
**Flexible cellular polymeric
materials — Determination of
antibacterial effectiveness**

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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.

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 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

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Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

Products with a label or marking tag of antibacterial treatment, such as kitchen sponge cleaners, mattresses, pillows and sofas, are available in markets worldwide. However, there is no common standard to evaluate the effectiveness of the antibacterial treatment. The material used for these products is usually a flexible cellular polymeric foam treated with antibacterial agents available in the markets. Because of the porosity of the material, efficient contact between a testing bacterial suspension and the material is critical in an evaluation of the effectiveness of antibacterial treatment. A specific procedure has been developed and adopted for this test method so that the test bacteria can efficiently make contact with the open cell surface of the flexible cellular polymeric test specimens. This document will help consumers to know whether these products have the appropriate quality of antibacterial effectiveness.

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Flexible cellular polymeric materials — Determination of antibacterial effectiveness

WARNING — Persons using this document should be familiar with microbiology. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices and to determine the applicability of any national regulatory conditions.

1 Scope

This document specifies a method of determining the antibacterial effectiveness of open-cell flexible cellular polymeric antibacterial treated materials, including their intermediate and final products.

This document is suitable for flexible cellular polymeric materials because the test procedure enables the test inoculum to efficiently contact with the surface of open cell in the flexible cellular polymeric materials.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 1923, *Cellular plastics and rubbers — Determination of linear dimensions*
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3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

surface of flexible cellular polymeric material

surface that is not only the outer peripheral surface but also the true surface of open-cell structure of flexible cellular polymeric material

3.2

antibacterial

condition suppressing the growth of bacteria on the surface of flexible cellular polymeric material

3.3

antibacterial agent

agent that inhibits the growth of bacteria on flexible cellular polymeric materials

3.4

antibacterial treatment

treatment with antibacterial agents

3.5

antibacterial treated material

flexible cellular polymeric material that is treated with antibacterial agents

3.6 antibacterial activity

difference in the logarithm of the viable cell counts found between an antibacterial-treated material and an untreated material after inoculation with bacteria and incubation

3.7 antibacterial effectiveness

ability of an antibacterial agent to inhibit the growth of bacteria on material treated with an antibacterial agent, as determined by the value of the antibacterial activity

4 Judgement criteria of antibacterial effectiveness

When the antibacterial activity value is not less than 2,0, the antibacterial effectiveness of antibacterial treated material is judged to be significant. An antibacterial activity value of more than 2,0 may be agreed between all interested parties.

5 Test methods

5.1 Bacteria to be used for the tests

Both of the following species of bacteria shall be used:

- a) *Staphylococcus aureus*;
- b) *Escherichia coli*.

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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 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	Culture collection
<i>Staphylococcus aureus</i>	ATCC 6538P	American Type Culture Collection
	FDA 209P	US Food and Drug Administration
	NBRC 12732	National Institute of Technology and Evaluation
	CIP 53.156	Collection des Bacteries del'Institut Pasteur Deutsche
	DSM 346	Sammlung von Mikroorganismen und Zellkulturen Gmbh
	NCIB 8625	National Collection of Industrial and Marine Bacteria Ltd
<i>Escherichia coli</i>	ATCC 8739	American Type Culture Collection
	NBRC 3972	National Institute of Technology and Evaluation
	CIP 53.126	Collection des Bacteries del'Institut Pasteur Deutsche
	DSM 1576	Sammlung von Mikroorganismen und Zellkulturen Gmbh
	NCIB 8545	National Collection of Industrial and Marine Bacteria Ltd

5.2 Reagents and materials

The following reagents and materials shall be used:

5.2.1 Water, an analytical grade for microbiological media preparation, which is freshly distilled, ion-exchanged, filtered with RO (reverse osmosis) or ultra-filtered, or a combination of these. It shall be free from all toxic or bacteria inhibitory substances.

5.2.2 Meat extract, for microbial test.

5.2.3 Peptone, for microbial test.

5.2.4 Sodium chloride, analytical grade, a grade appropriate for microbiological purposes or both.

5.2.5 Nonionic surfactant, polyoxyethylene sorbitan monooleate.

NOTE The generic name of polyoxyethylene sorbitan monooleate is polysorbate 80 (Tween 80[®]).

5.2.6 Sodium hydroxide, analytical grade, a grade appropriate for microbiological purposes or both.

5.2.7 Hydrochloric acid, analytical grade, a grade appropriate for microbiological purposes or both.

5.2.8 Agar, analytical grade, a grade appropriate for microbiological purposes or both.

5.2.9 Yeast extract, for microbial test.

5.2.10 Trypton, for microbial test.

5.2.11 Glucose, for microbial test.

5.2.12 Potassium dihydrogen phosphate (KH₂PO₄), analytical grade, a grade appropriate for microbiological purposes or both.

5.3 Equipment and apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

5.3.1 Inoculation loops, 4 mm in ring diameter, made of platinum.

5.3.2 Dry-heat sterilizer, capable of maintaining the temperature at a value between 160 °C and 180 °C.

5.3.3 Stopper, made of cotton, silicone, metal or molleton.

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

5.3.5 Clean bench, for microbial test.

5.3.6 Biological safety cabinet, for antibacterial test.

5.3.7 Balance, capable of weighing to ± 0,01 g.

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

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

5.3.10 Sterilized cup, with an outside diameter of 63 mm to 65 mm, a depth of 31 mm to 35 mm and an internal volume of 50 ml to 60 ml.

NOTE Sterilized cups with dimensions and volumes other than those specified can lead to different results.

