SLOVENSKI STANDARD
SIST EN 16437:2014+A1:2019

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Nadomešča:
SIST EN 16437:2014

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) (vključno z dopolnilom A1)

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)

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 - Mét

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

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EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

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English Version

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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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Contents

Page

[A1] European foreword [A1]

This document (EN 16437:2014+A1:2019) 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of an identical text or by endorsement, at the latest by April 2020 and conflicting national standards shall be withdrawn at the latest by April 2020.

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

This document includes Amendment 1 approved by CEN on 9 August 2019.

The start and finish of text introduced or altered by amendment is indicated in the text by tags [A1] [A1].

This document supersedes [A1] EN 6437:2014 [A1].

[A1] This document has been amended to update the scope and to correct a mistake in Tryptone Soya Agar and Broth formulations. The maximum contact time has also been extended and an obligatory contact time removed. The changes detailed above have no impact on the test results obtained using the previous version. Those results are still valid. [A1]

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, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

This document 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 document, 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 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 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, [A1) veterinary care facilities (A1], 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
[A1] Contact time	The contact time(s) shall be selected from the values given below [A1]
[A1] minimum	1 min ± 5 s [A1]
[A1] range	5 min ± 10 s, 15 min + 10 s, 30 min ± 10 s; 60 min ± 10 s and then up to 330 min ± 10 s at 30 min intervals [A1]
[A1] maximum	360 min ± 10 s [A1]
Interfering substance a) obligatory	3,0 g/l bovine albumin
b) additional	any relevant substance
[A1] Deleted text [A1] 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 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 neutralizer so that the action of the disinfectant is immediately neutralized. 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.

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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.

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

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 [5.3.2.1 a)]. Sterilization is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilized.

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
[A1] Deleted text [A1]	
Sodium chloride (NaCl)	5,0 g
Agar	15,0 g
Water (5.2.2.2)	to 1 000 ml

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization 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 neutralization (5.5.1.2 and 5.5.1.3), it may be necessary to add neutralizer to the TSA. Annex B gives guidance on the neutralisers 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

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

Sterilize 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 neutralizer

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
▌A1⟩ Glucose	2,5 g ⟨A1▌
Water (5.2.2.2)	to 1 000 ml

Sterilize in the autoclave [5.3.2.1 a)]. After sterilization the pH of the medium shall be equivalent to 7,3 ± 0,2 when measured at 20 °C ± 1 °C.

An adequate neutralizer shall be added according to its chemical properties before or after autoclaving (5.2.2.5). TSB with neutralizer 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 sterilize 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

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 [5.3.2.1 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 sterilization (moist and dry heat)

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

b) for dry heat sterilization, 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).

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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.