INTERNATIONAL STANDARD

ISO 22196

First edition
2007-10-15

Plastics — Measurement of antibacterial activity on plastics surfaces

Plastiques — Mesurage de l'action antibactérienne sur les surfaces en plastique

Reference number
ISO 22196:2007(E)

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Published in Switzerland

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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*.

Plastics — Measurement of antibacterial activity on plastics 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 plastic products (including intermediate products).

NOTE It may also be suitable for other non-porous materials.

It is not intended to be used to evaluate the effects and propagation of bacteria on plastics without antibacterial treatments. ISO 846 [6] describes tests to evaluate the effects and propagation of bacteria on plastics, which are different from those covered by this International Standard. Those who are interested are referred to ISO 846:1997, method C.

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 plastics. For biodegradation, refer to ISO 14851, ISO 14852 and ISO 14855 (see the Bibliography) and related standards.

This International Standard does not concern plastic building materials, such as PVC or composites, unless they act in the same way as treated articles.

Any results obtained with this International Standard should always refer to this standard and the conditions used. Results obtained with this International Standard indicate antibacterial activity under the specified experimental conditions used herein, 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*

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 by the use of an antibacterial surface treatment or a compounded agent

3.3
antibacterial activity
difference in the logarithm of the viable cell count 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 a plastic treated with the agent, as determined by the value of the antibacterial activity

4 Materials

4.1 Bacteria to be used for the tests

Both of the following species of bacteria shall be used:

a) *Staphylococcus aureus*;

b) *Escherichia coli*.

In addition to the above two species of bacteria, other species can be used, if required. If other species are used, the species and the reason for their use shall be included in the test report.

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

4.2 Reagents, culture media and solutions

Any water used 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

Use polyoxyethylene sorbitan monooleate.

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.

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. If so, it shall be prepared for use in accordance with the manufacturer's instructions.

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, preserve it at 5 °C to 10 °C. Never use a 1/500 NB that has been kept for one week or longer after preparation.

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, then preserve it at 5 °C to 10 °C. Never use a nutrient agar that has been kept for one month or longer after preparation.

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, preserve it at 5 °C to 10 °C. Never use a plate count agar that has been kept for one month or longer after preparation.

4.2.3.5 Slant culture medium

Pour 6 ml to 10 ml of nutrient agar, which has been warmed to dissolve, into a screw-capped test tube. Sterilize by autoclaving (see 6.2). After sterilization, place the test tube at a slant angle of about 15° to the horizontal and allow the contents to solidify. If it is not used immediately after preparation, preserve it at 5 °C to 10 °C. Never use a slant culture medium kept for one month or longer after preparation.

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, preserve it at 5 °C to 10 °C. Never use an SCDLP broth kept for one month or longer after preparation.

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 E 1054 [4] and EN 1040 [5].

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). Never use a phosphate buffer solution kept for one month or longer after preparation.

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, preserve it at 5 °C to 10 °C. Never use a phosphate-buffered physiological saline kept for one month or longer after preparation.

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.

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.

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.

7 Procedure

7.1 Pre-culture of bacteria

Using a sterile inoculating loop, transfer bacteria from the stock culture to the slant culture medium (4.2.3.5) and incubate at (35 ± 1) °C for 16 h to 24 h. From this culture, use a sterile inoculating loop to transfer bacteria onto fresh slant culture medium and incubate at (35 ± 1) °C for 16 h to 20 h.

7.2 Preparation of test specimens

Testing shall be performed on at least three specimens from each treated test material. At least six specimens of the untreated material are required. Half of the untreated test specimens are used to measure viable cells immediately after inoculation and half are used to measure viable cells after incubation for 24 h.

NOTE Use of more than three replicate specimens of the treated test material may help reduce variability, especially for materials that show smaller antimicrobial effects.

When testing a series of antibacterial treatments for a single polymer, each antibacterial treatment may be compared to a single set of untreated specimens if all tests are conducted at the same time using the same test inoculum.

