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Quantitative determination of antibacterial activity of ceramic tile surfaces — Test methods —

Part 1:
Ceramic tile surfaces with incorporated antibacterial agents

Détermination quantitative de l'activité antibactérienne des surfaces des carreaux céramiques — Méthodes d'essai —

Partie 1: Carreaux céramiques incorporant des agents antibactériens en surface

Reference number
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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).

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. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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.

This document was prepared by Technical Committee ISO/TC 189, *Ceramic tile*.

A list of all parts in the ISO 17721 series can be found on the ISO website.

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.

Quantitative determination of antibacterial activity of ceramic tile surfaces — Test methods —

Part 1:
Ceramic tile surfaces with incorporated antibacterial agents

1 Scope

This document specifies test methods for evaluating the antibacterial activity of glazed and unglazed ceramic tile surfaces with incorporated antibacterial agents.

Secondary effects on ceramic tile surfaces due to antibacterial treatments, such as changes in chemical resistance, stain resistance or small colour differences, are not covered by this document. For chemical resistance refer to ISO 10545-13, for stain resistance refer to ISO 10545-14 and for colour differences refer to ISO 10545-16.

Any results obtained with this document will always refer to this document and the conditions used. Results obtained with this document 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., are considered.

2 Normative references

There are no normative references in this document.

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 https://www.electropedia.org/

3.1
antibacterial
condition where growth of bacteria on the surfaces of product is suppressed or describing the effect of an agent which suppresses the growth of bacteria on the surface of products

3.2
antibacterial agent
agent that kills or inhibits growth of bacteria on the surfaces of products by the use of an *antibacterial* (3.1) 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 control after inoculation with and incubation of bacteria

4 Materials

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 laboratory hygiene must be strictly observed.

4.1 Bacterial strains

This test method was developed with the following bacteria:

a) *Escherichia coli*;

b) *Staphylococcus aureus*.

The test method shall be conducted using either of the above-mentioned bacteria. The bacterial strains to be used in the test are listed in Table 1 and are stored by entities that are registered under the World Federation for Culture Collections or of the Japan Society for Culture Collections. Other bacterial species may be tested with this test method, details of the species used, culture conditions and testing process shall be described in detail in the test report.

Transfer of cultures should be performed aseptically in safety cabinets. Inoculate each strain onto a nutrient agar medium slant using a sterile inoculation loop; incubate for 18 h - 24 h at 37 °C ± 1 °C, and then store at 5 °C – 10 °C. Incubation temperature and time suitable for the bacteria used in the testing shall be selected.

Subculture the strains by repeating the process within 30 d. The maximum number of subcultures from the original strain from the culture collection is 10. Discard slant cultures appropriately after 30 d.

NOTE In the case of bacteria stored at -80 °C and lyophilized cultures, the maximum number of subcultures from the original strain is 10.

If necessary, additional tests with other bacterial strains may be performed under the culture conditions specified for that particular species. Details of these conditions shall be included in the test report.

Table 1 — Bacterial strains and culture collections

Bacteria	Culture collections
E. coli	ATCC 8739, DSM 1576, NBRC 3972, CIP 53.126, NCIB 8545
S. aureus	ATCC 6538P, DSM 346, NBRC 12732, CIP 53.156, NCIB 8625

4.2 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 Non-ionic surfactant

The non-ionic surfactant shall be polyoxyethylene sorbitan monooleate (polysorbate 80).

4.2.2 Nutrient broth

For 1 000 ml of purified water, take 3,0 g of meat extract, 10,0 g of peptone and 5,0 g of sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly dissolved, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,1 ± 0,1) at 25 °C. Sterilize in an autoclave at 121 °C ± 2 °C for at least 15 min. After preparation, if nutrient broth is not used immediately, store it at 5 °C to 10 °C.

Other culture media may be selected based on the requirements for the bacteria selected for the testing. Details of the selected media shall be included in the test report under culture conditions.

Prepared culture media may be stored at 5 °C - 10 °C for up to one month.

