SLOVENSKI STANDARD
SIST EN 17126:2019

01-februar-2019

Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje sporocidnega delovanja kemičnih razkužil v humani medicini - Preskusna metoda in zahteve (faza 2, stopnja 1)

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

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der sporiziden Wirkung im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité sporicide des désinfectants chimiques utilisés dans le domaine médical - Méthodes d'essai et exigences (phase 2, étape 1)

Ta slovenski standard je istoveten z: **EN 17126:2018**

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 17126:2019 **en,fr,de**

EUROPEAN STANDARD
NORME EUROPÉENNE
EUROPÄISCHE NORM

EN 17126

December 2018

ICS 11.080.20

English Version

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

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Ref. No. EN 17126:2018 E

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European foreword

This document (EN 17126:2018) 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of an identical text or by endorsement, at the latest by June 2019, and conflicting national standards shall be withdrawn at the latest by June 2019.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

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Introduction

This European Standard specifies a suspension test for establishing whether a chemical disinfectant has a sporicidal activity in the area and fields described in the scope.

This laboratory test takes into account practical conditions of application of the product including contact time, temperature, test organisms, and interfering substances, i. e. conditions which may influence its action in practical situations.

Each utilization concentration of the chemical disinfectant found by this test corresponds to the chosen experimental conditions.

1 Scope

This document specifies a test method and the minimum requirements for sporicidal activity of chemical disinfectant 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 (97 % with a modified method for special cases) 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 in the fields of instrument disinfection by immersion, and surface disinfection by wiping, spraying, flooding or other means.

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.

EN 14885 specifies in detail the relationship of the various tests to one another and to “use recommendations”.

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.

EN 12353, *Chemical disinfectants and antiseptics — Preservation of test organisms used for the determination of bactericidal (including Legionella), mycobactericidal, sporicidal, fungicidal and virucidal (including bacteriophages) activity*

EN 14885, *Chemical disinfectants and antiseptics — Application of European Standards for chemical disinfectants and antiseptics*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 14885 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

4 Requirements

The product shall demonstrate at least 4 decimal log (lg) reduction, when tested in accordance with Table 1 and Clause 5.

Table 1 — Minimum and additional test conditions

Test Conditions	Surface disinfection	Instrument disinfection	Textile disinfection
Minimum spectrum of test organisms			
sporicidal activity against *Clostridium difficile*	*Clostridium difficile*	*Clostridium difficile*	*Clostridium difficile*
sporicidal activity	*Bacillus subtilis* and *Bacillus cereus*	*Bacillus subtilis* and *Bacillus cereus*	*Bacillus subtilis* and *Bacillus cereus*
Additional	Any relevant test organism		
Test temperature	according to the manufacturer´s recommendation, but between		
	4 °C and 30 °C	20 °C and 70 °C	20 °C and 80 °C
Contact time	according to the manufacturer's recommendation, but no longer than		
	15 min or 60 min[a]	60 min	60 min
Interfering substance			
clean conditions	0,3 g/l bovine albumin solution **and/or**	0,3 g/l bovine albumin solution **and/or**	0,3 g/l bovine albumin solution **and/or**
dirty conditions	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes	3,0 g/l bovine albumin solution plus 3,0 ml/l erythrocytes
Additional	any relevant substance	any relevant substance	any relevant substance

[a] The contact times for surface disinfectants stated in this table are chosen on the basis of the practical conditions of the product. The recommended contact time for the use of the product is within the responsibility of the manufacturer. Products intended to disinfect surfaces that are likely to come into contact with the patient and / or the medical staff and surfaces, which are frequently touched by different people, leading to the transmission of microorganisms to the patient, shall be tested with a contact time of maximum 15 min. The same applies where the contact time of the product shall be limited for practical reasons. Products for other surfaces than stated above may be tested with a contact time of maximum 60 min.

NOTE For the 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 sample of the product as delivered and/or diluted with hard water (or water for ready to use products) is added to a test suspension of spores in a solution of an interfering substance. The mixture is maintained at the temperature and the contact time specified in Clause 4 and 5.5.1.1. At the end of

this contact time, an aliquot is taken; the sporicidal 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 spores in each sample are determined and the reduction is calculated.

