SLOVENSKI STANDARD
SIST EN 16438:2014

01-julij-2014

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 - Test method and requirements (phase 2, step2)

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: **EN 16438:2014**

ICS:

11.220	Veterinarstvo	Veterinary medicine
71.100.35	Kemikalije za dezinfekcijo v industriji in doma	Chemicals for industrial and domestic disinfection purposes

SIST EN 16438:2014 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16438

February 2014

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 - Test method and requirements (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 - Méthode d'essai et prescriptions (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)

This European Standard was approved by CEN on 30 November 2013.

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. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

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 CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

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

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. EN 16438:2014 E

Contents

Page

Foreword

This document (EN 16438:2014) 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 August 2014 and conflicting national standards shall be withdrawn at the latest by August 2014.

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

According to the CEN/CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This European Standard specifies a surface test for establishing whether a chemical disinfectant or antiseptic has or does not have 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 utilisation 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.

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 documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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*

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

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 a) obligatory	*Aspergillus brasiliensis (conidiospores)* and *Candida albicans (vegetative cells)*
Test organism Yeasticidal activity a) obligatory	*Candida albicans (vegetative cells)*
b) additional	any relevant test organism
Test temperature a) obligatory	10 °C ± 1 °C
b) additional	4 °C ± 1 °C; 20 °C ± 1 °C; 40 °C ± 1 °C
Contact time a) obligatory	60 min ± 10 s
b) additional*	5 min ± 10 s; 30 min ± 10 s; 120 min ± 10 s
Interfering substance a) obligatory low level soiling high level soiling	3,0 g/l bovine albumin 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. *The recommended contact time for the use of the product is within the responsibility of the manufacturer. NOTE For the additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions.	

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.

5 Test method

5.1 Principle

A test suspension of fungal conidiospores 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 neutraliser so that the action of the disinfectant is immediately neutralised. 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 the conidiospores of *Aspergillus brasiliensis* (formerly niger) and the vegetative cells of *Candida albicans* (fungicidal activity) or only the vegetative cells of *Candida albicans* (yeasticidal

activity) 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.

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 *Aspergillus 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 °C ± 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

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 organism.

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.

Ready-to-use medium may be used if it complies with the required specification.

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

---

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.

5.2.2.2 Water

The water shall be freshly glass-distilled and not demineralised water.

Sterilise in the autoclave [5.3.2.1 a)].

NOTE 1 Sterilisation is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilised.

NOTE 2 If distilled water of adequate quality is not available, water for injections (see bibliographic reference [1]) can be used.

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 (e.g. Cristomalt powder from Difal) or equivalent that is not highly purified and not only based on maltose (Malt extract from OXOID)[2)]. However if there are problems producing at least 75 % spiny spores see 5.4.1.4.2.

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

In case of encountering problems with neutralisation (5.5.1.2 and 5.5.1.3), it may be necessary to add neutraliser to the MEA. Annex B gives guidance on the neutralisers that may be used. It is recommended not to use a neutraliser 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

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

5.2.2.5 Neutraliser

The neutraliser 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 neutralisers that have been found to be suitable for some categories of products is given in Annex B.

---

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.

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. Sterilise 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 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.

— prepare solution B: dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water (5.2.2.2) and dilute to 1 000 ml. Sterilise 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 °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 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.

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

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).

Sterilise 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 (5.3.2.12) and allow foam to collapse. Make up to the mark with water (5.2.2.2). Transfer to a clean dry bottle and sterilise in an autoclave [5.3.2.1 a)]. Allow to cool to 20 °C ± 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) sterilise 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.

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 2 000 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

Sterilise 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 [5.3.2.1 a)];

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

5.3.2 Usual microbiological laboratory equipment [4]

and, in particular, the following:

5.3.2.1 Apparatus for sterilisation (moist and dry heat)

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

b) for dry heat sterilisation, a hot air oven capable of being maintained at 180 $^{+5}_{0}$ °C for a minimum holding time of 30 min, at 170 $^{+5}_{0}$ C for a minimum holding time of 1 h or at 160 $^{+5}_{0}$ C for a minimum holding time of 2 h;

5.3.2.2 Water bath, capable of being controlled at 10 °C ± 1 °C, 20 °C ± 1 °C, and 45 °C ± 1 °C (to maintain melted MEA. If pour plate technique is used), and at additional temperatures ± 1 °C (5.5.1).

5.3.2.3 Incubator, capable of being controlled at 30 °C ± 1 °C and 37 °C ± 1 °C (for drying surfaces)

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

---

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.

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;

b) Mechanical shaker.

5.3.2.7 Membrane filtration apparatus, constructed of a material compatible with the substances to be filtered with a filter holder of at least 50 ml volume and suitable for use with filters of diameter 47 mm to 50 mm and 0,45 µm pore size for filtration of hard water (5.2.2.6) and bovine albumin (5.2.2.7.2, 5.2.2.7.3).

5.3.2.8 Refrigerator capable of being controlled at 2 °C to 8 °C.

5.3.2.9 Graduated pipettes, of nominal capacities 10 ml and 1 ml and 0,1 ml or calibrated automatic pipettes.

5.3.2.10 Petri dishes, (plates) of size 90 mm to 100 mm.

5.3.2.11 Glass beads, diameter: 3 mm to 4 mm.

5.3.2.12 Volumetric flasks

5.3.2.13 Temperature controlled cabinet, capable of being controlled at 10 °C ± 1 °C.

5.3.2.14 Glass screw top container, with a base diameter of 4 cm - 5 cm.

5.3.2.15 Fritted filter, with porosity of 40 µm to 100 µm according to **ISO 4793**.

5.3.2.16 Flasks with vented caps.

5.3.2.17 Microscope capable of x 400 magnification.

5.3.2.18 Vacuum desicator capable of achieving a vacuum of 20-25 in. mercury.

5.4 Preparation of test organism suspensions and product test solutions

5.4.1 Test organism suspension (test and validation suspension)

NOTE Test and validation suspension are the same in this standard.

5.4.1.1 General

For each test organism, one suspension shall be prepared: this is used as the fungal “test suspension” to perform the test and the “validation suspension” to perform the controls and method validation.

5.4.1.2 Preservation and stock cultures of test organisms

The test organisms and their stock cultures shall be prepared and kept in accordance with EN 12353.

---

[5] Vortex ® 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.