INTERNATIONAL STANDARD

**ISO 22612**

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# Clothing for protection against infectious agents — Test method for resistance to dry microbial penetration

*Vêtements de protection contre les agents infectieux — Méthode d'essai de la résistance à la pénétration microbienne par voie sèche*



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# Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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ISO 22612 was prepared by the European Committee for Standardization (CEN) in collaboration with Technical Committee ISO/TC 94, *Personal safety — Protective clothing and equipment*, Subcommittee SC 13, *Protective clothing*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

Throughout the text of this document, read "...this European Standard..." to mean "...this International Standard...".

For the purposes of this International Standard, the CEN annexes regarding the fulfilment of the European Council Directives have been removed.

# Contents

# Foreword

This document (EN ISO 22612:2005) has been prepared by Technical Committee CEN/TC 205 “Non-active medical devices”, the secretariat of which is held by BSI, in collaboration with Technical Committee ISO/TC 94 “Personal safety - Protective clothing and equipment”.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by August 2005, and conflicting national standards shall be withdrawn at the latest by August 2005.

This document has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EU Directive(s).

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

## Introduction

There are numerous examples of situations where bacteria may migrate through a barrier material in the dry state carried by organic or inorganic particles. The dry penetration of bacteria-carrying skin scales through an operating gown or a clean air suit is one example. Penetration through a packaging material during storage is another.

This document EN ISO 22612 describes a test method, with the associated equipment, that may be used to determine a material's resistance to dry penetration of bacteria on particles in the size range most typical for human skin scales

# 1 Scope

This test method provides a means for assessing the resistance to penetration through barrier materials of bacteria-carrying particles.

NOTE Due to its complexity, this EN ISO 22612 cannot be considered as a useful method for routine quality control but may suit the needs when a material is assessed for compliance with the requirements of current regulations such as EU Directive 93/42/EEC.

# 2 Normative references

The following referenced document is indispensable for the application of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 13795-1:2002, *Surgical drapes, gowns and clean air suits, used as medical devices for patients, clinical staff and equipment – Part 1: General requirements for manufacturers, processors and products.*

# 3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 13795-1:2002 apply.

# 4 Principle

The test is carried out on test pieces each fixed in a container. In every container except one a portion of talc contaminated with *Bacillus subtilis* is poured on the test piece. One container is left uncontaminated as a control. A sedimentation plate is inserted at the base of each container at a short distance below the test piece.

The apparatus supporting the containers is then vibrated by a pneumatic ball vibrator. The talc that penetrates is captured on the sedimentation plate. The sedimentation plates are removed and incubated.

The numbers of colonies produced are counted.

This document specifies two levels of challenge by means of giving two concentrations of bacterial cells on the talc particles and two times during which the barrier is subjected to vibration. The conditions for testing differ among product types and will be specified in other standards where this test method is applied such as in prEN 13795-3.



# 5 Testing conditions

Condition the samples and test at (20 ± 2) ºC and (65 ± 5) % relative humidity.

# 6 Equipment

## 6.1 General lay-out

NOTE See Figure 1.

**6.1.1** A 10 mm thick stone plate, such as marble, 40 cm x 40 cm, underneath which 4 rubber stoppers are mounted at the corners.

**6.1.2** A pneumatic ball vibrator[1)], able to generate 20 800 vibrations per minute with a force of 650 N.

**6.1.3** The vibrator is attached by means of screws to the upper surface of the marble plate along one of its sides.

**6.1.4** A compressed air flow meter capable of measuring the flow of air required to achieve a vibration frequency of 20 800 vibrations per minute.

**6.1.5** Six stainless steel test containers.

**6.1.6** A stainless steel plate with 6 retaining holes of suitable dimensions to fit the containers, the plate being held to the stone plate by means of clips.

**6.1.7** Stopwatch.

## 6.2 Test containers

NOTE See Figure 2.

**6.2.1** A suitable stainless steel container with a lid. The lid has a central aperture through which a metal plunger may be inserted to reach 10 mm underneath the lid to ensure that the test material is slack when inserted.

**6.2.2** Each container has a sedimentation plate insertion slot near the base.

**6.2.3** To ensure good contact between the containers and the stone plate by means of the fixing plate, each container is equipped with a rubber ring resting on its flanged base.

**6.2.4** The rim of the container is chamfered to prevent damage to the test piece when inserted.

**6.2.5** A supply of 9 cm diameter Petri dishes containing TGE agar (see Annex A).

## 6.3 Method to infect talc with spores

### 6.3.1 Materials

**6.3.1.1** 50 g ± 0,5 g of talc (95 % < 15 $\mu$ m)[2)]

**6.3.1.2** Purified spores of *Bacillus subtilis* ATCC 9372 at a concentration of ≥ $10^9$ /ml of ethyl alcohol [3)].

**6.3.1.3** TGE agar plates.

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1) e.g. K13, made by ERKALAITE OY, Helsinki, Finland. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

2) e.g. FINNTALC M15 from OMYA BENELUX S.A., Place Eug. Keym 43 B 27, B-1170, Bruxelles, tel.: +32 26 74 23 11, fax. +32 2672 92 68. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

3) e.g. SIMICON GmbH, Schuhmacherring 12, D-81737 München, fax +49 89 67 33 66 22. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of this product.

