INTERNATIONAL STANDARD

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Second edition
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Surgical drapes, gowns and clean air suits, used as medical devices, for patients, clinical staff and equipment — Test method to determine the resistance to wet bacterial penetration

Champs chirurgicaux, casaques et tenues de bloc, utilisés en tant que dispositifs médicaux, pour les patients, le personnel et les équipements — Méthode d'essai de résistance à la pénétration de la barrière bactérienne à l'état humide

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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 on 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 the following URL: www.iso.org/iso/foreword.html.

This document was prepared by ISO/TC 94, *Personal safety — Protective clothing and equipment*, SC 13, *Protective clothing*.

This second edition cancels and replaces the first edition (ISO 22610:2006), which has been technically and editorially revised.

Although the same principle applies as for ISO 22610:2006, this revised test method should be considered technically as a new test method.

The main differences between this document and ISO 22610:2006 are:

— Different strain of bacterial species;

— More detailed description of equipment, reagents and materials;

— Tighter tolerances on equipment requirements;

— Tighter tolerances on specimen (pre)treatment, preparation of the agar plates, bacterial inoculum and donor;

— Strict protocol for the test procedure.

Introduction

There are numerous examples of situations where bacteria carried by a liquid may migrate through a barrier material in the wet state. The wet penetration of skin flora through a covering material is one example (see Annex E).

European medical device regulations specifically place the responsibility for avoiding device-related infections on the manufacturer. In order to demonstrate compliance with this requirement and to describe a product to the user, there is a need to use harmonized and recognized international test methods.

The test method described in this international standard uses microbiological techniques and is therefore intended to be performed exclusively by laboratories experienced in and equipped for such work.

ISO 22610:2006 has been significantly revised in order to improve the precision of the test method.

The primary difference between this document and ISO 22610:2006 is that this document specifies a strain of a different bacterial species and tighter tolerances on material handling and procedure, resulting in more reproducible and accurate measurements.

In order to obtain accurate repeatable and reproducible results, not only does the equipment need to meet the requirements specified in this document, but also the material handling and test procedure need to be followed precisely and consistently. Minor deviations from the equipment requirements, procedure and/or specimen handling can result in considerable loss of repeatability, reproducibility and accuracy of the measurement.

Surgical drapes, gowns and clean air suits, used as medical devices, for patients, clinical staff and equipment — Test method to determine the resistance to wet bacterial penetration

WARNING — The use of this document may involve hazardous materials, operations and equipment. This document does not purport to address all of the safety problems associated with its use. It is the responsibility of the user of this document to establish appropriate safety and health practices prior to the application of this document and to comply with the legal requirements for this purpose.

IMPORTANT — This test method has been technically and editorially significantly revised. The equipment shall meet the requirements specified in this document and the measurements shall be carried out under the specified conditions with special attention being paid to specimen (pre) treatment, strictly following the procedure prescribed in this document. Minor deviations from the equipment requirements, procedure and/or specimen handling can result in considerable loss of repeatability, reproducibility and accuracy of the measurement.

1 Scope

This document specifies a test method, with associated test apparatus, which is used to determine the resistance of a material to the penetration of bacteria, carried by a liquid, when subjected to mechanical rubbing.

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 11607-1, *Packaging for terminally sterilized medical devices — Part 1: Requirements for materials, sterile barrier systems and packaging systems*

ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*

ISO 17665-1, *Sterilization of health care products — Moist heat — Part 1: Requirements for the development, validation and routine control of a sterilization process for medical devices*[1)]

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/

1) Under preparation. Stage at the time of publication: ISO/CD 17665-1:2018.

3.1
carrier material
material (paper sheet) used to support the donor (polyurethane film) during production and preparation

3.2
donor
material made of polyurethane film that has been inoculated with a known number of viable spores of a defined strain of test bacterium

3.3
cover film
HDPE material used to cover the donor and the test specimen during the test

3.4
finger
part of the apparatus for testing resistance to wet bacterial penetration, used to bring donor and test specimen into contact with the surface of an agar plate

3.5
replicate test
one complete evaluation of a single test specimen, cut from the sample (e.g. gowns, drapes), comprising of five plate counts directly in contact with the donor

3.6
test specimen
piece of material, for which the resistance to wet bacterial penetration is being determined

3.7
reference material
standardized material to assess the precision of the laboratory when performing the test for resistance to wet bacterial penetration

3.8
resistance to wet bacterial penetration
resistance of a barrier material to the penetration of bacteria carried by a donor, when subjected to mechanical rubbing and wetting

3.9
bacterial challenge
number of spores per ml of the suspension of *Bacillus atrophaeus* as used for inoculation of donor material

3.10
bacterial inoculum
suspension of *Bacillus atrophaeus* with a verified concentration

Note 1 to entry: The concentration is between $5{,}0 \times 10^3$ and $1{,}5 \times 10^4$ spores/ml.

