SLOVENSKI STANDARD
SIST EN 16437:2014

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

Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on porous surfaces without mechanical action - Test method and requirements (phase 2, step2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Oberflächenversuch zur Bestimmung der bakteriziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich auf 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 bactéricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine vétérinaire sur des surfaces poreuses sans action mécanique - Méthode d'essai et prescriptions (phase 2, étape 2)

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Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary area on porous surfaces without mechanical action - Test method and requirements (phase 2, step 2)

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COMITÉ EUROPÉEN DE NORMALISATION
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Foreword

This document (EN 16437:2014) 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of an identical text or by endorsement, at the latest by August 2014 and conflicting national standards shall be withdrawn at the latest by August 2014.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.

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Introduction

This European Standard specifies a surface test for establishing whether a chemical disinfectant or antiseptic, for use on porous surfaces without mechanical action, in the veterinary area. has or does not have bactericidal activity under the laboratory conditions defined by this European Standard, which influence the action of disinfectants in practical use.

The laboratory test takes into account practical conditions of application of the product including pre-drying test organisms and interfering substances on a surface, contact time and temperature, 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 can differ and therefore additional test conditions need to be used.

1 Scope

This European Standard specifies a test method and the minimum requirements for bactericidal activity of chemical disinfectants and antiseptic products that form a homogeneous, physically stable preparation when diluted with hard water - or in the case of ready-to-use products - with water.

This European Standard applies to products that are used in the veterinary area on porous surfaces without mechanical action 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.

NOTE 3 This method cannot be used to evaluate the activity of products against mycobacteria or bacterial spores.

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*

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 4 decimal log (lg) reduction from a water control, when tested in accordance with Table 1 and Clause 5 under simulated soiling (3,0 g/l bovine albumin).

Table 1 — Obligatory and additional test conditions

Test Conditions	Bactericidal activity on porous surfaces without mechanical action in the veterinary area
Test organism a) obligatory	*Enterococcus hirae* *Proteus vulgaris* *Pseudomonas aeruginosa* *Staphylococcus aureus*
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	1 min ± 5 s, 5 min ± 10 s, 15 min + 10 s, 30 min ± 10 s; 120 min ± 10 s
Interfering substance a) obligatory	3,0 g/l bovine albumin
b) additional	any relevant substance
NOTE 1 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 2 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 bactericidal 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 bacteria mixed with interfering substance is inoculated onto the test surface and dried. After the drying time the test surface is immersed into a sample of the product as delivered and/or diluted with hard water (for ready to use products: water) ensuring that the test surface is totally covered for one minute. The test surface is removed from the product solution and 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 test surface is transferred to a neutraliser so that the action of the disinfectant is immediately neutralised. The bacteria are removed from the surface by ultrasound treatment. The numbers of surviving bacteria which can be recovered from the surface is determined quantitatively.

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

The test is performed using *Enterococcus hirae*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* 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 organisms

The bactericidal activity shall be evaluated using the following strains[1)]:

Enterococcus hirae ATCC 10541;

Proteus vulgaris ATCC 13315;

Pseudomonas aeruginosa ATCC 15442;

Staphylococcus aureus ATCC 6538.

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

The required incubation temperature for these 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.

If additional strains do not grow on the media (5.2.2.3) or cannot be used with diluent (5.2.2.4) additional media shall be used and shall be reported as well as additional incubation conditions.

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 media may be used if it complies with the required specification.

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

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1) The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collections (ATCC). This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.

5.2.2.2 Water

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

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

NOTE See 5.2.2.6 for the procedure to prepare hard water.

5.2.2.3 Tryptone Soya Agar (TSA)

Tryptone soya agar, consisting of:

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of soybean meal	5,0 g
D(+)-Glucose	2,5 g
Sodium chloride (NaCl)	5,0 g
Agar	15,0 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 medium shall be equivalent to 7,2 ± 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 TSA. 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. The neutraliser shall be sterile. The neutraliser is added to diluent (5.2.2.4) and TSB (5.2.2.8).

NOTE Information on neutralisers 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 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. 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. 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 (CaC03) is in the test tube lower than 375 mg/l.

5.2.2.7 Interfering substances

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, detergents) shall be defined.

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

5.2.2.7.2 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 a refrigerator (5.3.2.8) and use within one month.

The final concentration of the bovine albumin in the test procedure (5.5) is 3 g/l.

5.2.2.8 Tryptone Soya Broth (TSB) with neutraliser

Tryptone Soya broth, consisting of:

Tryptone, pancreatic digest of casein	17,0 g
Soya peptone, papaic digest of soybean meal	3,0 g
Sodium chloride (NaCl)	5,0 g
Dipotassiumhydrogenphosphate (K_2HPO_4)	2,5 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 medium shall be equivalent to 7,3 ± 0,2 when measured at 20 °C ± 1 °C.

An adequate neutraliser shall be added according to its chemical properties before or after autoclaving (5.2.2.5). TSB with neutraliser should be filled into glass tubes in portions of 10 ml.

5.2.3 Test surface[2)]

Poplar wood: Size: 10mm wide, 20 mm long and 0,6 mm −1,0 mm thick with visually smooth cut edges. Cut from sliced veneer, stored at least one year before use, from untreated wood of the European poplar tree.

Prior to use put the surfaces into a glass Petri dish in a single layer and sterilise in the autoclave [5.3.2.1 a)] for 15 min.

Test surfaces should be kept in a sterile vessel until use. The surfaces should be used only once.

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[3)]

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 4 °C ± 1 °C, 10 °C ± 1 °C, 20 °C ± 1 °C, 40 °C ± 1 °C (5.5.1) and 45 °C ± 1 °C (to maintain melted TSA, 5.2.2.3, 5.5.2.2 and 5.5.2.3).

5.3.2.3 Incubator, capable of being controlled at 36 °C ± 1 °C or 37 °C ± 1 °C (5.2.1). The same temperature shall be used for incubation performed during a test and its control and validation.

5.3.2.4 pH-meter, having an inaccuracy of calibration of no more than ± 0,1 pH units at 20 °C ± 1 °C.

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

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2) DES-IN-TEST Supply Walbrunnenstrasse D-70599 Stuttgart Tel. ++49 (0) 711 45 54 06. This information is given for the convenience of the users of this European Standard and does not constitute an endorsement by CEN of this product.

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