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**Kemična razkužila in antiseptiki - Kvantitativni preskus na neporoznih površinah za vrednotenje baktericidnega in/ali fungicidnega delovanja in/ali delovanja kemičnih razkužil na kvasovke v humani medicini - Preskusna metoda in zahteve brez mehanskega delovanja (faza 2, stopnja 2)**

Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or yeasticidal and/or fungicidal activity of chemical disinfectants used in medical area - Test method and requirements without mechanical action (phase 2, step 2)

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Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächen-Versuch zur Bestimmung der bakteriziden und/oder levuroziden und/oder fungiziden Wirkung chemischer Desinfektionsmittel auf nicht porösen Oberflächen im humanmedizinischen Bereich - Prüfverfahren und Anforderungen ohne mechanische Behandlung (Phase 2, Stufe 2)

**Ta slovenski standard je istoveten z: prEN 17387**

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Chemical disinfectants and antiseptics - Quantitative non-porous surface test for the evaluation of bactericidal and/or yeasticidal and/or fungicidal activity of chemical disinfectants used in medical area - Part rev: Test method and requirements without mechanical action (phase 2, step 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächen-Versuch zur Bestimmung der bakteriziden und/oder levuroziden und/oder fungiziden Wirkung chemischer Desinfektionsmittel auf nicht porösen Oberflächen im humanmedizinischen Bereich - Prüfverfahren und Anforderungen ohne mechanische Behandlung (Phase 2, Stufe 2)

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 216.

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## European foreword

This document (prEN 13787:2019) has been prepared by Technical Committee CEN/TC 216 “Chemical disinfectants and antiseptics”, the secretariat of which is held by AFNOR.

This document is currently submitted to the CEN Enquiry.

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## **Introduction**

This document describes a surface test method for establishing whether a chemical disinfectant in the area and fields described in the scope (Clause 1) has or does not have bactericidal and/or fungicidal or yeasticidal activity on non-porous surfaces without mechanical action.

The laboratory test closely simulates practical conditions of application. Chosen conditions (contact time, temperature, and microorganisms on surfaces...) reflect parameters which are found in practical situations including conditions which may influence the action of disinfectants.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types.

However, for some applications the recommendations of use of a product can differ and therefore additional test conditions need to be used.

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## 1 Scope

This document specifies a test method and the minimum requirements for bactericidal and yeasticidal and additionally fungicidal 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.

NOTE 1 Dilutions are necessary as three concentrations in the active to non-active range are tested.

This document applies to products that are used in the medical area for disinfecting non-porous surfaces without mechanical action.

This document applies to areas and situations where disinfection or antiseptics 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 can occur in the workplace and in the home. It can also include services such as laundries and kitchens supplying products directly for the patients.

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

Using this document, it is possible to determine the activity of products like commercial formulations or active substances on bacteria and/or fungi in the conditions in which they are used and therefore it corresponds to a phase 2, step 2 test.

This method excludes the evaluation of the activity of products against mycobacteria and bacteria spores.

## 2 Normative references

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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.

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

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

## 4 Requirements

The product shall demonstrate at least a 5 decimal log (lg) reduction for bacteria and at least a 4 decimal log (lg) reduction for fungi, when tested in accordance with Table 1 and 5.5.

**Table 1 — Experimental conditions**

Test conditions	Bactericidal activity	Yeasticidal activity	Fungicidal activity
<b>Minimum spectrum of test organisms</b>	<i>Staphylococcus aureus</i> <i>Enterococcus hirae</i> <i>Pseudomonas aeruginosa</i>	<i>Candida albicans</i>	<i>Aspergillus brasiliensis</i> <i>Candida albicans</i>
additional	any relevant test organism		
<b>Test temperature</b>	according to the manufacturer's recommendation, but between 4 °C ± 1°C to 30 °C ± 1°C For tests performed at room temperature, the range shall be 20 °C ± 2,5°C		
<b>Contact time</b>	according to the manufacturer's recommendation, but at minimum 1 min and no longer than 5 min or 60 min <sup>a</sup> (from 1 min to 5 min at intervals of 1 min and from 5 min to 60 min at intervals of 5 min)		
<b>Interfering substance</b>			
clean conditions	0,3 g/l bovine albumin		
dirty conditions	3,0 g/l bovine albumin plus 3,0 ml erythrocytes		
additional	any relevant substance		
<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 5 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 minimum test conditions.			

