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Kemiska disinfektionsmedel och antiseptiska medel – Grundläggande sporocid effekt – Provningsmetod och krav (fas 1)

Chemical disinfectants and antiseptics – Basic sporicidal activity – Test method and requirements (phase 1)

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Désinfectants et antiseptiques chimiques - Activité sporicide de base - Méthode d'essai et prescriptions (phase 1)

Chemische Desinfektionsmittel und Antiseptika - Sporizide Wirkung (Basistest) - Prüfverfahren und Anforderungen (Phase 1)

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Management Centre: rue de Stassart, 36 B-1050 Brussels

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Contents

Foreword

This document (EN 14347:2005) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

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of an identical text or by endorsement, at the latest by September 2005, and conflicting national standards shall be withdrawn at the latest by September 2005.

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Introduction

This document describes a suspension test for establishing whether a chemical disinfectant or antiseptic has or does not have a sporicidal activity in the areas described in the scope.

If a product complies with the test requirements, it can be considered as possessing a sporicidal activity. The acceptibility of a product as a chemical disinfectant or antiseptic for a defined purpose cannot be determined from this test method. Chemical disinfectants or antiseptics are subjected to further testing by relevant tests according to European Standards to evaluate their activity under conditions appropriate to their intended use.

There is no evidence that the strains used in this document are virulent.

1 Scope

This document specifies a test method (phase 1) and the minimum requirements for sporicidal activity of chemical disinfectant or antiseptic products that form a homogeneous, physically stable preparation when diluted with water. Products can only be tested at a concentration of 80 % or less as some dilution is always produced by adding the test organisms.

This document applies to products that are used in agricultural (but not crop protection), domestic service, food hygiene and other industrial fields, institutional, medical and veterinary applications.

NOTE 1 This method cannot be applied for testing sporicidal activity of a product against spores of *Clostridium sp.*

NOTE 2 This document does not evaluate the activity of a product for an intended use. More specific test methods described in European Standards (see Introduction) are used for further assessement of the efficacy of chemical disinfectants and antiseptics for a defined purpose.

NOTE 3 This method corresponds to a phase 1 test (Annex E).

2 Normative references

The following referenced documents are 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 12353, *Chemical disinfectants and antiseptics – Preservation of microbial strains used for the determination of bactericidal and fungicidal activity*

EN 14079, *Non-active medical devices – Performance requirements and test methods for absorbent cotton gauze and absorbent cotton and viscose gauze*

3 Terms and definitions

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

3.1
product (for chemical disinfectants and/or antiseptics)
chemical agent or formulation used as chemical disinfectant or antiseptic

3.2
sporicide
product which kills dormant bacterial spores of relevant test organisms under defined conditions

NOTEThe adjective derived from "sporicide" is "sporicidal"

3.3
sporicidal activity
capability of a product to produce a reduction in the number of viable bacterial spores of relevant test organisms under defined conditions

3.4
sporistatic activity
capability of a product to inhibit the germination of dormant bacterial spores under defined conditions

4 Requirements

The product, when diluted with water (5.2.2.2) and tested in accordance with Clause 5 under the obligatory test conditions (20 °C; 30 min, 60 min or 120 min), shall demonstrate at least a decimal log (lg) reduction in viable counts of 4 and ONT (original neutralization tube) shows visible growth. It is possible to test also the product as delivered (highest test concentration is 80 %).

The sporicidal activity shall be evaluated using the dormant spores of: *Bacillus subtilis* and *Bacillus cereus.*

Where indicated, additional specific sporicidal activity shall be determined applying other contact times, temperatures and test organisms in accordance with 5.2.1 and 5.5.1.1 in order to take into account intended specific use conditions.

NOTE For these additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions.

5 Test method

5.1 Principle

5.1.1 A test suspension of bacterial spores is added to a sample of the product as delivered and/or diluted with water. The mixture is maintained at 20 °C ± 1 °C for a specific contact time chosen from one of the following 30 min ± 10 s, 60 min ± 10 s or 120 min ± 60 s. At the end of this contact time, an aliquot is taken and transferred into a original neutralization tube (ONT). This tube serves for neutralizing the sporicidal and/or the sporistatic action in this portion and, after incubation, is an indicator for a successful neutralization in the actual test. The numbers of surviving bacterial spores in each sample are determined (by counting the sporeforming bacteria) and the reduction is calculated.

5.1.2 The test is performed using dormant spores of *Bacillus subtilis* and *Bacillus cereus* as test-organisms.

5.1.3 Additional and optional contact times, temperatures and test organisms are specified. Further test organisms can be used.

5.2 Materials and reagents

5.2.1 Test organisms

The sporicidal activity shall be evaluated using the dormant spores of the following organisms[1)]:

— *Bacillus subtilis subsp. spizizenii* ATCC 6633

— *Bacillus cereus* ATCC 12826

1) The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collection (ATCC). This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of the product named.

If additional test organisms are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere, media) noted in the test report. If the additional test organisms selected do not correspond to the specified strains, their suitability for supplying the required inocula shall be verified. If these test organisms are not classified at a reference center, their identification characteristics shall be stated. In addition, they shall be held by the testing laboratory or national culture collection under a reference for 5 years.