5.1.2 The test is performed using spores of *Clostridium difficile* for a sporicidal activity against *Clostridium difficile* and/or *Bacillus subtilis* and *Bacillus cereus* for sporicidal activity (Clause 4, Table 1).

5.1.3 Additional and optional contact times and temperatures are specified (Clause 4, Table 1). Additional interfering substances and test organisms may be used.

5.2 Materials and reagents

5.2.1 Test organisms

The sporicidal activity shall be evaluated using the following strains as test organisms selected according to Clause 4 (Table 1)[1)].

a) Clostridium difficile R027	NCTC 13366
b) Bacillus subtilis	ATCC 6633
c) Bacillus cereus	CIP 105151

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

The required incubation temperature for these test organisms is 36 °C ± 1 °C or 37 °C ± 1 °C (5.3.2.3). The same temperature (either 36 °C or 37 °C) shall be used for all incubations performed during a test and its control and validation.

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 additional test organisms are not classified at a reference centre, their identification characteristics shall be stated. In addition, they shall be held by the testing laboratory or national culture collection under a reference for five years.

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this European 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.

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.

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1) The NCTC, CIP and ATCC numbers are the collection numbers of strains supplied by these culture collections. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of the product named.

For each culture medium and reagent, a time limitation for use should be fixed.

All specified pH values are measured at 20 °C ± 1 °C.

5.2.2.2 Water

The water shall be free from substances that are toxic or inhibiting to the bacterial spores or to the bacteria. It shall be freshly glass distilled water or deionized water.

Sterilize in the autoclave [5.3.2.1a)]. Sterilization is not necessary if the water is used e. g. for preparation of culture media and subsequently sterilized.

NOTE See 5.2.2.7 for the procedure to prepare hard water.

5.2.2.3 Culture media for spore forming bacteria

a) **BHIYT-L Agar for *Clostridium difficile***

BHIYT-L agar, consisting of:

Brain heart infusion	37,0 g
Yeast extract	5,0 g
L-Cysteine	1,0 g
Sodium taurocholate	1,0 g
Agar	15,0 g
Water (5.2.2.2.)	to 1000,0 ml

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization the pH (5.3.2.4) of the medium shall be equivalent to 7,0 ± 0,2. Let the medium cool down to 48 °C ± 2 °C. Dissolve 200 000 units of lysozyme in 10 ml water (5.2.2.2). Sterilize the enzymatic solution by membrane filtration (5.3.2.7).

In case of encountering problems with neutralization (5.5.1.2 and 5.5.1.3) it may be necessary to add neutralizer to BHIYT-L. Annex B gives guidance on the neutralizers that may be used. It is recommended not to use a neutralizer that causes opalescence in the agar.

b) **Tryptone Soya Agar (TSA) for *Bacillus* species**

Tryptone soya agar, consisting of:

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 1000,0 ml

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization the pH (5.3.2.4) of the medium shall be equivalent to 7,2 ± 0,2. This agar should be used for counting of viable *Bacillus* spores.

In case of encountering problems with neutralization (5.5.1.2 and 5.5.1.3) it may be necessary to add neutralizer to TSA. Annex B gives guidance on the neutralizers that may be used. It is recommended not to use a neutralizer that causes opalescence in the agar.

5.2.2.4 Diluent

Tryptone sodium chloride solution, consisting of:

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

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization the pH (5.3.2.4) of the medium shall be equivalent to 7,0 ± 0,2.

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product being tested in accordance with 5.5.1.2, 5.5.1.3 and 5.5.2. It 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 rinsing liquid shall be validated for the product being tested in accordance with 5.5.1.2, 5.5.1.3 and 5.5.3. It 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 Annex B.