4.2.3 1/500 nutrient broth

For 1 000 ml of purified water, take 3,0 g of meat extract, 10,0 g of peptone and 5,0 g of sodium chloride, put them in a flask and dissolve them thoroughly. Dilute this medium 500 times using distilled or deionized water, and set the pH to (7,0 ± 0,2) using hydrochloric acid solution or sodium hydroxide solution. Sterilize in an autoclave at 121 °C ± 2 °C for at least 15 min. After preparation, if 1/500 nutrient broth is not used immediately, store it at 5 °C to 10 °C.

Other culture media may be selected based on the requirements for the bacteria selected for the testing. Details of the selected media shall be included in the test report under culture conditions.

Prepared culture media may be stored at 5 °C - 10°C for up to one month.

4.2.4 Nutrient agar

For 1 000 ml of purified water, take 3,0 g of meat extract, 5,0 g of peptone and 15,0 g of agar powder, put them in a flask and mix. Heat the flask in boiling water to dissolve the contents thoroughly. Use a 0,1 mol/l solution of sodium hydroxide to bring the pH to (6,8 ± 0,2) at 25 °C. Sterilize in an autoclave at 121 °C ± 2 °C for at least 15 min. After preparation, if nutrient agar is not used immediately, store it at 5 °C to 10 °C. Keep the medium temperature between 45 °C and 48 °C when mixing with a bacterial suspension.

Other culture media may be selected based on the requirements for the bacteria selected for the testing. Details of the selected media shall be included in the test report under culture conditions.

Prepared culture media may be stored at 5 °C - 10 °C for up to one month.

4.2.5 Soybean-casein digest broth with lecithin and polysorbate 80 (SCDLP)

For 1 000 ml of purified water, take 17,0 g of casein peptone, 3,0 g of soybean peptone, 5,0 g of sodium chloride, 2,5 g of phosphoric acid monopotassium dehydrogenate, 2,5 g of glucose and 1,0 g of lecithin, put them in a flask and dissolve them. Add 7,0 g of non-ionic surfactant and dissolve it. Use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,0 ± 0,2) at 25 °C. Sterilize in an autoclave at 121 °C ± 2 °C for at least 15 min. After preparation, if SCDLP is not used immediately, store it at 5 °C to 10 °C.

Prepared SCDLP solution may be stored at 5 °C - 10 °C for up to one month.

4.2.6 Phosphate buffer saline (PBS)

For 1 000 ml of purified water, take 34,0 g of potassium dihydrogen phosphate, put it in a flask and dissolve it thoroughly. Use a solution of sodium hydroxide to bring the pH to (7,0 ± 0,2) at 25 °C. Sterilize in an autoclave at 121 °C ± 2 °C for at least 15 min. After preparation, if PBS is not used immediately, store it at 5 °C to 10 °C.

Prepared PBS solution may be stored at 5 °C - 10 °C for up to one month.

5 Test specimen

5.1 Size

Reduce samples to test pieces of a size approximately (50 ± 2) mm × (50 ± 2) mm square or as close to these dimensions as practical. Test specimen shall be sterilized based on the appropriate sterilization process selected for the test sample. All surfaces of the test specimen shall be sterilized.

NOTE When it is difficult or impossible to cut (50 ± 2) mm long (up to 10 mm thickness) squares, it is acceptable to use a different specimen size as long as the specimen surface can be covered with a 400 mm^2 to 1 600 mm^2 film. When the specimen surface is stained with organic contaminant, it is acceptable to first eliminate the contaminant by exposure to a 1,0 mW/cm^2 light source within the limit of 24 h.

5.2 Control

To explain the results and to validate the test, proper controls shall be included in the experimental set up. These controls should be devoid of any antibacterial properties and establish a baseline for technical performance by minimizing misleading results caused by the following.

— Day-to-day variations in laboratory conditions and test organism viability.

— Sample-to-sample variations within the formulation of commercial glazes where different sources are used for the glaze components and normal fluctuations occur in the mined materials. These variations frequently result in inconsistent effects on microbial proliferation, making it difficult to assess the efficacy of designed changes to the formula.