4 Principle

A sheet of donor material, of the same size as the test specimen and carrying the bacteria, is placed on the test specimen with the contaminated side facing down and covered by a sheet of HDPE cover film. Two conical metal rings, close-fitting into each other, hold the three sheets together. The assemblage of materials is placed on an agar plate with the steel rings hanging freely outside the brim, applying a tensile force. An abrasion-resistant finger is placed on top of the materials with a specified force to bring the test specimen into contact with the agar surface. The finger is moved over the entire surface of the plate in less than 15 min by means of a pivoted lever moved by an eccentric cam. The assemblage of materials, stretched by the weight of the steel rings, ensures that only a small area of the test specimen is brought into contact with the agar surface at any given time. Due to the combined effect of rubbing

and liquid migration up from the agar surface, bacteria may pass from the donor material through the test specimen down to the agar surface.

The test is carried out for 15 min. After 15 min the agar plate is replaced by a fresh one, and the test is repeated with the same assemblage, i.e. the same donor and test specimen. Five consecutive tests are performed with the same assemblage enabling an estimation of the penetration over time.

An estimation of the bacterial contamination on the upper side of the test specimen and the bacterial load remaining on the donor material may be determined by using the same technique.

The agar plates are incubated in order to grow the bacterial colonies, which are then counted.

The results are expressed as a percentage (%) of penetration compared to the bacterial load initially inoculated on the donor.

If the material tested contains an antimicrobial substance it should be inactivated before testing. If it is not possible to inactivate the antimicrobial substance or if there is an assumption that the material contains an antimicrobial substance, additionally the remaining antibacterial activity should be determined. Information about any antimicrobial treatment, the results of the penetration test and the additional test should be included in the report.

5 Equipment, reagents and materials

Normal laboratory equipment, reagents and materials, and the following shall be used.

Reagents shall be of analytical quality and/or suited for microbiological purposes.

Disposable equipment/materials apparatus are an acceptable alternative to re-usable glassware and plastic if having suitable specifications.

5.1 Biosafety cabinet class II.

5.2 Incubators, capable of maintaining the temperature at (56 ± 2) °C and (37 ± 2) °C.

5.3 Refrigerator, capable of maintaining the temperature at (5 ± 3) °C.

5.4 Water bath, capable of maintaining the temperature at (45 ± 2) °C.

5.5 Bacterial strain suspension, purified spores of *Bacillus atrophaeus* ATCC 9372 suspended in ethanol 90 % at a concentration of about 10^9 spores/ml[2)]. Other titers may be used however the maximum ethanol concentration after dilution shall be less than 0,02 %.

5.6 Purified water, freshly distilled and/or deionized and/or ultrafiltered and/or filtered by reverse osmosis, free of any toxic or bacteria inhibitory substances.

5.7 Peptone water, 10 g Peptone, 5 g NaCl and 1 g Polysorbate 80 in purified water, filled up to 1 000 ml, steam sterilized for 15 min at an autoclave temperature ($121\ ^{+3}_{0}$) °C.

5.8 TGE agar (TGEA), 3 g beef extract, 5 g Tryptone, 1 g dextrose, 15 g agar in purified water, filled up to 1 000 ml, steam sterilized for 15 min at ($121\ ^{+3}_{0}$) °C (see Annex A).

5.9 Petri dishes, sterile, 14 cm and 9 cm in diameter, with lid.

2) The spore suspension in alcoholic suspension can be bought at SIMICON Gmbh – München. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5.10 Donor material, five pieces of ~25 cm × 25 cm polyurethane (PU) film on siliconized carrier paper, film thickness 25 µm to 30 µm, each piece packed in a sterilization bag and steam sterilized at ($121\ ^{+3}_{0}$) °C for 15 min[3].