5.2.2.7 Hard water for dilution of products

For the preparation of 1 l of hard water, the procedure is as follows:

— prepare solution A: dissolve 19,84 g magnesium chloride ($MgCl_2$) and 46,24 g calcium chloride ($CaCl_2$) in water (5.2.2.2) and dilute to 1000 ml. Sterilize by membrane filtration (5.3.2.7) or in the autoclave [5.3.2.1 a)]. Autoclaving – if used - may cause a loss of liquid. In this case make up to 1000 ml with water (5.2.2.2) under aseptic conditions. Store the solution in the refrigerator (5.3.2.8) for no longer than one month;

— prepare solution B: dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water (5.2.2.2) and dilute to 1000 ml. Sterilize by membrane filtration (5.3.2.7). Store the solution in the refrigerator (5.3.2.8) for no longer than one week;

— place 600 ml to 700 ml of water (5.2.2.2) in a 1000 ml volumetric flask (5.3.2.12) and add 6,0 ml (5.3.2.9) of solution A, then 8,0 ml of solution B. Mix and dilute to 1000 ml with water (5.2.2.2). The pH of the hard water shall be 7,0 ± 0,2, when measured at 20 °C ± 1 °C (5.3.2.4). 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 h.

NOTE When preparing the product test solutions (5.4.2), the addition of the product to the hard water produces a different final water hardness in each test tube. In any case the final hardness expressed as calcium carbonate ($CaCO_3$) is in the test tube lower than 375 mg/l.

5.2.2.8 Interfering substance

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 (50 times in case of the modified method, 5.2.2.8.4).

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

NOTE 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 (5.2.2.4).

Sterilize by membrane filtration (5.3.2.7), keep in a refrigerator (5.3.2.8) and use within one month.

The final concentration of the bovine albumin in the test procedure (5.5) shall be 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 (5.2.2.4).

Sterilize by membrane filtration (5.3.2.7).

Prepare at least 8,0 ml fresh sterile defibrinated sheep blood (5.2.2.9). Centrifuge the erythrocytes at 800 g_N for 10 min (5.3.2.13). After discarding the supernatant, resuspend erythrocytes in diluent (5.2.2.4). Repeat this procedure at least 3 times, until the supernatant is colourless.

Resuspend 3,0 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 (5.3.2.8).

The final concentration of bovine albumin and sheep erythrocytes in the test procedure (5.5) shall be 3,0 g/l and 3,0 ml/l respectively.

5.2.2.8.4 Clean and dirty conditions for the modified method for ready-to-use products (5.5.4)

Follow in general the procedures for preparation according to 5.2.2.8.2 and 5.2.2.8.3, but prepare the interfering substance in fivefold higher concentrations, for the dirty conditions maximum 50 ml to avoid problems with the filtration

a) Clean conditions (5.2.2.8.2) – dissolve 1,50 g bovine albumin (instead of 0,3 g) in 100 ml of diluent (5.2.2.4);

b) Dirty conditions (5.2.2.8.3) – dissolve 7,5 g bovine albumin (instead of 1,5 g) in 42,5 ml of diluent (instead of 48,5 ml). Prepare at least 20 ml (instead of 4,0 ml) sheep blood. Resuspend 7,5 ml (instead of 1,5 ml) of the packed sheep erythrocytes in 42,5 ml of sterilized bovine albumin solution to obtain 50 ml.

5.2.2.9 Defibrinated sheep blood

The defibrinated sheep blood should be sterile (aseptic blood-letting and preparation), pooled from more than one sheep and can be acquired from a commercial supplier.

5.2.2.10 Sporulation media

5.2.2.10.1 Brain Heart Infusion

Brain heart infusion, consisting of:

Brain infusion solids	12,5 g
Beef heart infusion solids	5,0 g
Proteose peptone	10,0 g
Glucose ($C_6H_{12}O_6$)	2,0 g
Sodium chloride (NaCl)	5,0 g
Disodium hydrogen phosphate (Na_2HPO_4)	2,5 g
Water (5.2.2.2)	to 1000,0 ml

Sterilize in the autoclave [5.3.2.1. a)]. After sterilization the pH (5.3.2.4) of the medium shall be equivalent to 7,4 ± 0,2.