### 6.3.2 Procedure

**6.3.2.1** Prepare 50 g sterile talc in a suitable container and sterilise at 160°C with dry heat for (2 – 0/+ 1) h.

**6.3.2.2** Open an ampoule of 5 ml of the ethanolic spore solution.

**6.3.2.3** Spread the spore solution in 50 steps (50 X 100 $\mu$ l) over the talc.

**6.3.2.4** After every step shake the closed vessel with a vortex vibrator.

**6.3.2.5** Put the opened vessel in a desiccator with silica gel and dry it at room temperature for 2 days to 3 days.

**6.3.2.6** Weigh the vessel before and after drying to ensure complete drying.

**6.3.2.7** Estimate the bioburden expressed as cfu/g (3 fold, each fold two times repeated) of the spore talc mixture on TGE agar after incubation overnight at 35 °C.

**6.3.2.8** The final concentration should be $10^4$ or $10^8$ cfu/g talc. Ensure that the spores are homogeneously distributed in the talc.

## 7 Procedure

**7.1** Cut 12 test pieces 200 mm x 200 mm.

**7.2** Put test pieces in sterilising bags and sterilize by the method given by the manufacturer.

**7.3** Put containers in sterilising bags and sterilize.

**7.4** Fix the bases of the containers onto the stone plate by means of the fixing plate and secure with the clips.

**7.5** Aseptically remove the pieces of test material from the bags and place over the mouths of the test containers.

**7.6** With the plungers distended downwards, affix the lids to the containers thus fixing the test pieces with controlled slackness.

**7.7** Remove the plungers.

**7.8** Pour a 0,5 g ± 0,1 g portion of contaminated talc through each plunger orifice onto 5 of the test materials leaving the 6th one uncontaminated as a control.

**7.9** Seal the orifices with cling film.

**7.10** Put a small plastic bag over each container.

**7.11** A lidless sedimentation plate is inserted through the slot at base of each container.

**7.12** Close the slots with adhesive tape.

**7.13** Run the vibrator at an air flow that achieves vibration frequency of 20 800 vibrations per minute.

**7.14** Remove plastic bags and adhesive tape.

**7.15** Insert the lids of the sedimentation plates through the slots.

**7.16** Remove sedimentation plates and incubate at 35 °C for 24 h.

**7.17** Count the number of colonies produced. The control plate (6th) should read 0. If not the test should be aborted as there is extraneous contamination.

**7.18** For each material repeat steps 7.1 to 7.17.

**7.19** Calculate the arithmetic mean for the 10 valid results.

## 8 Test report

The test report shall include the following information:

a) identity of material tested;

b) test conditions, especially those variations chosen in 6.3.2.8 and 7.13;

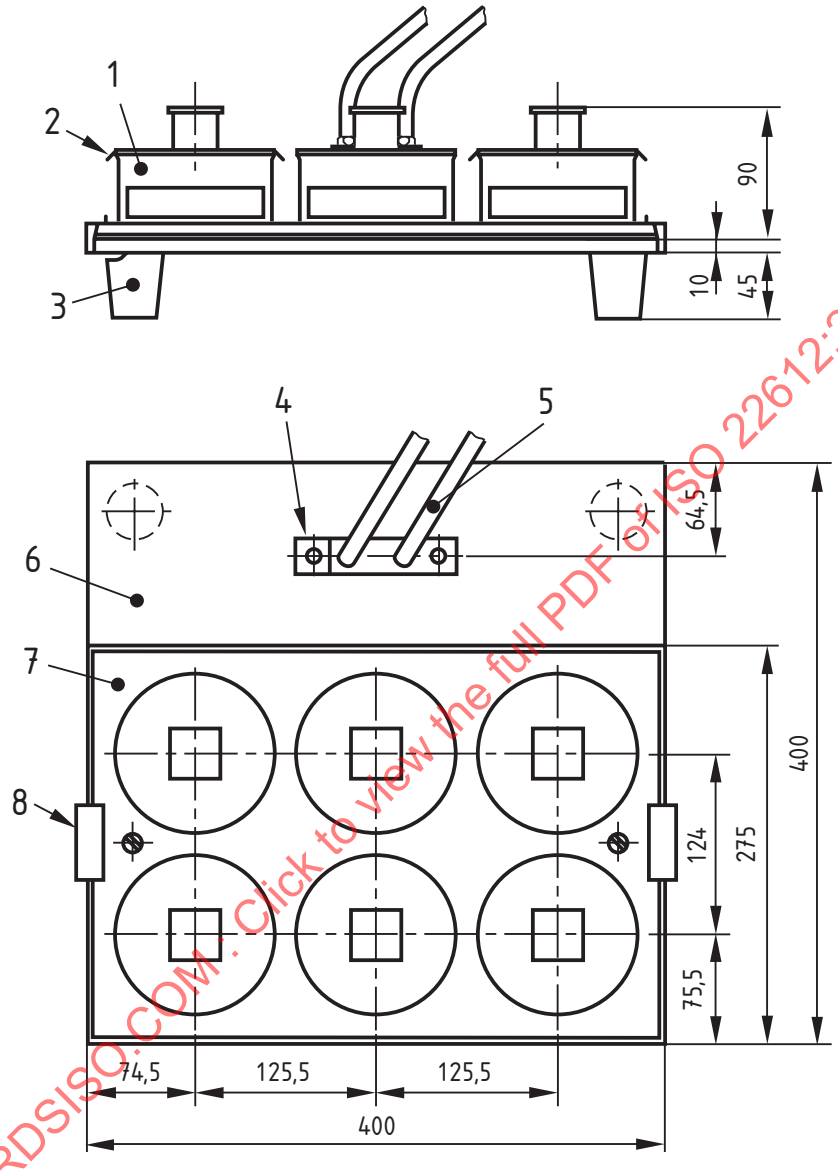
c) number of test pieces tested;

