
**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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Contents

Page

Foreword	iv
Introduction	v
1 Scope	1
2 Normative references	1
3 Terms and definitions	1
4 Principle	2
5 Equipment, reagents and materials	3
6 Test apparatus and accessories	5
7 Preparation of the agar plates	5
8 Bacterial inoculum	6
9 Procedure	6
9.1 General	6
9.2 Preparation of the agar plates, 14 cm in diameter	7
9.2.1 Drying of the plates	7
9.2.2 Determination of the distance agar-to-brim	7
9.3 Inoculation of the donor	7
9.4 Test material	8
9.5 Preparation of the test assembly	8
9.6 Test procedure	9
9.7 Control plates	12
9.7.1 Environmental controls	12
9.7.2 Bacterial challenge	12
10 Evaluation	13
10.1 Required conditions for a valid test	13
10.2 Determination of the number of colonies of <i>Bacillus atrophaeus</i>	13
11 Expression of the results	14
12 Precision	14
12.1 Reference material	14
12.2 Sample material	14
13 Test report	14
Annex A (normative) Media	16
Annex B (normative) Test apparatus and accessories	17
Annex C (normative) Equipment and laboratory performance monitoring	20
Annex D (informative) Estimation of the bacterial contamination on the upper side of the test specimen and the bacterial load remaining on the donor	21
Annex E (informative) Transmission of infective agents during invasive surgical procedures	22
Annex F (informative) Precision of the Wet Bacterial Penetration test	23
Bibliography	26

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.

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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

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 $(56 \pm 2) ^\circ\text{C}$ and $(37 \pm 2) ^\circ\text{C}$.

5.3 Refrigerator, capable of maintaining the temperature at $(5 \pm 3) ^\circ\text{C}$.

5.4 Water bath, capable of maintaining the temperature at $(45 \pm 2) ^\circ\text{C}$.

5.5 Bacterial strain suspension, purified spores of *Bacillus atrophaeus* ATCC 9372 suspended in ethanol 90 % at a concentration of about 10^9 spores/ml²⁾. 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 $(121^{+3}_0) ^\circ\text{C}$.

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 $(121^{+3}_0) ^\circ\text{C}$ (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 min³⁾.

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/cm³, (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 $500 \% \pm 125 \%$ (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 $0,95 \pm 0,095$ g/cm³⁴⁾.

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/m²;
- Finishing: no chemical treatment;
- Dyeing: not dyed;
- Air Permeability: ~10 mm/s according to ISO 9237:1995 at 0,1 KPa;
- Surface resistivity: max. $1\ 011\ \Omega$ 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.

3) Suitable donor material is available from Gerlinger Industries GmbH, Schwarzhamermühle, D-08491 Netzkau, 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, $\sim 25\text{ cm} \times 25\text{ cm}$ 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 $(60 \pm 1)\text{ r/min}$ 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 $(5,6 \pm 0,1)\text{ r/min}$. 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 $(3,00 \pm 0,05)\text{ N}$, 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, $(800 \pm 1)\text{ g}$ in total, for fixation of the material assembly (see [Annex B](#)).

6.4 Dynamometer capable of measuring a force of $(3,00 \pm 0,05)\text{ N}$.

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 $(3,0 \pm 0,5)\text{ mm}$ 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 $(3,0 \pm 0,5)\text{ mm}$. 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 $(121 \pm 3)^{\circ}\text{C}$ 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 $(5 \pm 3)^{\circ}\text{C}$ 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 $(37 \pm 2)^{\circ}\text{C}$ 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 $5,0 \times 10^3$ to $1,5 \times 10^4$ 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](#)).

9.2 Preparation of the agar plates, 14 cm in diameter

9.2.1 Drying of the plates

On the test day, check the 14 cm agar dishes and check for standing water on the plate and/or lid. If condensation is present, open the 14 cm agar plates to be used and let them dry in a biosafety cabinet until the droplets of condensate have evaporated. Check on the absence of any condensate before closing them.

9.2.2 Determination of the distance agar-to-brim

Open one plate for the distance agar-to-brim check and determine the distance by placing a razor blade, thickness $(0,10 \pm 0,01)$ mm, on the agar surface and a steel ruler lying across the dish (see [Figure 1](#)). The distance between the ruler and the blade is measured using a calliper (see [Figure 1](#)). Measure the distance at five points on the surface. The mean distance shall be $(3,0 \pm 0,5)$ mm. The distance shall be determined for each batch of plates poured at one time.