— The inherent water absorbing nature of ceramic surfaces that can cause organism death by desiccation and consequent misrepresentation of the antibacterial activity of the glaze itself.

Non-treated test specimens, i.e. test specimens which do not have any antibacterial treatment, should be used as controls.

When non-treated specimens cannot be provided, use glass panes instead. Care should be taken to avoid microbial contamination and cross-contamination among specimens.

5.3 Precondition

Control and test specimens should be appropriately sterilized before the precondition step. Prepare separate sample conditioning beds for the test samples and controls. Line the lower half of a sterile 150 mm × 150 mm Petri dish with a grade #1 Whatman filter paper size 150 mm or equivalent.

Wet the filter paper thoroughly with sterile deionized water. Place the test pieces together on the wet filter paper. Label the outside of each dish with sufficient sample information to maintain unique sample identity. Further condition the samples by placing these beds in an incubator maintained at 37 ± 2 °C and ≥75 % RH for 18 h - 24 h. Following this period, keep the filter paper wet through the time of incubation.

5.4 Number of test specimen

At a minimum, 6 non-treated and 3 treated test specimens will be included in the testing. Additional specimen may be included and details of the number of specimens used shall be included in the test report.

6 Procedure

6.1 Preparation of test inoculum

Transfer the stored bacteria to the nutrient agar slant using a sterile inoculating loop and incubate at 37 ± 1 °C for 16 h to 24 h. Transfer the bacteria to a new nutrient agar slant and incubate at 37 ± 1 °C for 16 h to 20 h. Uniformly disperse a small quantity of test bacteria in 1/500 NB with a platinum loop, and measure the bacteria count using the optical microscope observation method or any other adequate method. Suitably dilute this bacteria suspension with 1/500 NB to obtain a count of 6,7 × 10^5 cfu/ml – 2,6 × 10^6 cfu/ml and use the result as the bacterial suspension for the test. If the test bacteria suspension is not to be used immediately, store it at 4 °C and use it within 4 h.

6.2 Adhesive film

The adhesive film is inert and non-water absorbent with good sealing properties. The sheets are cut with dimensions of 40 mm ± 2 mm. The film should not affect bacterial growth and can be made of polyethylene, polypropylene or polyester [poly(ethylene terephthalate)]. Film that is 0,05 mm to 0,10 mm thick is recommended. Adhesive film pieces shall be sterilized by autoclave method or surface sterilization by 70 % ethyl alcohol prior to application.

NOTE Films cut from Stomacher bags are also suitable.

6.3 Inoculation of test specimens

Precondition non-treated control specimens and treated test specimens. Collect exactly 0,15 ml of test bacterial suspension with a sterilized pipette and drip it onto each test piece. Put a film on top of the dripped suspension and lightly push to get the suspension to spread to the whole film surface, while taking care that no suspension leaks out of the film edge.

The regulated suspension quantity can create leakage of suspension from the film edge or might not be enough to spread the suspension uniformly. In such a case, it is acceptable to reduce down to half the quantity of suspension or increase to twice the quantity of suspension. However, when the volume of test inoculum is changed, the concentration of the bacterial cells in the inoculum shall be adjusted to provide the same number of bacterial cells as when the normal volume of test inoculum is applied.

6.4 Recovery of bacteria from non-treated control specimens at time t = 0 h

Immediately after inoculation, process half of the untreated control test specimens by placing each test piece into 10 ml of either SCDLP broth (see 4.2.5) or a suitable, validated neutralizer. Shake vigorously for 60 s ± 5 s to ensure total and uniform removal of the test organisms from the film and test piece.

Other methods for recovery, such as the use of a stomacher bag, can be utilized. In addition, if a lower limit of detection is required, then the amount of neutralizer added can be reduced, however the entire sample shall be covered with neutralizer broth before proceeding.

