SLOVENSKI STANDARD
SIST EN 13727:2004

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Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za ocenjevanje baktericidnega delovanja kemičnih razkužil za instrumente, ki se uporabljajo v humani medicini - Preskusna metoda in zahteve (faza 2, stopnja 1)

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants for instruments used in the medical area - Test method and requirements (phase 2, step 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Prüfung der bakteriziden Wirkung chemischer Desinfektionsmittel für Instrumente im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité bactéricide des désinfectants chimiques pour les instruments utilisés en médecine - Méthode d'essai et exigences (Phase 2, Étape 1)

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Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants for instruments used in the medical area - Test method and requirements (phase 2, step 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité bactéricide des désinfectants chimiques utilisés pour les instruments en médicine - Méthode d'essai et prescriptions (phase 2, étape 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Prüfung der bakteriziden Wirkung chemischer Desinfektionsmittel für Instrumente im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

This European Standard was approved by CEN on 7 November 2003.

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This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Management Centre has the same status as the official versions.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 13727:2003) 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 June 2004, and conflicting national standards shall be withdrawn at the latest by June 2004.

A collaborative trial has been undertaken to provide a precision annex to this standard.

Other methods to evaluate the efficacy of chemical disinfectants and antiseptics for different applications in the medical field are in preparation.

Annexes A, B, C, D and E are informative.

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 Medical Devices Directive 93/42.

For relationship with EU Directive, see informative annex ZA, which is an integral part of this document.

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

Introduction

This European Standard describes a suspension test for establishing whether a chemical disinfectant for use on instruments (surgical instruments, anaesthesia material, endoscopes etc.) has or does not have a bactericidal activity in the area described in the scope.

In this laboratory test chosen conditions include contact time, temperature, test organisms and interfering substances, i.e. conditions which may influence the action of chemical disinfectants in practical situations.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each concentration of the chemical disinfectant found by this test corresponds to the chosen experimental conditions. However, for some applications the instructions of use of a product may differ and therefore additional test conditions need to be used.

1 Scope

This European Standard specifies a test method and the minimum requirements for bactericidal activity of chemical disinfectant products that form a homogeneous, physically stable preparation when diluted with hard water - or in the case of ready-to-use products - 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 and interfering substance.

This European Standard applies to products that are used in the medical area for disinfecting instruments by immersion - even if they are not covered by the EEC/93/42 Directive on Medical Devices.

This European Standard applies to areas and situations where disinfection is medically indicated. Such indications occur in patient care, for example:

— in hospitals, in community medical facilities and in dental institutions;

— in clinics of schools, of kindergartens and of nursing homes;

and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for the patients.

NOTE 1 The method described is intended to determine the activity of commercial formulations or active substances under the conditions in which they are used.

NOTE 2 This method corresponds to a phase 2 step 1 test (see annex E).

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 (including amendments).

EN 12353, *Chemical disinfectants and antiseptics – Preservation of microbial strains used for the determination of bactericidal and fungicidal activity.*

ISO 6710, *Single-use containers for venous blood specimen collection.*

3 Terms and definitions

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

3.1
product
chemical agent or formulation used as a chemical disinfectant or antiseptic

3.2
bactericide
product which kills vegetative bacteria under defined conditions

Adjective: bactericidal

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

3.4
bacteriostatic activity
capability of a product to inhibit the growth of bacteria under defined conditions

3.5
clean conditions
conditions representative of surfaces which have received a satisfactory cleaning programme and/or are known to contain minimal levels of organic and/or inorganic substances

3.6
dirty conditions
conditions representative of instruments which are known to or may contain organic and/or inorganic substances

4 Requirements

The product, when diluted with hard water or - in the case of ready-to-use products - with water and tested in accordance with clause 5 under simulated clean conditions (0,3 g/l bovine albumin solution), or dirty conditions (3 g/l bovine albumin solution, plus 3 ml/l washed sheep erythrocytes) according to its practical applications and under the obligatory test conditions (three selected test organisms, 20 °C, 60 min), shall demonstrate at least a decimal log (lg) reduction in counts of 5. It is also possible to test the product as delivered (highest test concentration = 80 %).

The bactericidal activity shall be evaluated using the following three test organisms : *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus hirae*.

Where indicated, additional specific bactericidal activity shall be determined applying other contact times, temperatures and interfering substances (see 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 bacteria in a solution of an interfering substance is added to a sample of the product as delivered and/or diluted with hard water (for ready to use products: water). The mixture is maintained at (20 ± 1) °C for 60 min ± 10 s (obligatory test conditions). At the end of this contact time, an aliquot is taken; the bactericidal and/or the bacteriostatic 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 numbers of surviving bacteria in each sample are determined and the reduction is calculated.

5.1.2 The test is performed using *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus hirae* as test-organisms (obligatory test conditions).

5.1.3 Additional and optional contact times and temperatures are specified. Additional interfering substances can be used.

5.2 Materials and reagents

5.2.1 Test organisms

The bactericidal activity shall be evaluated using the following three test organisms[1)]:

— *Pseudomonas aeruginosa* ATCC 15442;

— *Staphylococcus aureus* ATCC 6538;

— *Enterococcus hirae* ATCC 10541.

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

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 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. 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 (see 5.3.1).

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

NOTE 2 If distilled water of adequate quality is not available, water for injections (see EP) 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 (see 5.2.2.2)	to 1 000,0 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 (see 5.3.2.4).

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 (see B.3).

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

5.2.2.4 Diluent

Tryptone Sodium Chloride Solution:

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride (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 diluent 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 being tested in accordance with 5.5.1 and 5.5.2. The neutralizer shall be sterile.

