SLOVENSKI STANDARD
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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, é

Ta slovenski standard je istoveten z: **prEN 14204**

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EUROPEAN STANDARD

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English Version

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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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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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (prEN 14204:2024) 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 supersedes EN 14204:2012 and was revised to harmonize the structure and wording with other quantitative suspension tests of CEN/TC 216 (existing or in preparation).

Results obtained using the previous version of this standard are still valid.

Introduction

This document specifies a suspension test for establishing whether a chemical disinfectant or antiseptic has mycobactericidal activity in the area 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 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 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.

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 for equipment disinfection by immersion, surface disinfection by wiping, spraying or flooding or other means and teat disinfection in the veterinary area – i.e. in the breeding, husbandry, production, veterinary care facilities, 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 1 test.

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 and definitions

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

ISO and IEC maintain terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp/

— IEC Electropedia: available at https://www.electropedia.org/

4 Requirements

The product shall demonstrate at least a 4 decimal log (lg) reduction when diluted with hard water (5.2.2.7) 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 (3,0 g/l bovine albumin) or high-level soiling (10 g/l yeast extract and 10 g/l bovine albumin) or in additional test conditions.

Table 1 — Test conditions

Test conditions	Mycobactericidal activity
Minimum Spectrum of target organism	*Mycobacterium avium*
additional	any relevant test organism
Test temperature	
minimum	5 °C ± 1 °C
maximum	40 °C ± 1 °C
Contact time	
minimum	1 min ± 5 s
maximum	120 min ± 10 s
Interfering substance	
low level soiling	3,0 g/l bovine albumin
high level soiling	10 g/l yeast extract plus 10 g/l bovine albumin
additional	any relevant substance

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

5.1.1 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 the test temperature θ for the test contact time t. 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 method of choice is the dilution-neutralization. If a suitable neutralizer cannot be found, membrane filtration is used. The numbers of surviving mycobacteria in each sample are determined and the reduction in viable counts calculated.

5.1.2 For general disinfectant product, the test is performed using *Mycobacterium avium* as test organism.

5.1.3 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 mycobactericidal activity shall be evaluated using the following strain as test organisms:

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 (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 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.

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.

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.

For each culture medium and reagent, a shelf life should be fixed.

5.2.2.2 Water

The water shall be freshly glass distilled and not demineralized water

Sterilize in the autoclave [5.3.2.1 a)].

Refer to 5.2.2.7 for the procedure to prepare hard water.

NOTE 1 Sterilization is not necessary if the water is used e.g. for preparation of culture media and subsequently sterilized.

NOTE 2 If distilled water of adequate quality is not available, water for injections (see bibliographic reference [1] Pharmacopoeia) can be used.

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

7H10, consisting of:

middlebrook 7H10 agar	19 g;
glycerol	5 ml;
water (5.2.2.2) to	895 ml.

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

Heat to boiling to dissolve completely. Sterilize for 10 min in the autoclave [5.3.2.1 a)] 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 and 5.5.1.3), it can 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 5.3.2.1 a)].

— 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, 5.5.1.3 and 5.5.2. It shall be sterile.

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

5.2.2.6 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.

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

5.2.2.7 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 [5.3.2.1 a)]. Autoclaving- if used- can 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 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 ± 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 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.

5.2.2.8 Interfering substances

5.2.2.8.1 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.

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.

5.2.2.8.2 Low-level soiling (Bovine albumin solution)

Dissolve 3,0 g of bovine albumin fraction *V* (suitable for microbiological purposes) 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 3,0 g/l.

5.2.2.8.3 High-level soiling (mixture of bovine albumin solution with yeast extract)

Dissolve 50,0 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 [5.3.2.1 a)]. Allow to cool to (20 ± 1) °C.

Pipette 25 ml of this solution into a 50 ml volumetric flask and add 10 ml of water (5.2.2.2). Dissolve 5,0 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 (5.3.2.7) and keep in a refrigerator (5.3.2.8) and use within one month.

The final concentration in the test procedure (5.5) is 10,0 g/l yeast extract and 10,0 g/l bovine albumin.

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 [5.3.2.1 a)];

b) by dry heat, in the 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

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