# SLOVENSKI STANDARD
# SIST EN 14204:2004

**01-december-2004**

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**Kemična razkužila in antiseptiki - Kvantitativni suspenzijski preskus za ocenjevanje mikobaktericidnega delovanja kemičnih razkužil in antiseptikov, ki se uporabljajo 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)



Désinfectants et antiseptiques 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)

**Ta slovenski standard je istoveten z:** **EN 14204:2004**

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**ICS:**

| | | |
|---|---|---|
| 11.080.20 | Dezinfektanti in antiseptiki | Disinfectants and antiseptics |
| 11.220 | Veterinarstvo | Veterinary medicine |

**SIST EN 14204:2004** **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

# EN 14204

June 2004

ICS 11.080.20

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)

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)

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 20 February 2004.



CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the Central Secretariat or to any CEN member.



This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the Central Secretariat has the same status as the official versions.



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## Foreword

This document (EN 14204:2004) 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 December 2004, and conflicting national standards shall be withdrawn at the latest by December 2004.

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

## Introduction

This European Standard describes a suspension test for establishing whether a chemical disinfectant or antiseptic in the veterinary field has or does not have mycobactericidal activity under the laboratory conditions, defined by this European Standard, 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 mycobactericidal 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 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.

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 (Annex F).

## 2 Normative references

The following referenced documents are 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 1656, *Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics used in veterinary field - Test method and requirements (phase 2/step 1)*.



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 European Standard, the following terms and definitions apply.

**3.1**
**product**
chemical agent or formulation used as a chemical disinfectant or antiseptic [EN 1040]

**3.2**
**mycobactericide**
product that kills mycobacteria under defined conditions

NOTE The adjective derived from "mycobactericide" is "mycobactericidal".

**3.3**
**mycobactericidal activity**
capability of a product to produce a reduction in the number of viable mycobacterial cells of relevant organisms under defined conditions

# 4 Requirements

The product diluted in hard water when tested, in accordance with clause 5, shall demonstrate at least a lg reduction in viable counts of 4, when the test organism is *Mycobacterium avium.* The test is carried out under simulated low level soiling conditions (3.0 g/l bovine albumin) or simulated 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, 60 min, 1 referenced strain.

Where appropriate additional and optional contact times of 1 min ± 5 s, 5 min ± 10 s, 15 min ± 10 s, 30 min ± 10 s and 120 min ± 10 s 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 prepared sample of the product under test diluted in hard water is added to a test suspension of mycobacterial cells in a solution of interfering substances.

The mixture is maintained at 10 °C ± 1 °C. At a contact time of 60 min ± 10 s an aliquot is taken ; 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.



Preliminary tests leading to the choice of the inactivation method should be carried out before the actual test. However, it is necessary to check the neutralization of the carry over in parallel with the actual test.



## 5.2 Materials and reagents

### 5.2.1 Test organism

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

— *Mycobacterium avium* ATCC 15769[1)]

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

**5.2.1.2** If required for specific applications additional strains may be chosen and must 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.

If additional strains do not grow on the medium (see 5.2.2.3) and/or cannot be used with diluent (see 5.2.2.4) additional media shall be used and shall be reported as well as additional cultivation conditions.

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 standard and does not constitute an endorsement by CEN of the product named. Corresponding strains supplied by other culture collections may be used if they can be shown to lead to the same results.

### 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.

NOTE 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.

#### 5.2.2.2 Water

The water shall be free from substances that are toxic or inhibiting to the mycobacteria. It shall be freshly glass distilled and not demineralised water.

Sterilize in the autoclave (see 5.3.2.1a).

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 Pharmacopaeia) can be used.



#### 5.2.2.3 Middlebrook and Cohn 7H10 medium + 10 % 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.

#### 5.2.2.4 Diluent

Tryptone Sodium Chloride Solution :

— 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). 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 C.

#### 5.2.2.6 Phosphate buffer 0,25 mol/l

— Potassium Dihydrogen Phosphate ($KH_2PO_4$) 34 g ;

— water (see 5.2.2.2) to 500 ml.

Adjust the pH to 7,2 ± 0,1 with 1 mol/l sodium hydroxide (NaOH) ;

— water (see 5.2.2.2) to 1 000 ml.

Sterilize in the autoclave.

#### 5.2.2.7 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 1000 ml. Sterilize by membrane filtration (**5.3.2.7**) or in the autoclave [**5.3.2.1**a)]. Store the solution at 2 °C to 8 °C for no longer than one month; If necessary make up to 1 000 ml with water (**5.2.2.2**) under aseptic conditions.



— prepare solution B: dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water (**5.2.2.2**) and dilute to 1000 ml. Sterilize by membrane filtration (**5.3.2.7**). Store the solution at 2 °C to 8 °C for no longer than one week;



— place 600 ml to 700 ml of water (**5.2.2.2**) in a 1000 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 1000 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 hours.

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.

#### 5.2.2.8 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 composition shall be noted in the test report (see 5.8).

#### 5.2.2.8.2 Bovine albumin solution for low level soiling conditions

— Dissolve 3 g of bovine albumin (Cohn fraction V for Dubos Medium) in 90 ml of water (see 5.2.2.2) in a 100 ml volumetric flask. Make up to the mark with water (see 5.2.2.2) ;

— sterilize by membrane filtration.

The final concentration of the bovine albumin in the test procedure (see 5.5.2) is 3 g/l.

#### 5.2.2.8.3 Albumin/yeast extract mixture for high level soiling conditions

a) Dissolve 50 g yeast extract powder in 150 ml of water (see 5.2.2.2**)** in a 250 ml volumetric flask and allow foam to collapse. Make up to the mark with water (see 5.2.2.2). Transfer to a clean dry bottle and sterilize in the autoclave (see 5.3.2.1). 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 (see 5.2.2.2). Dissolve 5 g of the bovine albumin (Cohn fraction V for Dubos Medium) in the solution in the flask with shaking and allow foam to collapse. Make up to the mark with water (see 5.2.2.2) sterilize by filtration and keep in 10 ml portions in a refrigerator (see 5.3.2.15) until use.

The final concentration in the test procedure (see 5.5.2) is 10 g/l yeast extract and 10 g/l bovine albumin.

#### 5.2.2.9 7H9 broth + 10 % ADC (reported as 7H9 broth in the text)

— Ammonium Sulphate 0,5 g

— L. glutamic Acid 0,5 g

— Sodium Citrate 0,1 g

— Pyridoxine 0,001 g

— Biotin 0,0005 g

— Disodium phosphate 2,5 g

— Monopotassium Phosphate 1 g

— Ferric ammonium Citrate 0,04 g

— Magnesium Sulphate 0,05 g

— Calcium Chloride 0,0005 g

— Zinc Sulphate 0,001 g

— Copper Sulphate 0,001 g



Dissolve 4,7 g in 900 ml water (see 5.2.2.2) (containing 2 ml glycerol or 0,5 g Tween 80 ®[2)] if desired) sterilize at 121 °C to 124 °C for 10 min. Aseptically add 100 ml Middlebrook OADC enrichment to the medium at 45 °C. Final pH = 6,6 ± 0,2 at 25 °C.

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2) Analytical quality, non-hydrolized in accordance with European Pharmacopaeia volume 1. TWEEN 80 ® 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.