SLOVENSKI STANDARD
SIST EN 1040:2001

01-februar-2001

Kemična razkužila in antiseptiki - Osnovno baktericidno delovanje - Preskusna metoda in zahteve (faza 1)

Chemical disinfectants and antiseptics - Basic bactericidal activity - Test method and requirements (phase 1)

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

Antiseptiques et désinfectants chimiques - Activité bactéricide de base - Méthode d'essai et prescriptions (phase 1)

Ta slovenski standard je istoveten z: **EN 1040:1997**

ICS:

11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics
71.100.35	Kemikalije za dezinfekcijo v industriji in doma	Chemicals for industrial and domestic disinfection purposes

SIST EN 1040:2001 **en**

EUROPEAN STANDARD **EN 1040**

NORME EUROPÉENNE

EUROPÄISCHE NORM February 1997

ICS 11.080; 67.020

Descriptors: disinfectants, chemical compounds, tests, determination, antibacterial activity, preparation, filtration analysis, neutralization method, dilution, culture media

English version

Chemical disinfectants and antiseptics - Basic bactericidal activity - Test method and requirements (phase 1)

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

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CEN

European Committee for Standardization
Comité Européen de Normalisation
Europäisches Komitee für Normung

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Content

Foreword

This European Standard has been prepared by Technical Committee CEN/TC 216 "Chemical disinfectants and antiseptics" the secretariat of which is held by AFNOR.

Other methods to evaluate the efficacy of chemical disinfectants and antiseptics for different fields of application are in preparation.

Annex A is normative.

Annexes B, C, D, E, F and G are informative.

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of an identical text or by endorsement, at the latest by August 1997, and conflicting national standards shall be withdrawn at the latest by August 1997.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Denmark, Finland, France, Germany, Greece, Iceland, Ireland, Italy, Luxembourg, Netherlands, Norway, Portugal, Spain, Sweden, Switzerland and the United Kingdom.

Introduction

This European Standard describes a suspension test method for establishing whether a chemical disinfectant or antiseptic has or does not have a bactericidal activity under the laboratory conditions defined by this European Standard. If a product complies with the test requirements, it can be considered as possessing a bactericidal 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 and antiseptics are subjected to further testing by relevant tests according to European Standards to evaluate their activity under conditions appropriate to their intended use. [1)]

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

1 Scope

This European Standard specifies a test method (phase 1) and the minimum requirements for bactericidal activity of chemical disinfectant and antiseptic products that form a homogeneous physically stable preparation in water. This European Standard is applicable to products for use in agricultural (but not crop protection), domestic service, food hygiene and other industrial fields, institutional, medical and veterinary applications.

NOTE 1 : Using this European Standard it is not possible to determine the bactericidal activity of the undiluted product as some dilution is always produced by adding the inoculum.

NOTE 2 : This European Standard 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 assessment of the efficacy of chemical disinfectants and antiseptics for a defined purpose.

NOTE 3 : This method corresponds to a phase 1 test (see Annex G).

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies.

prEN 12353 Chemical disinfectants and antiseptics - Preservation of microbial strains used for the determination of bactericidal and fungicidal activity

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[1)] CEN/TC 216 will develop relevant tests.

3 Definitions

For the purposes of this European Standard the following definitions apply :

3.1 product (for chemical disinfection and/or antisepsis): Chemical agent or formulation used as a chemical disinfectant or antiseptic.

3.2 bactericide : Product which kills vegetative bacteria under defined conditions.

NOTE : The adjective derived from "bactericide" is "bactericidal".

3.3 bactericidal activity (EN 1040): Capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms under conditions defined by this European Standard.

4 Requirements

The product, when tested in accordance with clause 5, shall demonstrate at least a 10^5 log reduction in viable counts when the test organism is *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

5 Test method

5.1 Principle

5.1.1 A test suspension of bacteria is added to a prepared sample of the product under test. The mixture is maintained at 20 °C. At a specified contact time chosen from one of the following 1 min, 5 min, 15 min, 30 min, 45 min or 60 min ± 10 s, an aliquot is taken ; the bactericidal action in this portion is immediately neutralized or suppressed by a validated method. The method of choice is dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The number of surviving bacteria in each sample is determined and the reduction in viable counts calculated.

5.1.2 The test is performed using *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

5.2 Materials and reagents

5.2.1 Test organisms

The bactericidal activity shall be evaluated using two strains using the following two strains :

Pseudomonas aeruginosa	ATCC 15442 [2)]
Staphylococcus aureus	ATCC 6538 [2)]

[2)] ATCC 15442 and ATCC 6538 are the collection numbers of strains supplied by the American Type Culture Collection. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of the products named. Corresponding strains supplied by other culture collection may be used if they can be shown to lead to the same results

NOTE : See annex E for corresponding strain numbers in some other culture collections.

