SLOVENSKI STANDARD
oSIST prEN 14349:2023

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Kemična razkužila in antiseptiki - Kvantitativni preskus na neporoznih 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 the veterinary area 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 sur des surfaces non poreuses sans action mécanique -

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EUROPEAN STANDARD
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English Version

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 216.

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Recipients of this draft are invited to submit, with their comments, notification of any relevant patent rights of which they are aware and to provide supporting documentation.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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Contents

Page

European foreword

This document (prEN 14349:2023) 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.

This document will supersede EN 14349:2012.

Data obtained using the former version of EN 14349 may still be used.

It was revised to correct obvious errors and ambiguities, to harmonize the structure and wording with other tests of CEN/TC 216 (existing or in preparation), and to improve the readability of the standard and thereby make it more understandable.

Introduction

This document specifies a surface test for establishing whether a chemical disinfectant or antiseptic has bactericidal activity in the area and 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 defined experimental conditions. However, for some applications, the recommendations of use of a product may 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 when diluted with hard water, or – in the case of ready-to-use-products – with water.

The method described is intended to determine the activity of commercial formulations or active substances under the conditions in which they are used. This document applies to products that are used in the veterinary area for disinfecting non-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 This method corresponds to a Phase 2 Step 2 test.

This method excludes the evaluation of the activity of products against yeasts, fungal spores, mycobacteria and bacterial spores.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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 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, definitions and abbreviations

3.1 Terms and definitions

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

3.2 Symbols and abbreviations

c	is the sum of V_C-values taken into account
cfu	colony forming units
d	is the dilution taken into account, lower dilution factor
n	is the number of V_C-values taken into account
N	number of cells per ml in the test suspension
N_w	number of cfu recovered from the test surface in the water control
B	counting of the cfu in the neutralizer control
Na	number of cfu recovered from the test surface in the test
C	counting of the cfu in the method validation

N_{ts} number of colony forming units remaining on the test surface

R reduction

V is the volume of the inoculated into the plate expressed in ml

Nv is the validation suspension

4 Requirements

The product shall demonstrate at least a 4 decimal log (lg) reduction when diluted with hard water (5.2.2.6) or – in the case of ready-to-use products – with water (5.2.2.2) and tested in accordance with Table 1 and Clause 5 under simulated low level soiling (3 g/l bovine albumin) or high level soiling (10 g/l yeast extract and 10 g/l bovine albumin) on a surface.

Table 1 - Test conditions

Test conditions	**Bactericidal activity on non-porous surfaces without mechanical action in the veterinary area**
Minimum spectrum of test organisms	*Enterococcus hirae* *Pseudomonas aeruginosa* *Staphylococcus aureus*
additional	any relevant test organism
Test temperature Minimum Maximum	According to the manufacturer's recommendation but between 4 °C ± 1 °C 40 °C ± 1 °C At intervals of 5°C
Contact time Minimum Maximum	 1 min ± 5 s 120 min ± 10 s At intervals of 30 s from 30 s to 5 min and at intervals of 5 min from 5 min to 120 min
Interfering substance low level soiling high level soiling	 3,0 g/l bovine albumin 10 g/l yeast extract plus 10 g/l bovine albumin
additional	any relevant substance
The contact times for surface disinfectants stated in this table are chosen on the basis of the practical conditions of the product. 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 minimum 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 in a solution of interfering substances (5.2.2.7) is inoculated onto a test stainless steel surface and dried. A sample of the product under test is applied in a manner that completely covers the dried test organisms. The surface is maintained at the test temperature and contact time exemplified in Clause 4 and (5.5.2). At the end of the contact time the surface is transferred to a previously validated neutralization medium to suppress any product activity immediately. The number of surviving test organisms which can be recovered from the surface is determined quantitatively.

The number of bacteria on a test surface treated with hard water (or water in the case of ready to use products) in place of the product under test is also determined and the reduction in viable counts attributed to the product is calculated as the difference between the two test surfaces' results.

5.2 Materials and reagents

5.2.1 Test organisms

The bactericidal activity shall be evaluated using the following strains:

— Enterococcus hirae

— Pseudomonas aeruginosa

— Staphylococcus aureus

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

The required incubation temperature for these test 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 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 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.

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.

5.2.2.2 Water

The water shall be freshly glass-distilled 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 [5.3.2.1a)]. Sterilization is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilized.

NOTE The procedure to prepare hard water is described in 5.2.2.6.

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;

— Sodium chloride (NaCl) 5,0 g;

— Agar 15,0 g;

— Water (5.2.2.2) to1 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 ± 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 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.

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 ± 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.

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.

— 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 h.

NOTE When preparing the product test solutions (5.4.2), the addition of the product to the hard water produces different final water hardness in each test tube. In any case the final hardness expressed as calcium carbonate ($CaCO_3$) 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 any 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 100 ml of 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.

5.2.2.7.3 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 sterilize in the 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 (5.3.2.12) 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 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 (5.3.2.7) and keep in 10 ml portions in a refrigerator (5.3.2.8) and use within one month.

The final concentration in the test procedure (5.5) 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 (bibliography references [2] [3]) with grade 2b finish on both sides. The surfaces should be flat.

The surfaces should be used only once and only one side of the disc shall be used. The discs shall only be handled with forceps.

Prior to use the surfaces should be placed in a beaker (minimum size 50 ml) containing not less than 20 ml of 5 % (V/V) Decon®[1)] for 60 min. Immediately rinse the discs with water (5.2.2.2) for 10 s.

The surface shall not be allowed to dry to any extent. Rinse the discs with water for a further 10 s to ensure complete removal of the surfactant. To supply a satisfactory flow of water, a sterile 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 70 % (V/V) iso-propanol for 15 min. Remove the discs and dry by evaporation under laminar air flow (5.3.2.15)

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[2)]

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_{0}^{+3}) °C for a minimum holding time of 15 min;

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

5.3.2.2 **Water baths,** capable of being controlled at 4 and 40°C ± 1 °C as required by the test conditions, and 45 ± 1 °C (to maintain melted TSA,in case of pour plate technique).

5.3.2.3 **Incubator**, capable of being controlled either at (36 ± 1) °C or (37 ± 1) °C (5.2.1).

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

1) Decon® 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 endorsement by CEN of this product.

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