NOTE The sharp edges of the razor blade need to be made blunt before use.

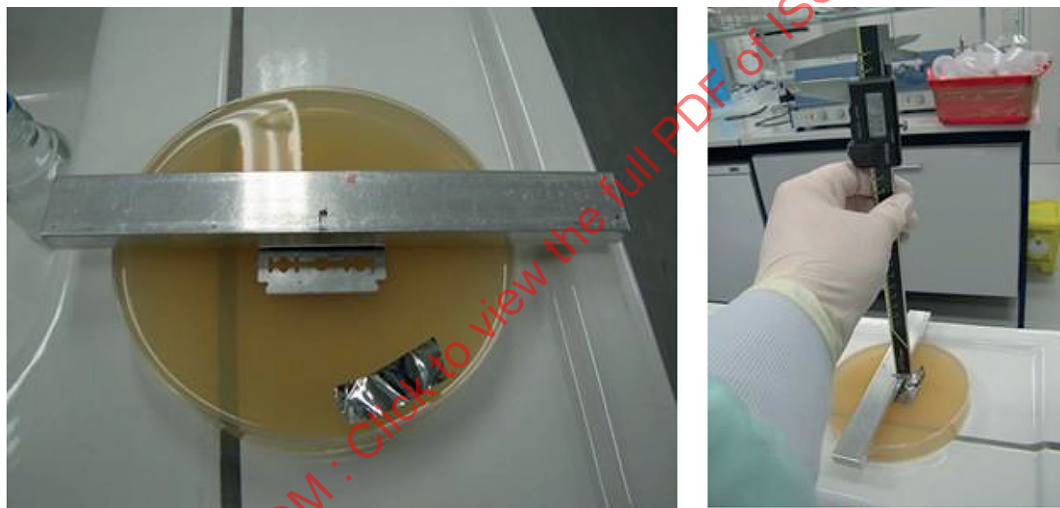


Figure 1 — Measurement of the distance agar-to-brim

9.3 Inoculation of the donor

The diluted *Bacillus atrophaeus* suspension (see [Clause 8](#)), adjusted to $5,0 \times 10^3$ to $1,5 \times 10^4$ spores per ml with peptone water is used for the inoculation of the donor.

For handling and preparation of the donor material wear sterile gloves. The gloves shall be changed after each step of the following procedures to avoid carry-over of bacterial spores.

- a) Open the sterilized bag containing the donor material aseptically. Keep the donor film on its paper carrier and place it on the disinfected bench (see [Figure 2](#)), with the paper side facing down and the PU side to be coated with spores, facing up. Using a permanent marker, mark the inoculation area on the donor corresponding to the area of an agar plate.

Using a 1,0 ml micropipette, distribute uniformly 1 ml of the of *Bacillus atrophaeus* suspension over that area. Spread the inoculum with a rake ([5.16](#)). The spreading does not result in an immediate formation of a film, but small drops form over the entire surface (see [Figure 2](#)).

Immediately after inoculation of the donor, the remaining inoculum is put on ice.

- b) Dry the donor in an incubator at 56 °C for approximately 30 min until completely dry, while spreading the *Bacillus atrophaeus* suspension on the donor every 2 min to ensure an even distribution (see [Figure 2](#)).

NOTE 1 By opening the incubator every 2 min, the temperature of 56 °C will decrease slightly during drying. The intention is to obtain a uniform distribution of the spores while not exceeding 56 °C in the incubator.

NOTE 2 To avoid loss of spores, between each step of spreading (every 2 min) the rake can be stored on a rake-holder with part of the rake being in contact with the spores upwards. Avoid contact of that part of the rake being in contact with the spores with any surface on which spores can be lost (e.g. the bottom of a beaker).

The donor shall be used immediately after drying.

9.4 Test material

The size of the test specimen shall be ~25 cm × 25 cm or approximately 25 cm in diameter.

Each test specimen shall be packed individually and sterilized, e.g. with steam or by means of ethylene oxide, according to the recommendations of the supplier.

In case un-sterilized materials are cut, prepared and tested at the test lab, it shall be reported whether or not the samples are sterilized before testing and if sterilized, the method of sterilization used.

