# SLOVENSKI STANDARD
# oSIST prEN 16438:2012

**01-oktober-2012**

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

Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action - (phase 2, step 2)

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Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächenversuch zur Bestimmung der fungiziden oder levuroziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich auf nicht-porösen Oberflächen ohne mechanische Wirkung - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Antiseptiques et désinfectants chimiques - Essai quantitatif de surface pour l'évaluation de l'activité fongicide ou levuricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine vétérinaire sur des surfaces non poreuses sans action mécanique (phase 2, étape 2)

**Ta slovenski standard je istoveten z:** **prEN 16438**

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**ICS:**

| | | |
|---|---|---|
| 71.100.35 | Kemikalije za dezinfekcijo v industriji in doma | Chemicals for industrial and domestic disinfection purposes |

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2003-01.Slovenski inštitut za standardizacijo. Razmnoževanje celote ali delov tega standarda ni dovoljeno.

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

**DRAFT**
**prEN 16438**

May 2012

ICS 71.100.35

English Version

# Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of fungicidal or yeasticidal activity of chemical disinfectants and antiseptics used in the veterinary area on non-porous surfaces without mechanical action - (phase 2, step 2)

Antiseptiques et désinfectants chimiques - Essai quantitatif de surface pour l'évaluation de l'activité fongicide ou levuricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine vétérinaire sur des surfaces non poreuses sans action mécanique - (phase 2, étape 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächenversuch zur Bestimmung der fungiziden oder levuroziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich auf nicht-porösen Oberflächen ohne mechanische Wirkung - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

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If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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## Foreword

This document (prEN 16438:2012) 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 European Standard specifies a surface test for establishing whether a chemical disinfectant or antiseptic has fungicidal or yeasticidal activity in the 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.

The conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant or antiseptic, 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.

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## 1 Scope

This European Standard specifies a test method and the minimum requirements for fungicidal or yeasticidal activity of chemical disinfectant and antiseptic products that form a homogeneous physically stable preparation in hard water or – in the case of ready-to-use products– with water.

This European Standard applies to products for use in the veterinary area i.e. in the breeding, husbandry, production, transport and disposal of all animals except when in the food chain following death and entry to the processing industry.

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

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 2 test.

## 2 Normative references

The following referenced documents are indispensable for the application 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.

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EN 12353 Chemical disinfectants and antiseptics - Preservation of test organisms used for the determination of bactericidal, mycobactericidal, sporicidal and fungicidal activity

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EN 14885 Chemical disinfectants and antiseptics - Application of European Standards for chemical disinfectants and antiseptics

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ISO 4793 Laboratory sintered (fritted) filters – Porosity grading, classification and designation

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## 3 Terms and definitions

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

## 4 Requirements

The product shall demonstrate at least a 3 decimal log (lg) reduction from a water control, when tested in accordance with Table 1 and clause 5 under simulated low level (3,0 g/l bovine albumin) or high level soiling (10 g/l yeast extract and 10 g/l bovine albumin) on a surface.

**Table 1 Obligatory and additional test conditions**

| **Test Conditions** | **Fungicidal or yeasticidal activity on non-porous surfaces without mechanical action in the veterinary area** |
|---|---|
| Test organism Fungicidal activity<br>a) obligatory | *Aspergillus brasiliensis (formerly niger)*<br>*Candida albicans* |
| Test organism Yeasticidal activity<br>a) obligatory | *Candida albicans* |

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| | |
|---|---|
| b) additional | any relevant test organism |
| Test temperature<br>a) obligatory | 10°C ± 1°C |
| b) additional | 4°C ± 1°C; 20°C ± 1°C; 40°C ± 1°C |
| Contact time<br>a) obligatory | 60 min ± 10 s |
| b) additional* | 5 min ± 10 s; 30 min ± 10 s; 120 min ± 10 s |
| Interfering substance<br>a) obligatory<br>low level soiling<br><br>high level soiling | <br><br>3,0 g/l bovine albumin<br><br>10 g/l yeast extract plus 10 g/l bovine albumin |
| b) additional | any relevant substance |
| The obligatory contact times for surface disinfectants stated in table 1 were chosen to enable comparison of standard conditions.<br><br>*The recommended contact time for the use of the product is within the responsibility of the manufacturer.<br><br>NOTE For the additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions. | |

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Any additional specific fungicidal activity shall be determined in accordance with 5.2.1 and 5.5.1.1 in order to take into account intended specific use conditions.

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## 5 Test method

### 5.1 Principle

A test suspension of fungal spores or yeast and interfering substance is inoculated onto the test surface and dried. After a drying time, an aliquot of the product under test is transferred to the surface, in a manner which covers the dried film. The surface is maintained at a specified temperature for a defined period of time specified in Clause 4 and 5.5.1.1. At the end of that contact time the surface is transferred to a neutralizer so that the action of the disinfectant is immediately neutralized. The numbers of surviving organisms which can be recovered from the surface is determined quantitatively.

The number of fungi or yeast on a surface treated with water in place of the disinfectant is also determined and the reduction is calculated.

The test is performed using *Aspergillus brasiliensis* and *Candida albicans* as test organisms (clause 4, table 1).

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

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## 5.2 Materials and reagents

### 5.2.1 Test organism

The fungicidal activity shall be evaluated using the following strains as the test organisms[1)] :

*Aspergillus brasiliensis* (formerly niger) ATCC 16404

*Candida albicans* ATCC 10231

The yeasticidal activity shall be evaluated using only *Candida albicans*.

NOTE See annex A for strain references in some other culture collections.

The required incubation temperature for these organisms is (30 ± 1)°C (5.3.2.3). The same temperature 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

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#### 5.2.2.1 General

All weights of chemical substances given in this 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.