NOTE Guidance for selecting a suitable donor material: wettable, solvent-cast polyurethane (PU) film of 25 µm, max. 30 µm, thickness, carried on siliconized carrier paper. The material has a density of 1,12 g/cm^3, (according to ISO 1183-1, Method A), a hardness (Shore-A) of 87 ± 1 (according to ISO 7619-1), a melting point of 160 to 175 °C (acc. to Kofler), a tear strength of (40 ± 10) MPa (measured on 50 µm thick films, measurement according to ISO 527-1, ISO 527-3) and an elongation at break of 500 % ± 125 % (measured on 50 µm thick films, measurement acc. to ISO 527-1, ISO 527-3).

5.11 Cover film, five pieces of ~25 cm × 25 cm, or ~25 cm in diameter High Density Polyethylene (HDPE) film, thickness (12 ± 2) µm, density 0,95 ± 0,095 g/cm^3[4].

NOTE One or two additional pieces are necessary if tests are also carried out in order to achieve an estimation of the bacterial contamination on the upper side of the test specimen and/or the bacterial load remaining on the donor material (see Clause 7, Annex D).

5.12 Reference material, "400.1.0.6 – ISO 22610 Reference Material"[5].

— Construction: plain weave, made from Polyester (PES);

— Fabric weight: (110 to 120) g/m^2;

— Finishing: no chemical treatment;

— Dyeing: not dyed;

— Air Permeability: ~10 mm/s according to ISO 9237:1995 at 0,1 KPa;

— Surface resistivity: max. 1 011 Ω according to DIN 54345-1:1992;

— Sterilisable with steam.

Another suitable reference material may also be used if proven that the WBP is in the same range and the precision of test method using this material is at least the same or has higher precision, compared with the reference material specified in this document.

5.13 Pipettes, 1 ml and 10 ml.

5.14 Micropipettes, 1 000 µl and 100 µl, with suitable sterile tips.

5.15 Sterile dilution tubes, 10 ml.

5.16 Rake, L- or triangle shape with horizontal side of 3 cm.

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3) Suitable donor material is available from Gerlinger Industries GmbH, Schwarzhammermühle, D-08491 Netzschkau, Germany (reference: 4220M or 4220). The material can also be ordered from schuett-biotec GmbH, Rudolf-Wissell-Str. 13, D-37079 Göttingen as part of a test kit. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

4) Suitable cover film is available from e.g. Mo Industri AB, Stråkenvägen 3, SE-56576 Bottnaryd, Sweden (reference: 1623-001). The material can also be purchased from schuett-biotec GmbH, Rudolf-Wissell-Str. 13, D-37079 Göttingen as part of a test kit. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5) Available from Rotecno AG, Via Vite 3, CH-6855 Stabio, Switzerland. The material can also be ordered from schuett-biotec GmbH, Rudolf-Wissell-Str. 13, D-37079 Göttingen. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

5.17 Test specimens, five pieces of the barrier material to be tested, ~25 cm × 25 cm or with a diameter of approximately 25 cm.

6 Test apparatus and accessories

6.1 Apparatus, as shown in Annex B[6].

The test apparatus is schematically shown in Figure A.1. The apparatus has an electrically-driven, timer-controlled turntable, able to hold a 14 cm diameter agar plate. A horizontal lever with a vertical steel 'finger' at its end, is fitted to a pivot, allowing sideways movement of the finger from the centre to the periphery and back to the brim of the rotating (60 ± 1) r/min agar plate. A weight can be slid along the lever to adjust the force exerted by the finger on the material assembly. The movement of the lever is guided by an eccentric cam rotating at (5,6 ± 0,1) r/min. The steel finger, having a semi-spherical polished end with a radius of 11 mm, is removable and shall be disinfected between tests.

NOTE The tip of the steel finger can become flat as a result of abrasion. The steel finger needs to be checked regularly to see if the tip has not flattened and be replaced if necessary.

The force exerted by the finger on the materials shall be (3,00 ± 0,05) N, measured by a dynamometer attached to the lever, or by a balance placed on the turntable, and if necessary adjusted using the slidable weight (see Annex B).

The material being tested shall only be in contact with the agar at one point, at any given time. To ensure that the finger moves over the entire surface, it shall be regularly monitored using the method described in Annex C. A quality record shall be maintained.