The side to be brought in contact with the inoculated donor shall be stated by the manufacturer of the material. If it is not stated on the labelling or instructions for use (IFU), then the side of the gown to be tested shall be the external surface of the gown or drape (i.e., the side which is not in contact with the wearer/patient).

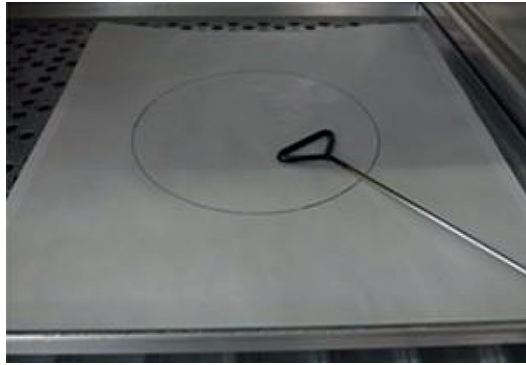
The test is carried out on 5 test specimens where each test specimen is brought 5 times into contact with a new 14 cm agar plate for 15 min.

NOTE 1 Woven materials are often sterilized at (134^{+3}_0) °C for 4 min. Non-woven materials are generally sterilized with ethylene oxide. Ethylene oxide (ETO) is prohibited in many countries.

NOTE 2 The delivery status of the materials to be tested should preferably be finalized and sterilized products.

9.5 Preparation of the test assembly

Sterilized forceps (or an equivalent instrument) are used to separate the donor PU-film partially from the paper sheet at one or two edges. The donor, with the PU-film still on the paper support, is placed with the inoculated side facing down onto the textile specimen. The PU-film is fixed onto the textile by hand, holding the film at one or two edges. The paper carrier is carefully removed and discarded. The cover film (HDPE-film) is placed on top of the PU-film to finish the test assembly (see [Figure 2](#)).

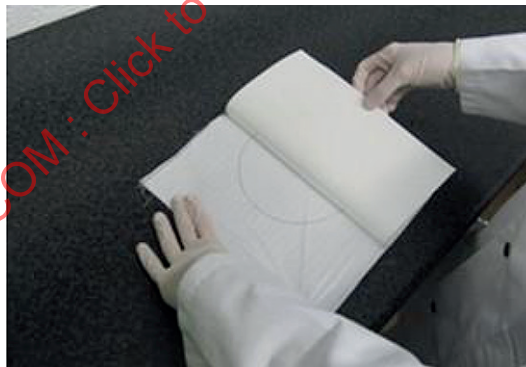


NOTE Spreading with a rake at intervals during drying at 56 °C leads to an even distribution of the spores.

a) Spore suspension distributed over the marked inoculation area of the donor foil in droplets



b) PU-foil separated partially from the paper support at one or two edges using sterilized forceps



NOTE The paper support at the back of the donor is removed.

c) Dried donor put with the spore coated PU-side on the fabric to be tested

Figure 2 — Inoculation of the donor and preparation of the test assembly

9.6 Test procedure

The force of $(3,00 \pm 0,05)$ N exerted by the finger on the materials shall be checked at least once a day before starting the preparations of the test and shall be adjusted if necessary (see [Figure B.3](#)).

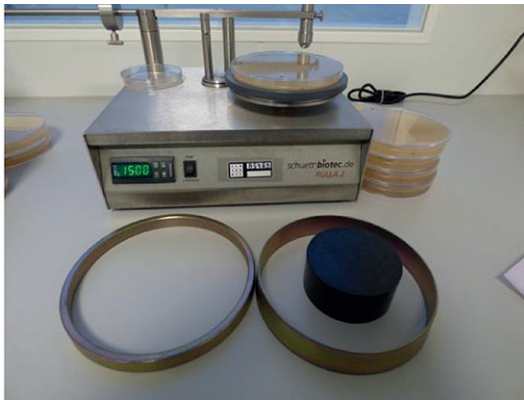
Place the first agar plate, with the lid, on the turntable.

