

Nederlandse norm

# NEN-EN 17126

(en)

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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**EN 17126**

NORME EUROPÉENNE

EUROPÄISCHE NORM

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)

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)

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

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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of an identical text or by endorsement, at the latest by June 2019, and conflicting national standards shall be withdrawn at the latest by June 2019.

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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.

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**EN 17126:2018 (E)****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
<b>Minimum spectrum of test organisms</b>			
sporicidal activity against <i>Clostridium difficile</i>	<i>Clostridium difficile</i>	<i>Clostridium difficile</i>	<i>Clostridium difficile</i>
sporicidal activity	<i>Bacillus subtilis</i> and <i>Bacillus cereus</i>	<i>Bacillus subtilis</i> and <i>Bacillus cereus</i>	<i>Bacillus subtilis</i> and <i>Bacillus cereus</i>
Additional	Any relevant test organism		
<b>Test temperature</b>	according to the manufacturer's recommendation, but between		
	4 °C and 30 °C	20 °C and 70 °C	20 °C and 80 °C
<b>Contact time</b>	according to the manufacturer's recommendation, but no longer than		
	15 min or 60 min <sup>a</sup>	60 min	60 min
<b>Interfering substance</b>	clean conditions		
	0,3 g/l bovine albumin solution <b>and/or</b>	0,3 g/l bovine albumin solution <b>and/or</b>	0,3 g/l bovine albumin solution <b>and/or</b>
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
<p><sup>a</sup> 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.</p>			
<p>NOTE For the additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions.</p>			

## 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

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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)<sup>1)</sup>.

- |                                      |            |
|--------------------------------------|------------|
| a) <i>Clostridium difficile</i> R027 | NCTC 13366 |
| b) <i>Bacillus subtilis</i>          | ATCC 6633  |
| c) <i>Bacillus cereus</i>            | CIP 105151 |

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

The required incubation temperature for these test organisms is  $36\text{ °C} \pm 1\text{ °C}$  or  $37\text{ °C} \pm 1\text{ °C}$  (5.3.2.3). The same temperature (either  $36\text{ °C}$  or  $37\text{ °C}$ ) 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.

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:

Bran 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 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:

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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  $7,0 \pm 0,2$

**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  $7,0 \pm 0,2$ , when measured at  $20^\circ C \pm 1^\circ C$  (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.

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