SLOVENSKI STANDARD
SIST EN 14204:2013

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Nadomešča:
SIST EN 14204:2004

Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za vrednotenje mikobaktericidnega delovanja kemičnih razkužil in antiseptikov v veterini - Preskusna metoda in zahteve (faza 2, stopnja 1)

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der mykobakteriziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

Antiseptiques et désinfectants chimiques - Essai quantitatif de suspension pour l'évaluation de l'activité mycobactéricide des antiseptiques et des désinfectants chimiques utilisés dans le domaine vétérinaire - Méthode d'essai et prescriptions (Phase 2, étape 1)

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Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants and antiseptics used in the veterinary area - Test method and requirements (phase 2, step 1)

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Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der mykobakteriziden Wirkung chemischer Desinfektionsmittel und Antiseptika für den Veterinärbereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

This European Standard was approved by CEN on 22 September 2012.

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COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (EN 14204:2012) 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of an identical text or by endorsement, at the latest by May 2013, and conflicting national standards shall be withdrawn at the latest by May 2013.

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.

This document supersedes EN 14204:2004.

Data obtained using the former version of EN 14204 may still be used.

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

The following technical changes have been made: Membrane filtration (5.5.3) method and associated media and equipment have been added.

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 suspension test for establishing whether a chemical disinfectant or antiseptic has mycobactericidal 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 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 mycobactericidal 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.

Products can only be tested at a concentration of 80 % or less, as some dilution is always produced by adding the test organisms and interfering substance.

This European Standard applies to products that are used 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 1 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, mycobactericidal, sporicidal and fungicidal 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 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).

Table 1 - Obligatory and additional test conditions

Test conditions	Mycobactericidal activity
Test organism a) obligatory	*Mycobacterium avium*
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[a]
b) additional	1 min ± 5 s; 5 min ± 10 s; 10 min ± 10 s, 15 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
NOTE For the additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions. [a] The obligatory contact time for disinfectants stated in Table 1 was chosen to enable comparison of standard conditions. The referenced test conditions are by no means intended as requirements for the use of a product. The recommended contact time for the use of the product is within the responsibility of the manufacturer.	

Any additional specific mycobactericidal 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 sample of the product as delivered and/or diluted in hard water (or water for ready to use products) is added to a test suspension of mycobacteria in a solution of an interfering substance.

The mixture is maintained at (10 ± 1) °C for 60 min ± 10 s. At the end of this contact time, an aliquot is taken and the mycobactericidal action in this portion is immediately neutralized or suppressed by a validated method.

The number of surviving mycobacteria in each sample is determined and the reduction in viable counts calculated.

The test is performed using *Mycobacterium avium* as the test organism (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

5.2.1.1 The mycobactericidal activity shall be evaluated using the following strain :

Mycobacterium avium ATCC 15769[1)]

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

The required incubation temperature for this test organism is (36 ± 1) °C or (37 ± 1) °C. 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 the 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 limitation for use should be fixed.

5.2.2.2 Water

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

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1) ATCC 15769 is the collection number of strain supplied by the American Type Culture Collections. 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.

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 See 5.2.2.6 for the procedure to prepare hard water.

5.2.2.3 Middlebrook and Cohn 7H10 medium + 10 % oleic acid albumin dextrose complex (OADC) (reported as 7H10 in the text).

For performance of viable counts:

— Middlebrook 7H10 agar 19 g ;

— Glycerol 5 ml ;

— Water (see 5.2.2.2) to 895 ml.

Heat to boiling to dissolve completely. Sterilize for 10 min in the autoclave and cool to 50 °C to 55 °C. Add 100 ml Middlebrook OADC enrichment under aseptic conditions. Final pH = 6,6 ± 0,2 at 25 °C.

In case of encountering problems with neutralization (5.5.1.2), it may be necessary to add neutralizer to the 7H10. Annex B gives guidance on the neutralizers that may be used.

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 (see 5.2.2.2) to 1 000 ml.

Sterilize in the autoclave [see 5.3.2.1a)]. After sterilization the pH of the medium 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 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.

Sterilize in the autoclave.

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)]. Store the solution at 2 °C to 8°C for no longer than one month.

NOTE 1 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 a 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 2 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 is lower than 375 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 10 times its final concentration in the test.

For all 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 (5.2.2.8).

5.2.2.7.2 Low-level soiling (Bovine albumin solution)

— Dissolve 3 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 (5.3.2.12). Make up to the mark with water (5.2.2.2) ;

— sterilize 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)

a) dissolve 50 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 an autoclave [5.3.2.1a)]. 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 5 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) sterilize 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) is 10 g/l yeast extract and 10 g/l bovine albumin.

5.2.2.8 Rinsing liquid (for membrane filtration)

The rinsing liquid shall be validated for the product being tested in accordance with 5.5.1.2, 5.5.1.3 and 5.5.3.

It shall be sterile, compatible with the filter membrane and capable of filtration through the filter membrane under the test conditions described in 5.5.3.

NOTE Information on rinsing liquids that have been found to be suitable for some categories of products is given in Annex B.

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 the autoclave [5.3.2.1a)];

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

5.3.2 Usual microbiological laboratory equipment[2] and, in particular, the following:

5.3.2.1 Apparatus for sterilization

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 baths, capable of being controlled at (4 ±1) °C, (10 ± 1) °C, (20 ± 1) °C, (40 ± 1) °C, (45 ± 1) °C and 50 °C to 55 °C.

5.3.2.3 CO_2 Incubator, capable of being controlled at either (36 ±1) °C or (37 ± 1) °C. An incubator at (36 ± 1) °C or (37 ± 1)°C may be used if a CO_2 incubator is not available.

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

5.3.2.6 Shaker

a) Electromechanical agitator, e.g. Vortex ® mixer[3]

b) Mechanical shaker

2) Disposable Sterile Equipment is an acceptable alternative to reusable glassware.

3) 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.