Place the inner ring of the two metal conical rings [see [Figure 3 a](#)] and [Figure B.2](#)] on the disinfected workbench and put the sterile cylindrical object into the centre. Place the assembly specimen-donor-HDPE [see [9.5](#), [Figure 3 b](#)] and [Figure 3 c](#)] on the cylindrical object with the specimen facing down

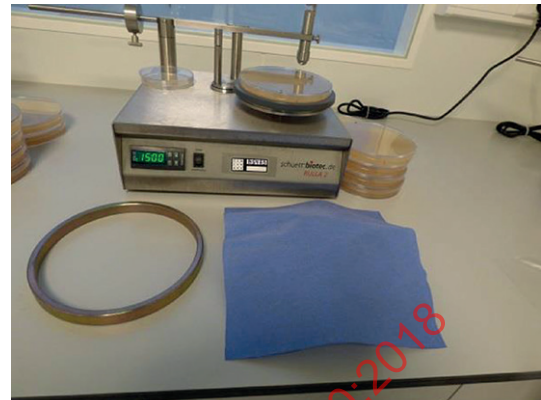
and the HDPE-side facing up. Centre the marked side carefully in the ring. Place the outer ring on top of the test assembly, aligned with the inner ring, and push the outer ring down firmly so that the three materials are clamped together and securely fixed between the two rings [see [Figure 3 d](#)]. Remove the lid from the agar plate and, with the materials slightly slack, place the rings over the agar plate on the turntable. The steel rings shall hang freely over the brim of the plate [see [Figure 3 e](#)].

NOTE 1 Due to the somewhat higher cylindrical object placed in the centre of the inner ring when preparing the test specimen, the assembly will be slightly slack when the rings are lifted from the cylindrical object.

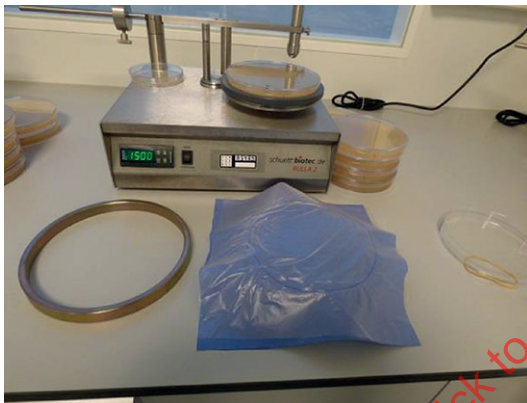
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a) Metal conical rings with sterile cylindrical object



b) Specimen on the Metal conical rings and sterile cylindrical object



c) Assembly specimen-donor-HDPE film on the Metal conical rings and sterile



d) Assembly specimen-donor-HDPE fixed between the two rings



e) Assembly specimen-donor-HDPE, fixed between the two rings placed over the Petri dish on the turntable and the steel finger placed on the test assembly just inside the brim of the petri dish

Figure 3 — Preparation of the test specimen and assembly of materials

Place the finger on the test assembly just inside the brim. The test specimen shall touch the agar surface. Run the test as described for 15 min with a finger pressure of $(3,00 \pm 0,05) \text{ N}$.

The rotation speed of the turntable shall be (60 ± 1) r/min. After 15 min, immediately remove the rings with the test assembly and place them on the disinfected workbench. Remove the first agar plate from the turntable, and seal it by putting the lid on. Immediately place the second agar plate on the turntable, place the test assembly on the agar plate, put the finger on the assembly just inside the brim and continue the test for 15 min.

Repeat the procedure on the same test assembly, using in total 5 agar plates.

Dry the agar plates for approximately 10 min on a clean bench, preferably under a laminar airflow, and incubate them with their lids on for 16 h to 24 h at (37 ± 2) °C until the growth is sufficient to enable accurate counting, but colonies on the plate are not confluent.

Repeat the procedure on the other four test specimens, using a freshly prepared donor for each test specimen.

The apparatus, including the finger, rings and the worktops shall all be cleaned with a sporicidal between tests.

NOTE 2 If e.g. for research purposes and/or material development an estimation needs to be achieved of the bacterial contamination remaining on the upper side of the test specimen and remaining on the donor, the procedure in [Annex D](#) applies.

9.7 Control plates

9.7.1 Environmental controls

Place an opened agar plate, 9 cm Petri dish, containing TGEA in the incubator at 56 °C during the drying of the donors.

Place an opened agar plate, 9 cm Petri dish, containing TGEA on the apparatus (see [Figure 3](#)) during the whole testing of one specimen.

The test of one specimen is finished after the specimen has been removed from the 5th agar plate.

Incubate the plates at (37 ± 2) °C for 16 h to 24 h. If no colonies are visible, incubate the plates at (37 ± 2) °C for an additional 16 h to 24 h.