5.2.2 Culture media and reagents

5.2.2.1 General

The reagents shall be of analytical grade and/or appropriate for microbiological purposes.

NOTE : 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.

5.2.2.2 Water

The water shall be free from substances that are toxic or inhibiting to the bacteria. It shall be freshly glass distilled and not demineralized water.

Sterilize in the autoclave (see 5.3.1).

NOTE 1 : If the water is sterilized during the sterilization of the reagents this is not necessary.

NOTE 2 : If distilled water of adequate quality is not available, water for injectable preparations (see European Pharmacopoeia) can be used.

5.2.2.3 Tryptone soya agar (TSA)

For maintenance of bacterial strains and performance of viable counts.

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of Soybean meal	5,0 g
NaCl	5,0 g
Agar	15,0 g
Water (see 5.2.2.2)	to 1000,0 ml

Sterilize in the autoclave (see 5.3.1). After sterilization the pH of the medium shall be equivalent to 7,2 ± 0,2 when measured at 20 °C.

5.2.2.4 Diluent

Tryptone Sodium Chloride Solution :

Tryptone, pancreatic digest of casein	1,0 g
NaCl	8,5 g
Water (see 5.2.2.2)	to 1000,0 ml

Sterilize in the autoclave (see 5.3.1). After sterilization the pH of the medium shall be equivalent to 7,0 ± 0,2 when measured at 20 °C.

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product under test in accordance with annex A. The neutralizer shall be sterile.

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

5.2.2.6 Rinsing liquid (for membrane filtration)

The liquid shall be sterile, compatible with the filter membrane and capable of filtration through the filter membrane under the test conditions described in annex A.

NOTE : Information on rinsing liquids that have been found to be suitable for some categories of products is given in annex C.

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) in the autoclave (see 5.3.2.1) by maintaining it at 121 $^{+3}_{0}$ °C for a minimum holding time of 15 min ;

b) in the dry heat sterilizer (see 5.3.2.1) by maintaining it at 180 °C for a minimum holding time of 30 min, at 170 °C for at least 1 h, or at 160 °C for at least 2 h.

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.

b) For dry heat sterilization, a hot air oven capable of being maintained at a temperature of 180 °C for a minimum holding time of 30 min, at 170 °C for a minimum holding time of 1 h or at 160 °C for a minimum holding time of 2 h.

5.3.2.2 Water baths, capable of being controlled at 20 °C ± 1 °C and 45 °C ± 1 °C.

5.3.2.3 Incubator, capable of being controlled at either 36 °C ± 1 °C or 37 °C ± 1 °C. An incubator at 37 °C ± 1 °C may be used if an incubator at 36 °C ± 1 °C is not available.

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[3] Disposable equipment is an acceptable alternative to reusable glassware.

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

5.3.2.5 **Stopwatch**

5.3.2.6 **Agitator (electromechanical agitator, i.e. Vortex® mixer[4])**

5.3.2.7 **Membrane filtration apparatus** (if this method is used), constructed of a material compatible with the product under test, with a filter holder which shall have a usable volume of 50 ml minimum, and suitable for use with filters of diameter 47 mm to 50 mm, of 0,45 µm pore size.

The vacuum source used shall give an even filtration flow rate. In order to obtain a uniform distribution of the microorganisms over the membrane and in order to prevent overlong filtration, the device shall be set so as to obtain the filtration of 100 ml of rinsing liquid in 20 s to 40 s.

5.3.2.8 **Container** : test tubes or flasks of suitable capacity.

5.3.2.9 **Graduated pipettes**, of nominal capacities 10 ml and 1 ml and 0,1 ml. Calibrated automatic pipettes may be used.

5.3.2.10 **Petri dishes** of size 90 mm to 100 mm.

5.3.2.11 **Glass beads** (Diameter : 3 mm to 4 mm)

5.3.2.12 **Volumetric flasks** calibrated at 20 °C

5.3.2.13 **Mechanical shaker**

5.4 Preparation of bacterial suspensions and test solutions

5.4.1 Bacterial suspensions

5.4.1.1 Stock cultures of test organisms

Stock cultures shall be kept in accordance with the requirements of prEN 12353.

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[4] Vortex® is an example of a suitable product available commercially. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of this product.

5.4.1.2 Working culture of test organisms

In order to prepare the working culture of *Pseudomonas aeruginosa,* subculture from the stock culture (see 5.4.1.1) by streaking onto TSA slopes and incubate (see 5.3.2.3). After 18 h to 24 h prepare a second subculture from the first subculture in the same way and incubate for 18 h to 24 h. From this second subculture, a third subculture may be produced in the same way.

NOTE : The second and/or third subculture are the working culture(s).

If it is not possible to prepare the second subculture on a particular day, a 48 h subculture may be used for subsequent subculturing, provided that the subculture has been kept in the incubator during the 48 h period. In these circumstances, prepare a further 24 h subculture after proceeding. Do not take a fourth subculture.