NOTE See Annex A for corresponding strain reference in some other culture collections.

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this standard refer to the anhydrous salts. Hydrated forms may be used as an alternative, but the weights required shall be adjusted to allow for consequent molecular weight differences.

The reagents shall be of analytical grade and/or appropriate for microbiological purposes. They shall be free from substances that are toxic or inhibitory to the test organisms.

NOTE 1 To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

NOTE 2 For each culture medium and reagent a limitation for use should be fixed.

5.2.2.2 Water

The water shall be freshly glass distilled water and not demineralized water.

Sterilize in the autoclave [5.3.2.1 a)].

NOTE 1 Sterilization is not necessary if the water is used - e.g. for preparation of culture media - and subsequently sterilized.

NOTE 2 If water of adequate quality is not available, water for injections (see bibliographic reference [2]) can be used.

5.2.2.3 Tryptone Soya Agar (TSA)

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of Soybean meal	5,0 g
Sodium chloride (NaCl)	5,0 g
Agar	15,0 g
Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at 20 °C.

NOTE In special circumstances (problems with neutralization - see 5.5.1.2 and 5.5.1.3) it may be necessary to add neutralizer to TSA (Annex B.3).

5.2.2.4 Blood agar[2)]

Protease peptone	15,0 g
Liver digest	2,5 g
Yeast extract	5,0 g
Sodium chloride (NaCl)	5,0 g
Agar	12,0 g
Water (5.2.2.2)	to 1 000,0 ml
Defibrinated sterile sheep blood	70 ml to 100 ml

Sterilize in the autoclave excluding the sheep blood [5.3.2.1 a)]. After sterilization cool down to 45 °C to 50 °C and add the sterile defibrinated sheep blood and mix thoroughly. Fill into Petri dishes.

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product being tested in accordance with 5.5.2.4. The neutralizer shall be sterile.

NOTE 1 Information on neutralizers that have been found to be suitable for some categories of products is given in Annex B.

NOTE 2 The neutralizer has to form a homogenous, clear, not cloudy preparation when added to TSB [5.2.2.6 b)].

5.2.2.6 Tryptone Soya Broth (TSB)

a) Composition of Tryptone Soya Broth (TSB)

Tryptone, pancreatic digest of casein	17,0 g
Soya peptone, papaic digest of Soybean meal	3,0 g
Sodium chloride (NaCl)	5,0 g
Dipotassiumhydrogenphosphat (K_2HPO_4)	2,5 g
Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization the pH of the medium shall be equivalent to 7,3 ± 0,2 when measured at 20 °C.

b) Trypone Soya Broth (TSB) with neutralizer.

TSB [5.2.2.6 a)]. An adequate neutralizer shall be added according to its chemical properties before or after autoclaving (5.2.2.5). TSB plus neutralizer should be filled into glass tubes both in portions of 9 ml and 10 ml.

2) OXOID blood agar base in an example of a suitable product available commercially.

5.2.2.7 Sporulation Agar (Manganese-Sulfate-Agar):

Peptone USP 10,0 g

Yeast extract 2,0 g

Manganese sulfate ($MnSO_4$) 0,04 g

Agar 15,0 g

Water (5.2.2.2) to 1 000,0 ml

Sterilize in autoclave [5.3.2.1 a)] at 121 °C ± 1 °C for 20 min. Fill 150 ml into 1 000 ml Roux-bottle (5.3.2.15).

5.3 Apparatus and glassware

5.3.1 General

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents or the sample, except those which are supplied sterile, by one of the following methods:

a) by moist heat, in the autoclave [5.3.2.1 a)];

b) by dry heat, in the hot air oven [5.3.2.1 b)].

5.3.2 Usual microbiological laboratory equipment [3] and in particular, the following:

5.3.2.1 Apparatus for sterilization:

a) for moist heat sterilization, an autoclave capable of being maintained at (121 $^{+3}_{0}$) °C for a minimum holding time of 15 min;

a) for dry heat sterilization, a hot air oven capable of being maintained at (180 $^{+5}_{0}$) °C for a minimum holding time of 30 min, at (170 $^{+5}_{0}$) °C for a minimum holding time of 1 h or at (160 $^{+5}_{0}$) °C for a minimum holding time of 2 h.

5.3.2.2 Water baths capable of being controlled at 20 °C ± 1 °C, at 45 °C ± 1 °C (to maintain melted TSA in case of pour plate technique) and at additional test temperatures ± 1 °C (5.5.1.1).

5.3.2.3 Incubator, capable of being controlled either at 36 °C ± 1 °C or at 37 °C ± 1 °C and at 30 °C ± 1 °C.

The same temperature shall be used for all incubations performed during a test and its control and validation.

5.3.2.4 pH-meter, having an accuracy of calibration of ± 0,1 pH units at 25 °C.

For measuring the pH of the agar media (5.2.2.3, 5.2.2.4 and 5.2.2.7) a puncture electrode or a flat membrane electrode shall be used.

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3) Disposable sterile equipment is an acceptable alternative to reusable glassware.