NOTE Spores can need more than 24 h to form visible colonies.

The number colonies of the control plates should be for the incubator <4 and for the duration of the test <25 .

9.7.2 Bacterial challenge

Perform a viable count using standard microbiological techniques on the inoculum placed on ice, i.e. a serial dilution of the suspension with peptone water and spreading of 100 µl on 9 cm TGEA plates. The suspension shall be diluted sufficiently to enable accurate counting of the colonies on the final plate.

Incubate the plates at (37 ± 2) °C for 16 h to 24 h until the growth is sufficient to enable accurate counting, but colonies on the plate do not confluent.

Determine at least in triplicate the bacterial challenge 'I' brought on the donor, expressed as the number of colonies in 1 ml of inoculum.

10 Evaluation

10.1 Required conditions for a valid test

- The Wet Bacterial Penetration, $P(\%)$, of the reference material shall be determined on a regular basis and documented in accordance with the Quality Management System, in which the test frequency is justified.
- The Wet Bacterial Penetration, $P(\%)$, of the reference material shall be within the range of 0,64 % to 3,04 %.
- Equipment requirements:
 - Rotating speed agar plate (60 ± 1) r/min;
 - Movement of the lever by an eccentric cam rotating at $(5,6 \pm 0,1)$ r/min;
 - Steel finger, semi-spherical polished end, radius of 11 mm, removable;
 - Force $(3,00 \pm 0,05)$ N exerted by the steel finger on the materials, adjustable by a slidable weight.
- Distance to brim $(3,0 \pm 0,5)$ mm, to be determined for each batch of plates poured at one time.
- Inoculum concentration of $5,0 \times 10^3$ to $1,5 \times 10^4$ spores per ml.
- A maximum countable number of colonies for a 14 cm diameter agar plate is 760 colonies.
- If any of the 5 test plates cannot be counted, the % Penetration cannot be calculated.
- For materials with plates showing excessive growth of >760 colonies the bacterial load shall be lowered until the number of colonies is <760 colonies where the bacterial challenge will be at least of 5 000 spores per ml. For materials with plates still showing excessive growth of >760 colonies this test is not applicable as the relation bacterial challenge versus passed bacteria is out of balance.

NOTE 1 Excessive growth on the plates can be an indication of low barrier performance.

NOTE 2 Invalid test results can be reported for information only, especially in view of the background why the test was not valid.

10.2 Determination of the number of colonies of *Bacillus atrophaeus*

Count the number of colonies of *Bacillus atrophaeus* on each plate of the test. Disregard the count in the area with a radius of 15 mm from the centre of the plate. Plates with excessive growth and many confluent colonies cannot be counted and shall be disregarded (see 10.1). The counting can be carried out visually or by using an automatic colony counter, provided that a correlation between visual and automatic counting has been established.

11 Expression of the results

Calculate the percentage of penetration in three digits for every test specimen as P_n , using the [Formula \(1\)](#):

$$P_n(\%) = \frac{[S \times 100]}{I} \quad (1)$$

where

- P is the percentage of penetration for each test specimen n ;
- S is the total of the counted colonies on the 5 plates, excluding a centre circle with a radius of 15 mm;
- I is the bacterial challenge of *Bacillus atrophaeus* spores on the donor defined as the number of bacteria in 1 ml of inoculum as determined in [9.7.2](#).

Calculate the mean percentage of penetration $P(\%)$ of all 5 specimens.

NOTE A penetration of 1 spore and a bacterial challenge of 15 000 spores on the donor results in 0,006 7 % of penetration. The percentage of penetration reported is 0,01 %.

EXAMPLE Report of penetration in three significant digits: 12,1 %, 1,21 %, 0,12 %, 0,01 %

12 Precision

12.1 Reference material

Precision data have been determined by analysis of a woven reference material.

Mean	1,84 $P(\%)$
Standard deviation	0,60 $P(\%)$
95,4 % Confidence Interval	0,64 to 3,04 $P(\%)$
Number of measurements N	35

12.2 Sample material

Precision data have been determined by analysis of a woven reference material and four commercially available materials, two woven and 2 non-woven materials.