5.3.11 Bacteria spreader, for microbial test and with a tip width of 20 mm or more.

5.3.12 Glass rod, with a diameter of approximately 20 mm and a flat tip.

5.3.13 Shaker with thermostatic chamber, capable of shaking at (150 ± 10) rpm with (30 ± 5) mm in amplitude of horizontal direction, and chamber with temperature control accuracy within ± 1 °C.

NOTE A mechanical shaker can be used in a thermostatic chamber.

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

5.3.15 Petri dishes, made of glass, sterilized plastics or both, with an inner diameter of approximately 90 mm.

5.4 Sterilization methods

5.4.1 General

Glass and plastic apparatus are thoroughly washed with alkali or neutral detergent, rinsed thoroughly with water, dried and then sterilized. The method of sterilization is according to [5.4.2](#) or [5.4.3](#). In the case of flame sterilization of inoculation loops, follow [5.4.4](#).

The plastic apparatus shall have heat resistance capable of withstanding the sterilization treatment temperature, or sterile apparatus may be used. When sterile apparatus is used, another sterilization is not necessary.

5.4.2 Dry-heat sterilization

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

Temperature	Minimum sterilization time
170 °C	60 min
160 °C	120 min

If the cotton stopper or wrapping paper of the apparatus to be sterilized gets wet with water after completion of dry-heat sterilization, the apparatus shall not be used.

5.4.3 High-pressure steam sterilization

Pour water into an autoclave and then place the objects to be sterilized in a wire mesh basket on a wire mesh shelf. After locking the lid of the autoclave, increase the temperature and maintain at a temperature of 121 °C and a pressure of 103 kPa for 15 min to 20 min. After sterilization, naturally cool down to 100 °C or lower, before removing the objects from the autoclave. If further cooling is necessary, use a clean bench or a biological safety cabinet. An autoclave should be cleaned with neutral detergent and rinsed with water to prevent contamination by medium and processing chemicals.

5.4.4 Flame sterilization

Flame the whole apparatus with gas or alcohol flames. In the case of an inoculation loop, flame it until it glows. In the case of test tubes, flame them for 2 s to 3 s.

5.5 Medium and buffer

5.5.1 General

As the medium and buffer solution, those having the following composition are used. Commercially available products can be used as long as they have the same composition.

5.5.2 1/500 nutrient broth medium (1/500 NB)

Add 5,0 g of meat extract, 10,0 g of peptone and 5,0 g of sodium chloride to 1 000 ml of water, mix and dissolve, and prepare nutrient broth medium. 800 ml of water is added to 2 ml of nutrient broth medium and 0,5 g of non-ionic surfactant that has been weighed, mixed and dissolved, and water is added to make the total volume 1 000 ml. Adjust the solution with sodium hydroxide solution or hydrochloric acid solution to pH 6,8 to 7,2 (25 °C) using a pH meter, sterilized by autoclaving, 1/500 nutrient broth medium (1/500 NB). If it is not used immediately after preparation, store it at a temperature of 5 °C to 10 °C. 1/500 NB that is one week old or more shall not be used.

5.5.3 Slant culture medium

Add 5,0 g of scaled meat extract, 10,0 g of peptone and 5,0 g of sodium chloride to 1 000 ml of water and mix them. Adjust the solution with sodium hydroxide solution or hydrochloric acid solution to pH 7,0 to 7,2 (25 °C) using a pH meter. Add 15,0 g of agar powder, dissolve it by heating. Pour 6 ml to 10 ml of the mixture into a test tube, close with a cotton stopper and sterilize by autoclaving. After sterilization, place the test tube at an angle of about 15 degrees with respect to the horizontal plane in a clean room and solidify the contents to make the slant culture medium. If it is not used immediately after preparation store it at a temperature of 5 °C to 10 °C. If the condensed water runs out, dissolve it, coagulate again and use it. Slant culture medium that is over one month old shall not be used.

5.5.4 Standard nutrient agar medium (SA medium)

2,5 g of weighed yeast extract, 5,0 g of tryptone and 1,0 g of glucose are added to 1 000 ml of water and mixed, and the mixture is adjusted with sodium hydroxide solution or hydrochloric acid solution to pH 7,0 to 7,2 (25 °C) using a pH meter. 15,0 g of the agar powder is added and dissolved by heating. Sterilize in autoclave with high-pressure steam. Use this as standard nutrient agar medium. If it is not used immediately after preparation, store it at a temperature of 5 °C to 10 °C. SA media that is over one month old shall not be used.

5.5.5 Phosphate-buffered physiological saline

Dissolve 34,0 g of potassium dihydrogen phosphate in 500 ml of water and mix, then adjust the solution with a sodium hydroxide solution to pH 6,8 to 7,2 (25 °C) using a pH meter. Dilute the mixture with water to 1 000 ml. Take 1,25 ml of this solution and dilute with sodium chloride aqueous solution with a mass fraction of 0,85 % (physiological saline) to 1 000 ml. If necessary, aliquot it into a test tube or Erlenmeyer flask, cotton plug it and sterilize by autoclaving to phosphate-buffered physiological saline. If it is not used immediately after preparation, store it at a temperature of 5 °C to 10 °C. Phosphate-buffered physiological saline that is more than one month old shall not be used.

5.6 Preservation of test strain

Inoculation of bacteria shall be carried out aseptically. Use a safety cabinet if necessary. Hold the test tube of the slant culture medium prepared in 5.5.3 and the container of original strain in one hand; hold an inoculation loop handle, by which the cotton stopper is pulled out to sterilize the mouth of the test