## 5 Test methods

### 5.1 Principle

A test suspension of bacteria or fungi in a solution of interfering substances is inoculated onto a test stainless steel surface and dried. A sample of the product under test is applied in a manner that completely covers the dried test organisms. The surface is maintained at the temperature and contact time exemplified in Clause 4 and 5.5.1. At the end of the contact time the surface is transferred to a previously validated neutralization medium to suppress any product activity. The number of surviving test organisms which can be recovered from the surface is determined quantitatively.

The number of bacteria or fungi on a test surface treated with hard water in place of the product under test is also determined and the reduction in viable counts attributed to the product is calculated as the difference between the two test surfaces' results.



## 5.2 Materials and reagents

### 5.2.1 Test organisms

The bactericidal activity shall be evaluated using the following strains<sup>1</sup>:

- *Staphylococcus aureus* ATCC 6 538;
- *Enterococcus hirae* ATCC 10 541;
- *Pseudomonas aeruginosa* ATCC 15 442.

The fungicidal activity shall be evaluated using the following strains<sup>1</sup>:

- *Aspergillus brasiliensis* ATCC 16 404.
- *Candida albicans* ATCC 10 231;

The yeasticidal activity shall be evaluated using the following strain<sup>1</sup>:

- *Candida albicans* ATCC 10 231.

If required for specific applications, additional strains may be chosen.

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

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.

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

All specified pH values are measured at  $(20 \pm 1)$  °C.

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<sup>1</sup> The 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

**prEN 13787:2019 (E)****5.2.2.2 Water**

The water shall be freshly glass-distilled water and not demineralized water. If distilled water of adequate quality is not available, water for injections (see bibliographic reference [1]) may be used.

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 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
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 (5.3.2.6) of the medium shall be equivalent to  $7,2 \pm 0,2$ .

**5.2.2.4 Malt extract agar (MEA)**

For maintenance of fungal strains, sporulation and performance of viable counts.

Malt extract [food grade (e.g. Christomalt powder from Difal) or an equivalent extract that is not highly purified and not only based on maltose (e.g. Malt extract from OXOID)] <sup>2</sup>	30,0 g
Agar	15,0 g
Water (5.2.2.2)	to 1 000,0 ml

However, if there are problems producing at least 75 % spiny spores see 5.4.1.3.

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization, the pH (5.3.2.6) of the medium shall be equivalent to  $5,6 \pm 0,2$ .

In case of an encountering problems with neutralization (5.5.2.3 and 5.5.2.4), it may be necessary to add neutralizer to MEA. Annex B gives guidance on the neutralizers that may be used. It is recommended not to use neutralizer that causes opalescence in the agar.

**5.2.2.5 Diluent**

Tryptone sodium chloride solution:

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride	8,5 g
Water (5.2.2.2)	to 1 000,0 ml

<sup>2</sup> This Malt extracts from Difal and OXOID are examples of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization, the pH (5.3.2.6) of the diluent shall be equivalent to  $7,0 \pm 0,2$ .

### 5.2.2.6 Neutralizer

The neutralizer shall be validated for the product being tested in accordance with 5.5.2.3 and 5.5.2.4. 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.

### 5.2.2.7 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 1 000 ml. Sterilize by membrane filtration (5.3.2.19) or in the autoclave [5.3.2.1 a)]. Autoclaving – if used – may cause a loss of liquid. In this case make up to 1 000 ml with water (5.2.2.2) under aseptic conditions. Store the solution in the refrigerator (5.3.2.15) 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 (5.3.2.19). Store the solution in the refrigerator (5.3.2.15) for no longer than one week;
- place 600 ml to 700 ml of water (5.2.2.2) in a 1 000 ml volumetric flask (5.3.2.13) and add 6,0 ml of solution A, then 8,0 ml of solution B. Mix and dilute to 1 000 ml with water (5.2.2.2). The pH (5.3.2.6) of the hard water shall be  $7,0 \pm 0,2$ . 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 h.

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

### 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 2 times its final concentration in the test.

For any additional interfering substance, the ionic composition (e.g. pH, calcium and/or magnesium hardness) and chemical composition (e.g. mineral substances, protein, carbohydrates, lipids, detergents) shall be fully defined.