The investigation showed a different percentage of Wet Bacterial Penetration ($P(\%)$) for each material with a different precision, with the result that the five materials tested are not considered to be representative of the materials currently on the market. Due to this, the different $P(\%)$ levels and the corresponding different precision levels, a realistic and uniform precision statement for this document covering all materials, cannot be determined on the basis of the available data (see [Annex F](#)). Investigations are currently being carried out on further improvement of the reproducibility of this test method.

13 Test report

The test report shall include at least the following information:

- a) a reference to this document, i.e. ISO 22610:2018;

- b) all details necessary for the complete identification of the test specimen;
- c) results of the performance monitoring according to [Annex C](#);
- d) test conditions; temperature and humidity;
- e) distance from agar to brim of the Petri dish;
- f) individual colony counts of all plates;
- g) percentage of penetration for each test specimen;
- h) the mean percentage of penetration $P(\%)$ of all 5 specimens;
- i) strain number;
- j) concentration inoculum;
- k) any deviation from the test method in this document shall be reported and justified.

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Annex A (normative)

Media

A.1 TGE agar (TGEA)

Beef extract	3 g
Tryptone	5 g
Dextrose	1 g
Agar	15 g
Distilled water	add up to a final volume of 1 000 ml

Suspend the dry ingredients in water and heat while swirling to dissolve and mix. Sterilize at $(121 \pm 3)^\circ\text{C}$ for 15 min, swirl thoroughly and dispense.

After sterilization, the pH shall be $7,0 \pm 0,25$. If the media pH is out of range after sterilization, discard the media and use or prepare a new batch.

Commercially suitable TGEA media can be used. TGEA qualities may differ. Inferior TGEA qualities may lead to petri dishes that show cracks, pits or indentation on the surface after conducting the test procedure.

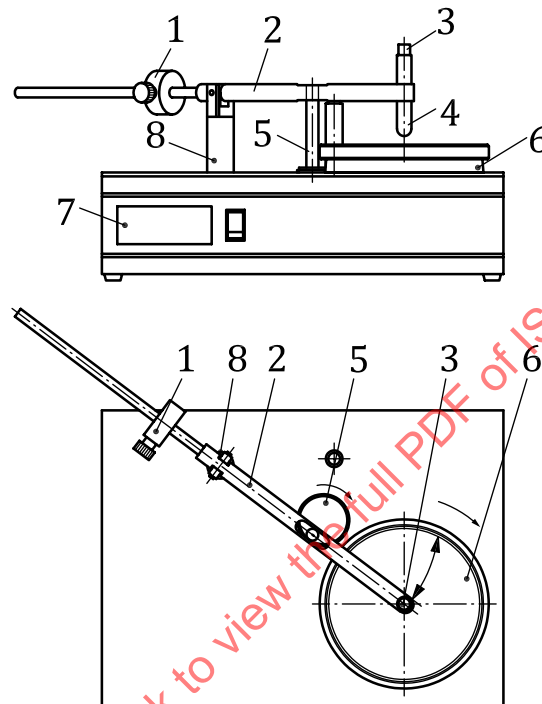
A.2 Peptone water

Peptone	10 g
Sodium chloride	5 g
Polysorbate 80	1 g
Distilled water	add up to a final volume of 1 000 ml

Suspend the dry ingredients in water and heat while swirling to dissolve and mix. Sterilize at $(121 \pm 3)^\circ\text{C}$ for 15 min.

Annex B (normative)

Test apparatus and accessories



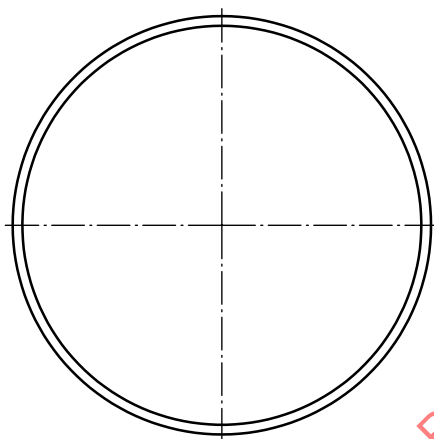
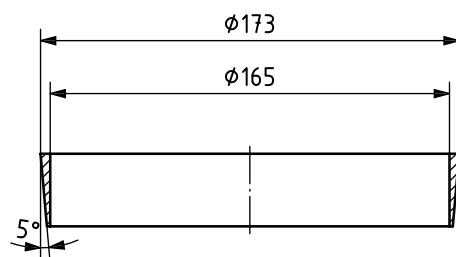
Key