d) any deviations from the standard method;

e) details of the contaminant used;

f) geometric mean of bacterial count (see 7.19).

Dimensions in millimetres



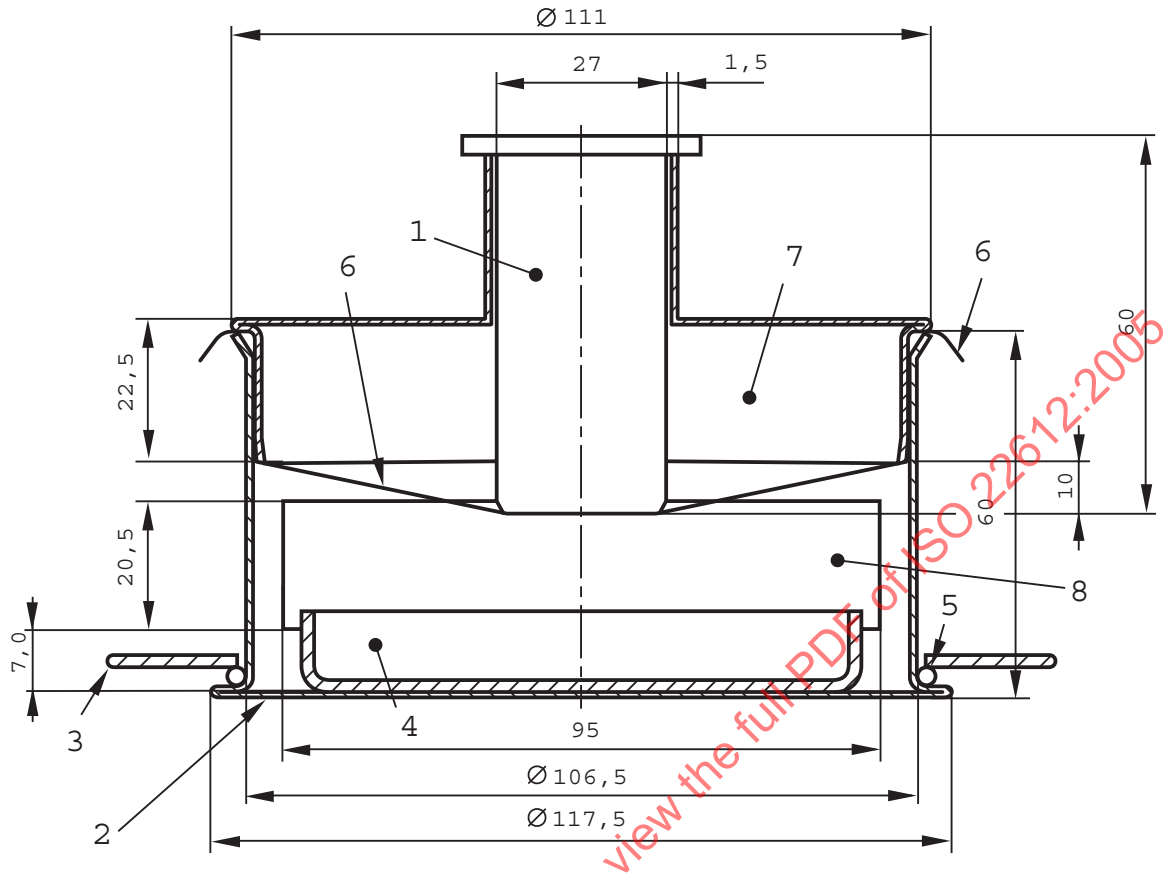



**Key**

1 Test container
2 Test material
3 Rubber stoppers
4 Pneumatic ball vibrator
5 Pneumatic hoses
6 Marble plate
7 Fixing plate
8 Clips

**Figure 1 — General layout of test equipment**

Dimensions in millimetres




**Key**

1 Metal plunger
2 Base of container
3 Fixing plate
4 Sedimentation plate
5 Rubber ring
6 Test material
7 Lid
8 Sedimentation plate insertion slot

**Figure 2 — Test container**