Prepare flat (50 ± 2) mm × (50 ± 2) mm specimens of the treated and untreated test materials. Specimens should be no more than 10 mm in thickness. If it is difficult or impossible to cut the product into a square of this size, then test specimens of other sizes and shapes may be used, as long as they can be covered with a film of surface area between 400 mm^2 and 1 600 mm^2. It is preferable to prepare test specimens from the final product itself. However, if the shape of the product prevents this, then the test specimens may be prepared in a format suitable for testing using the same raw materials and processing methods as are normally used for the product. If the test specimen differs from the 50 mm × 50 mm square dimensions, the actual dimensions used shall be stated in the test report.

When preparing specimens, take care to avoid contamination with microorganisms or extraneous organic debris. Similarly, do not allow specimens to come into contact with each other. If metal apparatus is used to avoid cross-contamination, it is necessary to ensure that the metal does not have any antibacterial effect. If necessary, test specimens can be cleaned/disinfected/sterilized prior to testing (e.g. by wiping with 70 % ethanol in water).

Cleaning of the test specimen can cause softening, dissolution of the surface coating or elution of components so should be avoided. If cleaning is required due to gross contamination, the cleaning method shall be stated in the test report.

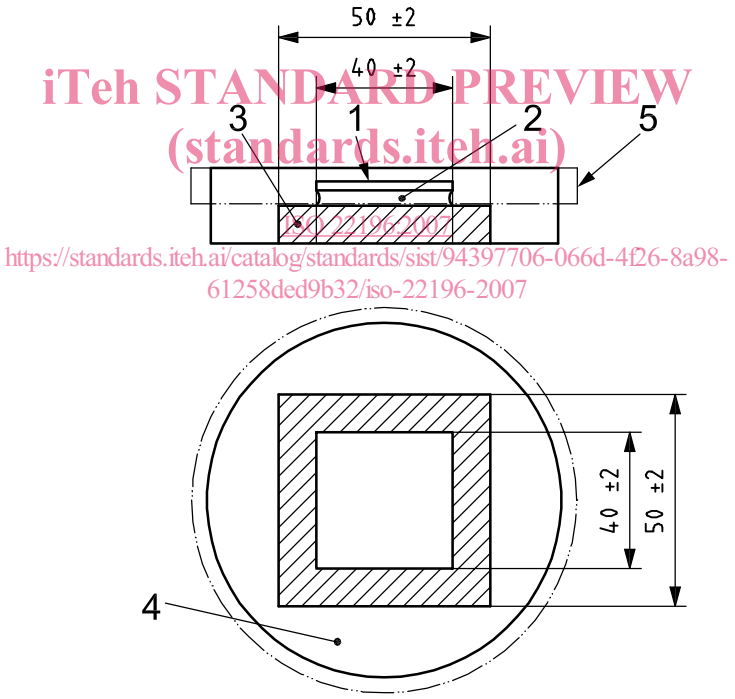
7.3 Preparation of test inoculum

Using a sterile inoculating loop, transfer one loop of the test bacteria pre-incubated as specified in 7.1 into a small amount of 1/500 NB prepared in accordance with 4.2.3.2. Make sure that the test bacteria are evenly dispersed, and estimate the number of bacteria using direct microscopic observation and a counting chamber or another appropriate method (e.g. spectrophotometrically). Dilute this suspension with 1/500 NB, as appropriate for the estimated bacterial concentration, to obtain a bacterial concentration that is between $2{,}5 \times 10^5$ cells/ml and 10×10^5 cells/ml, with a target concentration of 6×10^5 cells/ml. Use this solution as the test inoculum. If the test inoculum is not used immediately, then chill it on ice (0 °C) and use it within 2 h of preparation.

7.4 Inoculation of test specimens

The surface to be tested is the exposed outer surface of the product. Do not test cross-sections of the product. Place each test specimen prepared in 7.2 into a separate sterile Petri dish with the test surface uppermost. Pipette 0,4 ml of the test inoculum prepared in 7.3 onto the test surface. Cover the test inoculum with a piece of film (5.11) that measures 40 mm × 40 mm and gently press down on the film so that the test inoculum spreads to the edges. Make sure that the test inoculum does not leak beyond the edges of the film. After the specimen has been inoculated and the cover film applied, replace the lid of the Petri dish (see Figure 1).

Dimensions in millimetres



Key

1 cover film
2 test inoculum (0,4 ml)
3 test specimen
4 Petri dish
5 lid of Petri dish

Figure 1 — Inoculation of test specimen and placement of cover film