- 1 counterweight
- 2 balancing arm with finger
- 3 loop for spring balance/dynamometer
- 4 stainless steel finger
- 5 eccentric cam
- 6 turntable
- 7 electronic timer
- 8 ball bearing

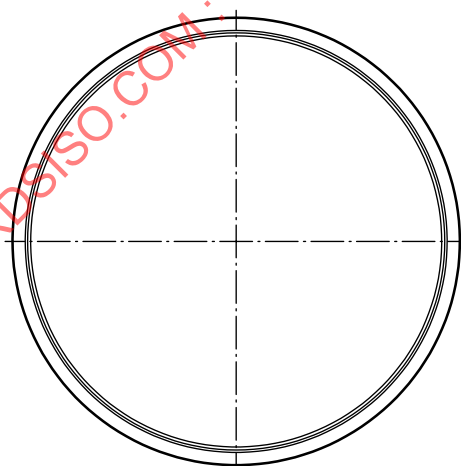
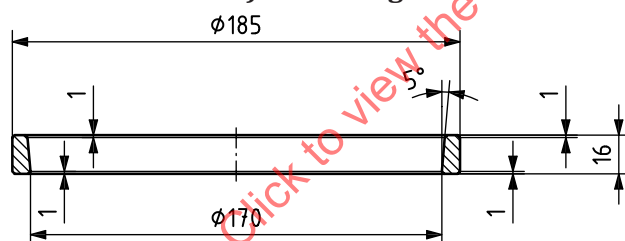
Figure B.1 — Apparatus

The conical steel rings (see Figure B.2) shall have a total weight of (800 ± 1) g for fixation of the material assembly.

Dimensions in millimetres



a) Inner ring



b) Outer ring

Figure B.2 — Conical steel rings for fixation of the material assembly

The force of $(3,00 \pm 0,05)$ N exerted by the finger on the materials shall be checked at least once a day before starting the preparations of the test and adjusted if necessary.

The force exerted by the steel finger on the materials is measured by using a dynamometer attached to the lever, or by a balance placed on the turntable. If necessary adjust the force by sliding the weight on the lever (see [Figure B.3](#)).

