SLOVENSKI STANDARD
SIST EN 14349:2004

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Kemična razkužila in antiseptiki - Kvantitativni preskus na neporoznih površinah brez mehanskega delovanja za ocenjevanje 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 bacterial activity of chemical disinfectants and antiseptics used in veterinary field on non-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 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 bactéricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine vétérinaire - Méthode d'essai et exigences (phase 2, étape 2)

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Chemical disinfectants and antiseptics - Quantitative surface test for the evaluation of bacterial activity of chemical disinfectants and antiseptics used in veterinary field 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 bactéricide 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 bakteriziden 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 16 January 2004.

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Contents

Page

Foreword

This document (EN 14349:2004) 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 February 2005, and conflicting national standards shall be withdrawn at the latest by February 2005.

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

Introduction

This document describes a surface test for establishing whether a chemical disinfectant or antiseptic in the veterinary field, on non-porous surfaces without mechanical action, 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 type and level of interfering substance can be selected as well as contact times and temperatures in addition to the levels specified in order to support recommendations for use under particular conditions. The method involves neutralization of the bactericidal activity at the moment of sampling by dilution into a previously validated neutralizer.

The conditions that shall be tested are intended to cover general purposes and to allow reference between laboratories and product types. For some applications, however, the recommendations of use of a product can differ and therefore additional test conditions need to be used.

1 Scope

This document specifies a test method and the minimum requirements for bactericidal 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 – with water.

This document is applicable to products for use in the veterinary field 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.

NOTE 1 Mycobacteria are the subject of a separate standard.

NOTE 2 This method corresponds to a Phase 2 Step 2 test (Annex E).

2 Normative references

The following referenced document is 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.

EN 12353, *Chemical disinfectants and antiseptics — Preservation of microbial strains used for the determination of bactericidal and fungicidal activity*.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
product
chemical agent or formulation used as chemical disinfectant or antiseptic

3.2
bactericide
product which kills vegetative bacteria under defined conditions

NOTE The adjective derived from 'bactericide' is 'bactericidal'.

3.3
bactericidal activity
capability of a product to produce a reduction in the number of viable bacterial cells of relevant organisms under defined conditions

4 Requirements

The product diluted in hard water (5.2.2.6) when tested, in accordance with Clause 5, shall demonstrate at least a lg 4 reduction in viable counts from a water control when the test organisms are *Enterococcus hirae*, *Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus.* The test is carried out under simulated low level (3,0 g/l bovine albumin) or high level soiling conditions (10 g/l yeast extract and 10 g/l bovine albumin) according to its practical applications and under the required test conditions (10 °C, 30 min, 4 referenced strains). Where appropriate additional and optional contact times of 1 min, 5 min and 60 min and additional and optional temperatures of 4 °C, 20 °C and 40 °C are specified.

5 Test method

5.1 Principle

5.1.1 A test suspension of bacteria and interfering substance is inoculated onto the test surface and dried. After a drying time, 0,1 ml of the product is transferred to the surface. The surface is maintained at a specified temperature for a defined period of time. The surface is transferred to a previously validated neutralization medium so that the action of the disinfectant is immediately neutralized. The number of surviving organisms 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 in viable counts calculated by difference.

5.2 Materials and reagents

All weights of chemical substances given in this document 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.

5.2.1 Test organisms

The bactericidal activity shall be evaluated using the following strains :

Enterococcus hirae ATCC 10541[1)];

Proteus vulgaris ATCC 13315[1)];

Pseudomonas aeruginosa ATCC 15442[1)];

Staphylococcus aureus ATCC 6538[1)].

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

If required for specific applications, additional strains may be chosen and shall be noted in the test report. Their suitability for supplying inocula of sufficient concentration shall be verified. If the additional strains tested are not classified at a reference centre, identification characteristics shall be given. In addition they shall be held by the testing laboratory under a reference for 5 years (see EN 12353 for storage of strains).

5.2.2 Culture media and reagents

5.2.2.1 General

All weights of chemical substances given in this document refer to 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.

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1) ATCC 10541, ATCC 13315, ATCC 15442 & ATCC 6538 are the collection numbers of strains supplied by the American type Culture Collection. The information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of the product named. Corresponding strains supplied by other culture collections can be used if they can be shown to lead to the same result.

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.

NOTE To improve the reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media. The manufacturers' instructions relating to the preparation of these products should be rigorously followed.

5.2.2.2 Water

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

Sterilize in the autoclave [5.3.2.1 a].

NOTE 1 If the water is sterilized during the sterilization of the reagents this is not necessary.

NOTE 2 If distilled water of adequate quality is not available, water for injectable preparations (European Pharmacopoeia) can be used.

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 ;

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.

5.2.2.4 Diluent

Tryptone Sodium Chloride Solution :

Tryptone, pancreatic digest of casein 1,0 g ;

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 medium shall be equivalent to (7,0 ± 0,2), when measured at 20 °C.

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product under test in accordance with Annex A. The neutralizer 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 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]. Store the solution in the refrigerator (5.3.2.8) for no longer than one month ;

NOTE In the case of loss of volume during sterilization by autoclave, make up to 1 000 ml with water (5.2.2.2) under aseptic conditions before storage.

— 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 (5.3.2.9) 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 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 300 mg/l of calcium carbonate ($CaCO_3$) in the test tube.

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.

The ionic composition e.g. pH, calcium and/or magnesium hardness and chemical composition e.g. mineral substances, protein, carbohydrates, liquids 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 (Cohn fraction V for Dubos Medium) 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 (5.3.2.1).

Keep in a refrigerator (5.3.2.13) 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)

a) Dissolve 10 g yeast extract powder in 150 ml of water (5.2.2.2) in a 250 ml volumetric flask 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 the autoclave (5.3.1 a). Allow to cool to 20 °C ± 5 °C ;

b) 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 the bovine albumin in the solution in the flask 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 10 ml portions in a refrigerator (5.3.2.13) 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[2)] 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

Sterilize all glassware and parts of the apparatus that will come into contact with the culture media and reagents of 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 a].

5.3.2 Usual microbiological laboratory equipment[3)] and, in particular, the following :

5.3.2.1 Apparatus for sterilization

a) For moist heat sterilization, an autoclave (hot air oven) 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 ;

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2) Decon concentrate is obtained from Decon Laboratories Ltd, Convway St, Hovem BN3 3Lym UK Tel. 01273 756598. Studies have shown that this method of cleaning is satisfactory. A suitable ‘Generic’ will be specified at a later stage.

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