Use the same procedure for *Staphylococcus aureus.*

5.4.1.3 Bacterial test suspensions

Take 10 ml of diluent (see 5.2.2.4) and place in a 100 ml flask with 5 g of glass beads (see 5.3.2.11). Take the working cultures (see 5.4.1.2) and transfer loopfuls of the cells into the diluent. The cells should be suspended in the diluent by immersing the loop in the diluent and rubbing it against the side of the flask to dislodge the cells. Shake the flask for 3 min using a mechanical shaker (see 5.3.2.13). Aspirate the suspension from the glass beads and transfer to another tube. Adjust the number of cells in the suspension to 1,5 x 10^8 cfu/ml [5)] to 5 x 10^8 cfu/ml using the diluent, estimating the numbers of units by any suitable means. Maintain this suspension in the water bath at 20 °C ± 1 °C and use within 2 h.

For counting of the bacterial test suspension prepare 10^{-6} and 10^{-7} dilutions of the test suspension (see 5.4.1.3) using diluent (see 5.2.2.4). Mix (see 5.3.2.6). Take a sample of 1,0 ml of each dilution in duplicate and transfer each 1,0 ml sample into separate Petri dishes (see 5.3.2.10) and add 12 ml to 15 ml melted TSA (see 5.2.2.3), cooled to 45 °C ± 1 °C.

5.4.1.4 Counting of bacterial test suspensions

Incubate the plates at 36 °C ± 1 °C (or 37 °C ± 1 °C) (see 5.3.2.3) for 24 h. Discard any plates which are not countable (for any reason). Count the plates and determine the number of colony forming units for each plate. Incubate the plates for a further 24 h. Do not recount plates which no longer show well-separated colonies. Recount the remaining plates. Determine the higher number of colonies for each 1,0 ml sample. Calculate the number of cfu/ml in the test suspension (N) using the method given in 5.6.1.1.

5.4.2 Product test solution

Details of samples of the product as received shall be recorded.

Product test solutions shall be prepared at three different concentrations to include at least two concentrations in the active range. The concentrations shall decrease in a geometrical progression with a factor of at least 2 (i.e. at least doubling the dilutions). The concentration of the product test solution shall be 1,25 times the required test concentration.

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5) cfu/ml : Colony Forming Unit per ml.

NOTE : The product as received may be used as one of the product test solutions.

For solid products, dissolve the product as received by weighing at least 1g ± 10 mg of the product in a volumetric flask and filling up with water (see 5.2.2.2.). Subsequent dilutions shall be prepared in volumetric flasks (see 5.3.2.12) on a volume/volume basis.

For liquid products, dilutions of the product shall be prepared in water (see 5.2.2.2) on a volume/volume basis using volumetric flasks (see 5.3.2.12).

The concentration of the product stated in the test report shall be the test concentration. Record the test concentration in terms of volume per volume or mass per volume.

The product test solutions shall be prepared freshly and used for not more than one working day (or less if of low stability).

5.5 Procedure

5.5.1 General

The method of choice is the dilution-neutralization method. To determine a suitable neutralizer the following procedure shall be adopted. The validation of the dilution-neutralization method (see A.4.1) shall be carried out using a suitable neutralizer, chosen according to laboratory experience and published data.

If this neutralizer is not valid, repeat the validation test using an alternative neutralizer containing a combination of polysorbate 80 30 g/l, saponin 30 g/l, L-histidine 1 g/l, lecithin 3 g/l, sodium thiosulphate 5 g/l in either diluent (see 5.2.2.4.) or in phosphate buffer 0,0025 mol/l. If both neutralizers are found to be invalid, the membrane filtration method may be used in place of the dilution-neutralization method.

5.5.2 Dilution-neutralization method

5.5.2.1 General

Prior to testing, equilibrate all reagents (product test solution, water, bacterial test suspension, neutralizer) to the test temperature of 20 °C ± 1 °C using the water bath (see 5.3.2.2). Check that the temperature of the reagents is stabilized at 20 °C ± 1 °C.

5.5.2.2 Test procedure for bactericidal activity of products

Pipette 8,0 ml of one of the product test solutions into a container of suitable capacity and add 1,0 ml of water (see 5.2.2.2). Add 1,0 ml of one of the bacterial test suspensions containing $1,5 \times 10^8$ to 5×10^8 cfu/ml (see 5.4.1.3). Immediately start the stopwatch (see 5.3.2.5), mix (see 5.3.2.6) and place the container in a water bath controlled at 20 °C ± 1 °C.

NOTE : When adding bacterial suspensions to containers, care should be taken to avoid touching the upper part of the container sides.

The activity of the product shall be determined for a contact time chosen from one of the following : 1 min, 5 min, 15 min, 30 min, 45 min or 60 min ± 10 s.