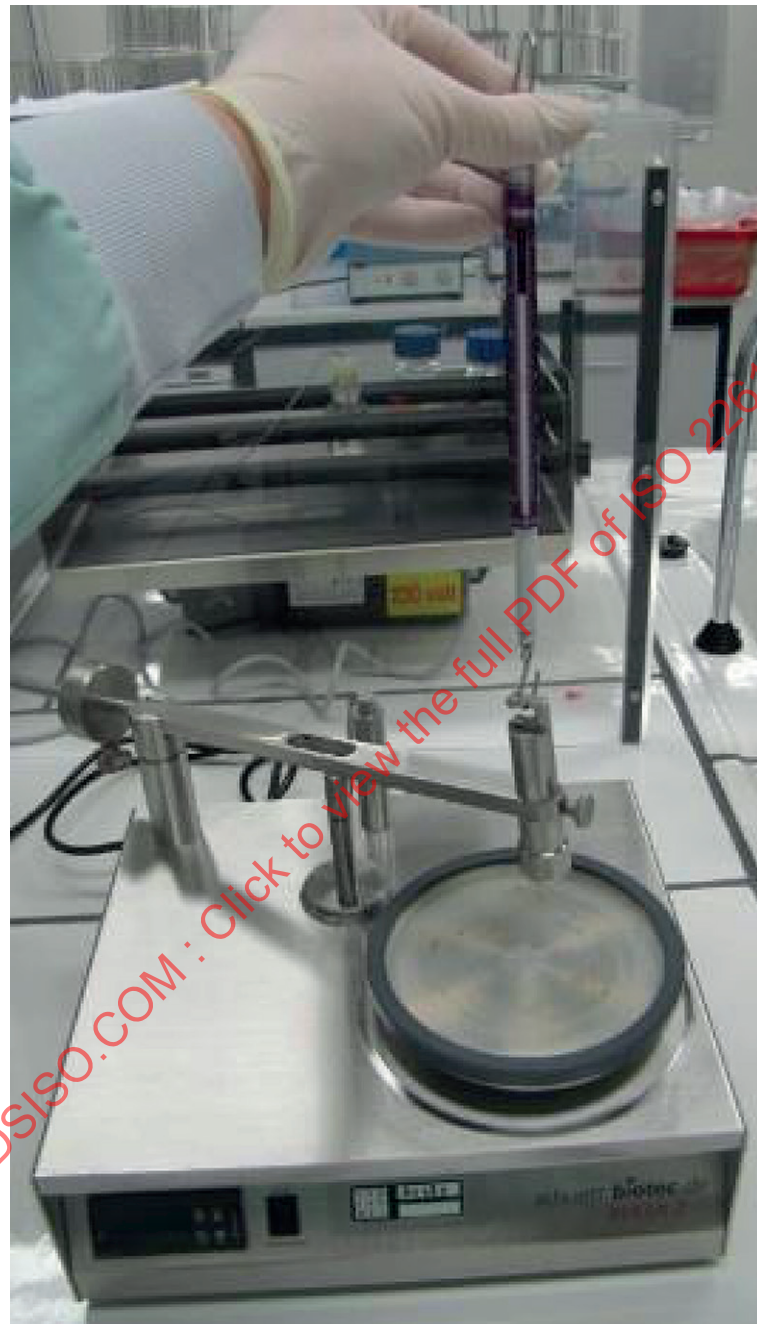


Figure B.3 — Force adjustment of the finger on the agar plate

Annex C (normative)

Equipment and laboratory performance monitoring

C.1 General

The two methods below shall be used to assess the performance of the laboratory. There shall be a documented programme of performance monitoring and the date of the latest assessment shall be recorded.

C.2 Equipment monitoring

Prepare an assembly using the steel rings specified in [Annex B](#), consisting of one sheet of white paper, one sheet of carbon paper and one sheet of HDPE film. Put the bottom part of a 14 cm Petri dish upside down on the turntable and the assembly over it, as described in [9.6](#). Apply the finger to the HDPE film and run the apparatus for 15 min. Extract the white paper and ensure that the finger has left an even contact pattern over the whole surface of the plate.

C.3 Laboratory monitoring

The precision of the laboratory shall be assessed using the reference material specified in [5.12](#), in combination with the test method described in this document. The frequency of assessments shall be determined in accordance with ISO/IEC 17025. This will allow the laboratory to check the precision and bias of its own operators. It will not be possible to infer any information about the test method in general use, without conducting further inter-laboratory trials. The reference material shall be packed in a sterilizer bag that conforms to ISO 11607-1 and sterilized with moist heat at $(134 \pm 2)^\circ\text{C}$ for 4 min to 5 min, in accordance with ISO 17665-1.

Annex D (informative)

Estimation of the bacterial contamination on the upper side of the test specimen and the bacterial load remaining on the donor

D.1 General

The intention of this document is to determine the wet bacterial penetration where the result is expressed as a percentage of the bacterial load brought on the donor.

If e.g. for research purposes and/or material development, it is considered to be useful to achieve an estimation of the bacterial load remaining on the upper side of the test specimen and the load remaining on the donor, the following procedure should be applied.

D.2 Preparation of additional Petri dishes

If after the five consecutive tests on the test specimen, additional tests are being carried out in order to achieve an estimation of the remaining bacterial load on the upper side of the test specimen and the bacterial load remaining on the donor material (see 9.6), 2 more Petri dishes, 14 cm in diameter, need to be prepared for one complete test.

D.3 Procedure

- a) Disassemble the test set up, remove the donor aseptically, store it on an empty sterile Petri dish for determination of the bacterial load remaining on the donor.
- b) Turn the test specimen upside down on the inner steel ring, cover the material with a new HDPE film, and fix the assembly between the steel rings.
- c) Perform the test 15 min, as described above, using the sixth agar plate.
- d) Disassemble the test set up, remove the test specimen and place the donor with the contaminated side facing downwards on the inner ring, cover the donor with the HDPE film, and fix the assembly between the rings.
- e) Perform the test 15 min, as described above, using the seventh agar plate.
- f) Dry the agar plates for 10 min on a clean bench, preferably under laminar flow, and incubate them with their lids on for 16 to 24 h at $(37 \pm 2) ^\circ\text{C}$ until the growth is sufficient to enable accurate counting, but colonies on the plate do not confluent
- g) Repeat the procedure on the other four test specimens.

The apparatus, including the finger, rings and the worktops shall all be cleaned with a sporicidal between tests.