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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 organism.

NOTE 1 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.

NOTE 2 Ready-to-use media may be used if it complies with the required specification.

NOTE 3 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 and not demineralised water.

Sterilize in the autoclave [5.3.2.1a)].

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 bibliographic reference [1]) can be used.

1) The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collection (ATCC). 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.

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NOTE 3 See 5.2.2.6 for the procedure to prepare hard water.

#### 5.2.2.3 Malt extract agar (MEA)

Malt extract agar, consisting of:

| | |
|---|---|
| Malt extract* | 30,0 g |
| Agar | 15,0 g |
| Water (5.2.2.2) | to 1 000 ml |

*The malt extract should be food grade (Cristomalt powder from Difal is recommended) or equivalent that is not highly purified and not only based on maltose (Malt extract from OXOID is recommended)[2)] .

Sterilize in the autoclave [5.3.2.1a)]. After sterilization, the pH of the medium shall be equivalent to 5,6 ± 0,2 when measured at (20 ± 1) °C.

NOTE In case of encountering problems with neutralization (5.5.1.2 and 5.5.1.3), it may be necessary to add neutralizer to the MEA. 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 1 000 ml |

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Sterilize in the autoclave [5.3.2.1a)]. After sterilization, the pH of the diluent shall be equivalent to 7,0 ± 0,2 when measured at (20 ± 1) °C.

#### 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 Hard water for dilution of products

For the preparation of 1 000 ml 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 (5.3.2.7)) or in the autoclave [5.3.2.1a)]. 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 (5.3.2.8) for no longer than one month.

2) 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. Equivalent products may be used if they can be shown to lead to the same results.

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— prepare solution B: dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water (5.2.2.2) and dilute to 1 000 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 1 000 ml volumetric flask (5.3.2.12) 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 of the hard water shall be 7,0 ± 0,2, when measured at 20 ± 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 hours.

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 is lower than 375 mg/l of calcium carbonate ($CaCO_3$) in the test tube.

#### 5.2.2.7 Interfering substance

##### 5.2.2.7.1 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 the additional interfering substances 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.

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NOTE The term ‘interfering substance’ is used even if it contains more than one substance.

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##### 5.2.2.7.2 Low level soiling (bovine albumin solution)

Dissolve 0,6 g of bovine albumin V (suitable for microbiological purposes) in 90 ml of water (5.2.2.2) in a 100 ml volumetric flask. Make up to the mark with water (5.2.2.2).

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

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

##### 5.2.2.7.3 High level soiling (mixture of bovine albumin solution with yeast extract)

Dissolve 10 g yeast extract powder in 150 ml of water (5.2.2.2) in a 250 ml volumetric flask (5.3.2.13) and allow foam to collapse. Make up to the mark with water (5.2.2.2). Transfer to a clean dry bottle and sterilize in an autoclave [5.3.2.1a)]. Allow to cool to (20 ± 5) C.

pipette 25 ml of this solution into a 50 ml volumetric flask and add 10 ml of water (5.2.2.2). Dissolve 1 g of bovine albumin fraction V (suitable for microbiological purposes) in the solution with shaking and allow foam to collapse. Make up to the mark with water (5.2.2.2) sterilize by membrane filtration and keep in a refrigerator (5.3.2.8) and use within one month.

The final concentration in the test procedure (5.5.2) is 10 g/l yeast extract and 10 g/l bovine albumin.

#### 5.2.3 Test surface

Stainless steel discs (2 cm diameter discs) 304 with grade 2 finish on both sides. The surfaces should be flat. The surfaces should be used only once.

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Prior to use the surfaces should be placed in a beaker (minimum size 50 ml) containing not less than 20 ml of 5 % Decon®[3] for 60 min. Immediately rinse the discs with running freshly distilled water for 10 s.

The surface shall not be allowed to dry to any extent. The discs shall only be handled with forceps. Rinse the discs with flowing water for a further 10 s to ensure complete removal of the surfactant. To supply a satisfactory flow of water, a fluid dispensing pressure vessel with suitable hose and connectors or other suitable method can be used and regulated to supply approximately 2000 ml per min. Place the clean discs in a bath containing 95 % 2-propanol for 15 min. Remove the discs and dry by evaporation.

## 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 an autoclave [5.3.2.1a)];

b) by dry heat, in a hot air oven [5.3.2.1b)].

### 5.3.2 Usual microbiological laboratory equipment [4]

and, in particular, the following:

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

a) for moist heat sterilization, an autoclave capable of being maintained at 121 °C for a minimum holding time of 15 min;

b) for dry heat sterilization, a hot air oven capable of being maintained 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;

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**5.3.2.2 Water bath,** capable of being controlled at (4 ± 1) °C, (10 ± 1) °C, (20 ± 1)° C, (40 ± 1)°C and (45 ± 1) C (to maintain melted MEA, 5.5.2.2, 5.5.2.3, 5.5.2.4 and 5.5.2.5).

**5.3.2.3 Incubator,** capable of being controlled at (30 ± 1) °C

**5.3.2.4 pH-meter,** having an accuracy of calibration of 0,1 pH units at (20 °C ± 1) °C.

NOTE A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar media (5.2.2.3)

#### 5.3.2.5 Stopwatch

#### 5.3.2.6 Shakers

a) Electromechanical agitator, e.g. Vortex® [5] mixer .

3) Decon concentrate is obtained from Decon Laboratories Ltd, Conway Street, Hove, East Sussex, BN3 3LY, UK Tel. 01273 756598. Studies have shown that this method of cleaning is satisfactory. A suitable 'Generic' will be specified at a later stage.

4) Disposable sterile equipment is an acceptable alternative to reusable glassware.