.2). If it is not used immediately after preparation, store it at 5 °C to 10 °C. Plate count agar that has been kept for one month or longer after preparation shall not be used.

4.2.3.5 Slant culture medium

Warm 6 ml to 10 ml of nutrient agar and pour into a screw-capped test tube. Sterilize by autoclaving (see 6.2). After sterilization, place the test tube at an angle of about 15° to the horizontal and allow the contents to solidify. If it is not used immediately after preparation, store it at 5 °C to 10 °C. Slant culture medium kept for one month or longer after preparation shall not be used.

4.2.3.6 Soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate (SCDLP broth)

Prepare SCDLP broth by dissolving 17,0 g of casein peptone, 3,0 g of soybean peptone, 5,0 g of sodium chloride, 2,5 g of disodium hydrogen phosphate, 2,5 g of glucose and 1,0 g of lecithin in 1 000 ml of distilled or deionized water. Mix thoroughly and add 7,0 g of nonionic surfactant. Adjust the pH to a value between 6,8 and 7,2 (at 25 °C) with sodium hydroxide or hydrochloric acid. Sterilize by autoclaving (see 6.2). If it is not used immediately after preparation, store it at 5 °C to 10 °C. SCDLP broth kept for one month or longer after preparation shall not be used.

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NOTE SCDLP is the default neutralizer in the majority of circumstances. Information about selection and evaluation of alternative antibacterial neutralizing agents can be found in ASTM E1054[7] and EN 1040[8].

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4.2.3.7 Phosphate buffer solution

Prepare phosphate buffer solution by placing 34,0 g of potassium dihydrogen phosphate in a 1 000 ml volumetric flask. Add 500 ml of distilled or deionized water and mix to dissolve. Adjust the pH to a value between 6,8 and 7,2 (at 25 °C) with sodium hydroxide. Add distilled or deionized water to make up to 1 000 ml. Sterilize by autoclaving (see 6.2). Phosphate buffer solution kept for one month or longer after preparation shall not be used.

4.2.3.8 Phosphate-buffered physiological saline

Prepare physiological saline by placing 8,5 g of sodium chloride in 1 000 ml of distilled or deionized water and mixing to dissolve. Dilute the phosphate buffer solution prepared in 4.2.3.7 with the physiological saline to an 800-fold volume. Sterilize the phosphate-buffered physiological saline solution by autoclaving (see 6.2). If this solution is not used immediately after preparation, store it at 5 °C to 10 °C. Phosphate-buffered physiological saline kept for one month or longer after preparation shall not be used.

5 Apparatus

Unless otherwise specified, use the following apparatus and materials:

5.1 Dry-heat sterilizer, capable of maintaining the temperature at a value between 160 °C and 180 °C within ±2 °C of the set point at equilibrium conditions.

5.2 Autoclave, capable of maintaining a temperature of (121 ± 2) °C and a pressure of (103 ± 5) kPa.

5.3 Hotplate with stirrer, or **hot-water bath**.

5.4 pH-meter, capable of measuring to ±0,2 units.

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5.5 **Balance**, capable of weighing to ±0,01 g.

5.6 **Pipetters**, sterile, with 1 000 µl tips.

5.7 **Incubator**, capable of maintaining the temperature within ±1 °C of the set point at equilibrium conditions.

5.8 **Vortex mixer**, if required (see 7.6.1).

5.9 **Sonicator**, if required (see 7.6.1).

5.10 **Inoculating loops**, 4 mm in ring diameter, sterile.

5.11 **Cover film**, that does not affect bacterial growth or absorb water, made of polyethylene, polypropylene or polyester [poly(ethylene terephthalate)]. Film that is 0,05 mm to 0,10 mm thick is recommended.

NOTE Films cut from Stomacher bags are also suitable.

5.12 **Screw-capped test tubes**.

5.13 **Petri dishes**, sterile, 90 mm to 100 mm in diameter.

5.14 **Gauze** or **absorbent cotton**.

5.15 1 000 ml **volumetric flask**.

5.16 **Stoppered Erlenmeyer flasks** or **media bottles**, as required for preparation of media.

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6 Sterilization of apparatus and storage of stock cultures

6.1 Dry-heat sterilization

Place objects to be sterilized in a dry-heat sterilizer, using the following minimum times for the given temperature:

Temperature	Minimum sterilization time
180 °C	30 minutes
170 °C	60 minutes
160 °C	120 minutes

6.2 High-pressure steam sterilization

Put the objects to be sterilized in an autoclave and maintain at (121 ± 2) °C for at least 15 min.

6.3 Preparation of glassware

Wash well with alkali or neutral detergent, then rinse well with distilled or deionized water. Sterilize using dry heat or an autoclave prior to use.

6.4 Maintenance of stock cultures

Stock cultures shall be stored at 5 °C to 10 °C on an appropriate medium and transferred monthly. After five transfers or if more than one month has passed between transfers, the stock culture shall be discarded and replaced with a fresh culture obtained from the institute or culture collection concerned.

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