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

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 5.5.3.

NOTE Information on rinsing liquids that have been found to be suitable for some categories of products is given in B.2.

5.2.2.7 Hard water for dilution of products

Prepare:

— Solution A: Dissolve 19,84 g anhydrous magnesium chloride ($MgCl_2$) or an equivalent of hydrated magnesium chloride and 46,24 g anhydrous calcium chloride ($CaCl_2$) or an equivalent of hydrated calcium chloride in water (see 5.2.2.2) and dilute to 1000 ml . Sterilize in the autoclave (see 5.3.1). Store the solution at 2 °C to 8 °C for no longer than one month.

— Solution B: Dissolve 35,02 g sodium hydrogencarbonate ($NaHC0_3$) in water (see 5.2.2.2) and dilute to 1000 ml. Sterilize by membrane filtration (see 5.3.2.7). Store the solution at 2 °C to 8 °C for no longer than one week.

— Hard water: For the preparation of 1 litre, place 600 ml to 700 ml water (see 5.2.2.2) in a 1000 ml volumetric flask (see 5.3.2.12) and add 6,0 ml of solution A, then 8,0 ml of solution B. Mix and dilute to 1000 ml with water (see 5.2.2.2). The pH of the hard water shall be 7,0 ± 0,2. If necessary adjust the pH by using a solution of approximately 40 g /l (about 1 mol/l) of sodium hydroxide (NaOH) or approximately 36,5 g/l (about 1 mol/l) of hydrochloric acid (HCl). The hard water shall be freshly prepared under aseptic conditions and used within 12 hours.

NOTE When preparing the product test solutions (see 5.4.2) the addition of the product to this hard water produces a different final water hardness in each test tube. In any case the final hardness is lower than 300 mg/l of calcium carbonate ($CaC0_3$) in the test tube.

5.2.2.8 Interfering substances

5.2.2.8.1 General

The interfering substance shall be chosen according to the conditions of use laid down for the product.

The interfering substance shall be sterile and prepared at 10 times its final concentration in the test.

The ionic composition e.g. pH, calcium and/or magnesium hardness and chemical composition e.g. mineral substances, protein, carbohydrates, lipids, detergents shall be defined.

NOTE In the following the term "interfering substance" is used even if it contains more than one substance.

5.2.2.8.2 Clean conditions (bovine albumin solution – low concentration

Dissolve 0,30 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of diluent (see 5.2.2.4).

Sterilize by membrane filtration (see 5.3.2.7), keep in a refrigerator at 2 °C to 8 °C and use within 1 month.

The final concentration of the bovine albumin in the test procedure (see 5.5) is 0,3 g/l.

5.2.2.8.3 Dirty conditions (Mixture of bovine albumin solutions – high concentration with sheep erythrocytes)

Dissolve 3,00 g of bovine albumin fraction V (suitable for microbiological purposes) in 97 ml of diluent (see 5.2.2.4).

Sterilize by membrane filtration (see 5.3.2.7).

Prepare at least 8,0 ml fresh sterile defibrinated sheep blood according to ISO 6710 or purchase such blood from a commercial supplier (see 5.2.2.9). Centrifuge the erythrocytes at 800 g for 10 min. After discarding the supernatant, resuspend erythrocytes in diluent (see 5.2.2.4). Repeat this procedure at least 3 times, until the supernatant is colourless.

Resuspend 3 ml of the packed sheep erythrocytes in the 97 ml of sterilized bovine albumin solution (see above). To avoid later contamination this mixture should be split in portions probably needed per day and kept in separate containers for a maximum of 7 days in a refrigerator at 2 °C to 8 °C.

The final concentration of bovine albumin and sheep erythrocytes in the test procedure (see 5.5) shall be 3 g/l and 3 ml/l respectively.

5.2.2.9 Sterile defibrinated sheep blood

The sterile defibrinated sheep blood can be acquired from a commercial supplier or prepared according to ISO 6710.

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

b) by dry heat, in the hot air oven (see 5.3.2.1 b)) by maintaining it at 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 Usual microbiological laboratory equipment[2] and in particular, the following:

5.3.2.1 Apparatus for sterilization:

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

b) for dry heat sterilization, a hot air oven capable of being maintened at 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 ± 1) °C, at (45 ± 1) °C (to maintain melted TSA in case of pour plate technique) and at additional test temperatures ± 1 °C (see 5.5.1).

5.3.2.3 Incubator

capable of being controlled either at (36 ± 1) °C or at (37 ± 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 inaccuracy of calibration of not more than ± 0,1 pH units at 20 °C. The additional inaccuracy of the electrodes shall not be more than ± 0,1 pH units.

NOTE For measuring the pH of the agar-media (see 5.2.2.3) a puncture electrode or a flat membrane electrode should be used.

5.3.2.5 Stopwatch

5.3.2.6 Electromechanical agitator, e.g. Vortex® mixer[3]

5.3.2.7 Membrane filtration apparatus constructed of a material compatible with the product, with a filter holder of at least 50 ml volume, and suitable for use of filters with diameter 47 mm to 50 mm and 0,45 µm pore size [or 0,22 µm pore size for sterilization of hard water (see 5.2.2.7) and bovine albumin (see 5.2.2.8)].

The vacuum source used shall give an even filtration flow rate. In order to obtain a uniform distribution of the micro-organisms 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 Containers: Test tubes, culture bottles 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.

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2) Disposable equipment is an acceptable alternative to reusable glassware.

3) Vortex® in 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.