5.2.2.10.2 Columbia Broth

Columbia broth, consisting of:

Pancreatic digest of casein	10,0 g
Yeast extract	5,0 g
Proteose peptone No. 3	5,0 g
Tryptic digest of beef heart	3,0 g
L-cysteine HCl	0,1 g
Dextrose (D-glucose)	2,5 g
Sodium chloride (NaCl)	5,0 g
Magnesium Sulfate ($MgSO_4$) (anhydrous)	0,1 g
Ferrous sulfate ($FeSO_4$)	0,02 g
Sodium carbonate (Na_2CO_3)	0,6 g
Tris (hydroxmethyl) aminomethane ($C_4H_{11}NO_3$)	0,83 g
Tris (hydroxmethyl) aminomethane HCL	2,86 g
Water (5.2.2.2)	to 1000,0 ml

Sterilize in the autoclave [5.3.2.1. a)]. After sterilization the pH (5.3.2.4) of the medium shall be equivalent to 7,5 ± 0,2.

5.2.2.10.3 Liquid Sporulation Medium

Liquid sporulation medium for preparation of *Clostridium difficile* spores, consisting of:

Prepare 1 L of the medium in a 2 L Erlenmeyer flask by adding the following in order given:

Water (5.2.2.2.)	700,0 ml
Special peptone[2)]	10,0 g

2) Special peptone is a commercial available medium with a specially designed mixture of peptones, consisting of: Total nitrogen 11,7 g, Amino nitrogen 3,8 g, Sodium chloride (NaCl) 3,5 g.

Potassium dihydrogenphosphate (KH_2PO_4)	2,60 g
Ammonium sulfate [$(NH_4)_2SO_4$]	0,60 g
Calcium chloride monohydrate ($CaCl_2 \times H_2O$)	0,08 g
Yeast extract powder	10,0 g
Potassium carbonate (K_2CO_3)	3,48 g
Magnesium sulfate ($MgSO_4$)	0,12 g
Water (5.2.2.2.)	to 1000,0 ml

The pH (5.3.2.4) of the medium shall be 7,9 ± 0,2 before sterilization. If needed the adjustment should be performed with KOH or HCl. Sterilize in the autoclave [5.3.2.1. a)] for 15 min at 121 °C.

5.2.2.10.4 Sodium Phosphate Buffer (0,1 M)

Sodium phosphate buffer (1 M), consisting of:

Disodium hydrogen phosphate (Na_2HPO_4)	8,19 g
Sodium dihydrogen phosphate monohydrate (NaH_2PO_4)	5,84 g
Water (5.2.2.2.)	to 1000,0 ml

Sterilize in the autoclave [5.3.2.1. a)]. After sterilization the pH (5.3.2.4) of the medium shall to equivalent to 7,0 ± 0,2.

5.2.2.10.5 Enzymatic Buffer

Enzymatic buffer, consisting of:

800 units of lysozyme and 250 units of trypsin per mg wet weight to 25 ml of 0,1 M Sodium phosphate buffer (5.2.2.10.4.).

Sterilize by membrane filtration (5.3.2.7).

5.2.2.10.6 Tryptone Glucose Broth (TGB)

Tryptone glucose broth for preparation of the inoculum of *Bacillus* species, consisting of:

Yeast extract	2,5 g
Tryptone	5,0 g
Glucose ($C_6H_{12}O_6$)	1,0 g
Water (5.2.2.2)	to 1000,0 ml

Distribute in test tubes at a rate of 10 ml per tube. Sterilize in the autoclave [5.3.2.1. a)]. After sterilization the pH (5.3.2.4) of the medium shall be equivalent to 7,2 ± 0,2 .

5.2.2.10.7 Meat Yeast extract Agar (MYA)

Meat yeast extract agar for preparation of *Bacillus* species spores consisting of:

Meat extract	10,0 g
Yeast extract	2,0 g
Manganese sulfate tetrahydrate ($MnSO_4 \times 4H_2O$)	0,053 g
Agar	15,0 g