6.2 Cylindrical object, stainless steel, or another appropriate material suitable for sterilization, approximately 9 cm in diameter and 4 cm in height.

6.3 Conical steel rings, (800 ± 1) g in total, for fixation of the material assembly (see Annex B).

6.4 Dynamometer capable of measuring a force of (3,00 ± 0,05) N.

7 Preparation of the agar plates

Standard Petri dishes, of 9 cm in diameter, are used for the determination of bacterial challenge on the donor (see 9.7), the inoculum concentration check (see Clause 8) and the environmental controls, (see 9.7).

Prepare the following agar plates for one test specimen:

— 5 Petri dishes, 14 cm in diameter, per test (see 9.2);

— 1 Petri dish, 14 cm in diameter, for the measurement of the distance agar to brim (see 9.2.2).

The 14 cm in diameter agar plates shall be prepared on a horizontal surface and filled with TGE agar, prepared according to Annex A, to (3,0 ± 0,5) mm from the brim using the following procedure.

NOTE 1 For the 9 cm diameter plates the distance agar-to-brim does not need to be (3,0 ± 0,5) mm. A standard volume of TGEA can be used.

NOTE 2 If more than one test is carried out in one run, the inoculum concentration check and the measurement of the distance agar-to-brim need to be carried out only once and hence the number of petri dishes can be lowered accordingly.

6) Available from schuett-biotec GmbH, Rudolf-Wissell-Str. 13, D-37079 Göttingen, Germany. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

The height of Petri dishes may differ from supplier to supplier and/or batch to batch. The determination of the volume, or mass, of agar that will result in each dish being filled to the correct distance from the brim by measuring the height and diameter, has to be carried out previously.

Pour the determined amount of TGE agar into a suitable glass flask and sterilize at (121 $^{+3}_{0}$)°C for 15 min. Volumetric or gravimetric methods shall be used when pouring the agar into the Petri dishes.

The flasks with TGE agar may be stored in the refrigerator at (5 ± 3) °C for a maximum period of 3 months, however, freshly prepared agar is preferred.

In case of using not freshly prepared TGEA, the TGEA is re-liquefied by heating the flasks in boiling water, microwave or any other suitable means. Avoid overheating and remove directly when the medium is liquefied.

Subsequently the liquid TGEA is cooled down to approximately 45 °C and poured into the Petri dishes; take care to avoid air bubbles and irregularities on the surface. The solidified agar surface shall be as flat as possible. In case of dispute, freshly prepared agar shall be used.

Each plate is left to dry without its lid on at room temperature in a biosafety cabinet class II for 20 min. No visible fluid (condensate) shall be present on the agar surface. The plates are closed and stored in the biosafety cabinet or in a clean, aseptic, closed plastic bag and used within (24 ± 4) h.

8 Bacterial inoculum

The inoculum is prepared using the following procedure:

— The day before the test, 1 ml of the stock suspension of *Bacillus atrophaeus* ATCC 9372 10^9 spores/ml, stored in the refrigerator, is diluted with 9 ml ethanol 96 %. This 10 fold diluted suspension is stored in the refrigerator until the next day. Its concentration is checked by performing a viable count using standard microbiological techniques, i.e. a serial dilution of the suspension in peptone water and spreading of 100 µl on TGEA 9 cm plates.

— Incubate the plates at (37 ± 2) °C during 16 h to 24 h. The colonies on the plate shall not be confluent.

— At the test day determine the concentration of the 10 fold diluted suspension of *Bacillus atrophaeus* by evaluating the incubated plates. Based on the concentration obtained dilute the suspension with peptone water in order to obtain a final concentration of 5,0 × 10^3 to 1,5 × 10^4 spores per ml.

NOTE It can be helpful to repeat the quantification of the 10 fold diluted suspension on several subsequent days.

9 Procedure

9.1 General

Before starting the test procedure, all laboratory surfaces shall be disinfected with a sporocide and dried.

The procedure should be carried out under aseptic conditions. A biosafety cabinet is recommended for plating.

NOTE One or two additional petri dishes are necessary if tests are also carried out in order to achieve an estimation of the bacterial contamination on the upper side of the test specimen and/or the bacterial load remaining on the donor material (see Clause 7 and Annex D).