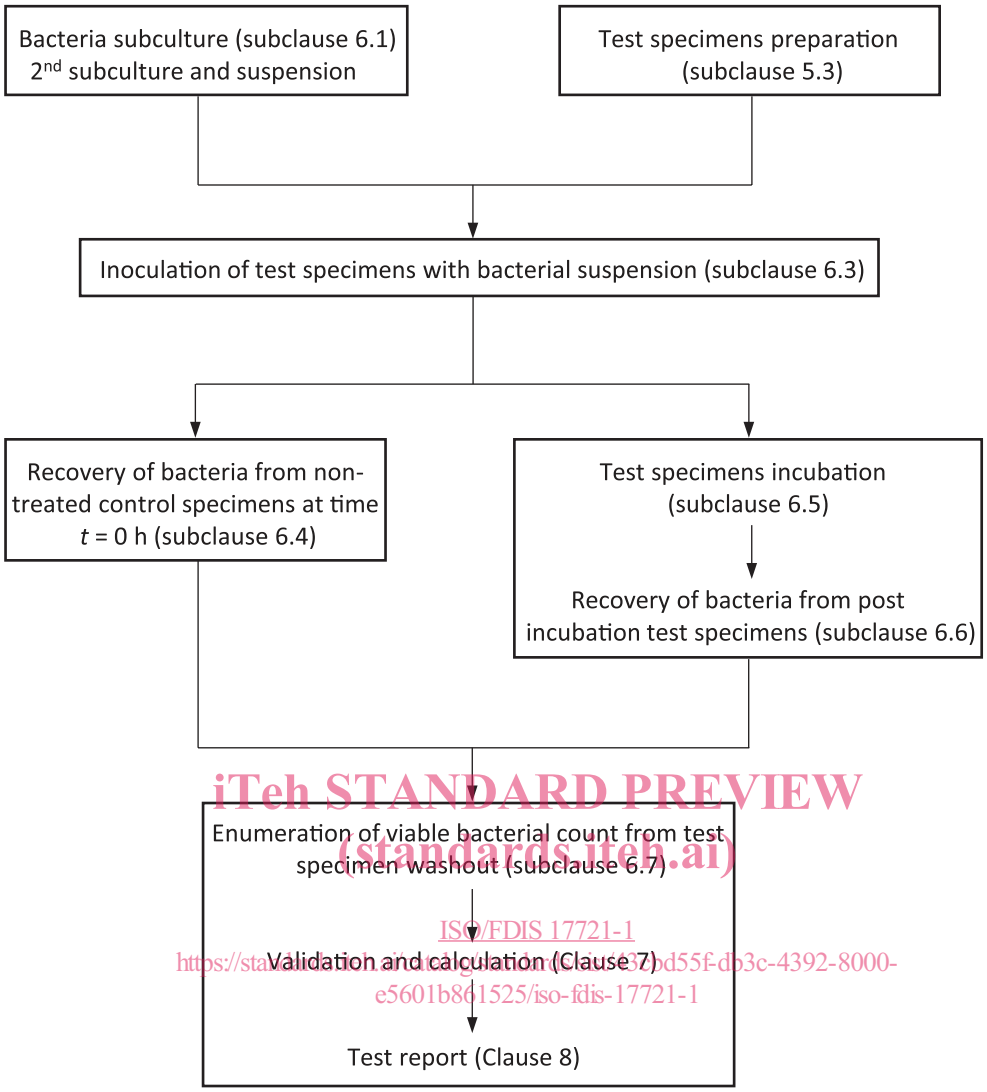

Figure 1 — Flowchart of test procedure

6.5 Incubation

Incubate the Petri dishes containing the test specimens (3 control non-treated and 3 treated) at 37 °C ± 1 °C and ≥75 % RH for the desired contact time period (see sections below for different contact times). One or more of the contact time points may be selected for the testing. Each time point shall be considered as one test. Controls should also be included for different time points. Selected time point(s) and all results shall be included in the test report.

Section A: contact time of 30 min

Section B: contact time of 1 h

Section C: contact time of 4 h

Section D: contact time of 8 h

Section E: contact time of 24 h