NOTE The term “interfering substance” is used even if it contains more than one substance.

The method of preparation and sterilization together with the composition shall be noted in the test report (5.7).

**prEN 13787:2019 (E)****5.2.2.8.2 Bovine albumin solution**

Bovine albumin solutions for the test conditions shall be prepared as follows.

## a) Preparation for clean conditions

- dissolve 0,3 g of bovine albumin (Cohn fraction V for Dubos medium) in 100 ml of diluent (5.2.2.5)
- sterilize by membrane filtration (5.3.2.19)
- Dilute to the 1/5th with diluent (5.2.2.5)
- Keep in the refrigerator and use within one month

The final concentration of bovine albumin in the test procedure (5.5.2) is 0,3 g/l.

## b) Preparation for dirty conditions

- Dissolve 3,00 g of bovine albumin fraction V (suitable for microbiological purposes) in 97 ml of diluent (5.2.2.5)
- Sterilize by membrane filtration (5.3.2.19)
- Prepare at least 8,0 ml fresh sterile defibrinated sheep blood. Centrifuge the sheep blood at 800  $g_N$  (5.3.2.14) for 10 min. After discarding the supernatant, resuspend erythrocytes in diluent (5.2.2.5)
- 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 (above)
- Dilute to the 1/5th with diluent (5.2.2.5)
- To avoid 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 (5.3.2.15)

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

**5.2.3 Test surface**

The test surface is a flat stainless steel 304 disc (2 cm diameter) with grade 2b finish on both sides. Only one side of a disc shall be used. The discs shall be used only once.

For cleaning, the discs shall be placed in a beaker (minimum size: 50 ml) containing not less than 20 ml of 5 % (V/V) Decon<sup>®3</sup> for 60 min. Immediately rinse the discs twice with water (5.2.2.2) for 10 s to completely remove the cleanser. Do not let the discs dry.

**NOTE** To supply a satisfactory flow of water, a sterilized fluid dispensing pressure vessel with suitable hose and connectors or other suitable method can be used and regulated to supply approximately 2 000 ml per min.

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<sup>3</sup> Decon<sup>®</sup> 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.

For decontamination, place the clean disc in a bath containing 70 % (V/V) iso-propanol for 15 min. Remove the disc and dry by evaporation under laminar air flow.

### 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<sup>4</sup>** and, in particular, the following:

#### 5.3.2.1 Apparatus for sterilization (moist and dry heat)

- a) For moist heat sterilization, an autoclave capable of being maintained 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 maintained at  $(180_0^{+5})$  °C for a minimum holding time of 30 min, at  $(170_0^{+5})$  °C for a minimum holding time of 1 h or at  $(160_0^{+5})$  °C for a minimum holding time of 2 h.

**5.3.2.2 Temperature controlled cabinet or chamber** capable of being controlled at test temperatures  $\theta \pm 1$  °C (for test temperatures others than ambient).

**5.3.2.3 Water baths**, capable of being controlled at  $(20 \pm 1)$  °C, at  $(45 \pm 1)$  °C and at additional test temperatures  $(\theta \pm 1)$  °C (5.5.1)

**5.3.2.4 Incubator (for bactericidal activity)**, capable of being controlled either at  $(36 \pm 1)$  °C or  $(37 \pm 1)$  °C. An incubator at  $(37 \pm 1)$  °C may be used if an incubator at  $(36 \pm 1)$  °C is not available. The same temperature shall be used for incubations performed during a test and its control and validation

**5.3.2.5 Incubator (for fungicidal or yeasticidal activity)**, capable of being controlled at  $(30 \pm 1)$  °C

**5.3.2.6 pH-meter**, having an inaccuracy of calibration of no more than  $\pm 0,1$  pH units at  $(20 \pm 1)$  °C

#### 5.3.2.7 Stopwatch

#### 5.3.2.8 Shakers

- a) Electromechanical agitator, i.e. Vortex<sup>®</sup> mixer<sup>5</sup>;
- b) Mechanical shaker

**5.3.2.9 Containers: Test tubes**, culture bottles or flasks of suitable capacity

<sup>4</sup> Disposable sterile equipment is an acceptable alternative to reusable glassware.

<sup>5</sup> Vortex<sup>®</sup> is an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.