SLOVENSKI STANDARD
SIST EN 14563:2009

01-marec-2009

Kemiļ na razkuģila in antiseptiki - Kvantitativni preskus s steklenim nosilcem za vrednotenje mikobaktericidnega ali tuberkulocidnega delovanja kemiļ nih razkuģil za instrumente, ki se uporabljajo v humani medicini - Preskusna metoda in zahteve (faza 2, stopnja 2)

Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area - Test method and requirements (phase 2, step 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Keimträgerversuch zur Prüfung der mykobakteriziden oder tuberkuloziden Wirkung chemischer Desinfektionsmittel für Instrumente im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Désinfectants chimiques et antiseptiques - Essai quantitatif de porte germe pour l'évaluation de l'activité mycobactéricide ou tuberculocide des désinfectants chimiques utilisés pour instruments en médecine humaine - Méthode d'essai et prescriptions (phase 2, étape 2)

Ta slovenski standard je istoveten z: **EN 14563:2008**

ICS:

11.080.20	Dezinfektanti in antiseptiki	Disinfectants and antiseptics

SIST EN 14563:2009 **en,fr,de**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 14563

November 2008

ICS 11.080.20

English Version

Chemical disinfectants and antiseptics - Quantitative carrier test for the evaluation of mycobactericidal or tuberculocidal activity of chemical disinfectants used for instruments in the medical area - Test method and requirements (phase 2, step 2)

Désinfectants et antiseptiques chimiques - Essai quantitatif de porte-germe pour l'évaluation de l'activité mycobactéricide ou tuberculocide des désinfectants chimiques utilisés pour instruments en médecine humaine - Méthode d'essai et prescriptions (phase 2, étape 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Keimträgerversuch zur Prüfung der mykobakteriziden oder tuberkuloziden Wirkung chemischer Desinfektionsmittel für Instrumente im humanmedizinischen Bereich - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

This European Standard was approved by CEN on 18 October 2008.

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 CEN Management Centre 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 CEN Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland and United Kingdom.

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

Management Centre: rue de Stassart, 36 B-1050 Brussels

Ref. No. EN 14563:2008: E

Contents

Page

Foreword

This document (EN 14563:2008) 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 2009, and conflicting national standards shall be withdrawn at the latest by May 2009.

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 has been prepared under a mandate given to CEN by the European Commission and the European Free Trade Association, and supports essential requirements of EC Directive(s).

For relationship with EU Directive 93/42/EEC, see informative annex ZA, which is an integral part of this document.

Other methods to evaluate the efficacy of chemical disinfectants and antiseptics for different applications in the medical field are in preparation.

A collaborative trial will be undertaken to provide a precision annex to this standard.

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

Introduction

This European Standard specifies a carrier test for establishing whether a chemical disinfectant for use on instruments (surgical instruments, anaesthesia material, endoscopes etc.) has a mycobactericidal or tuberculocidal activity in the area described in the scope.

The laboratory test closely simulates practical conditions of application including pre-drying mycobacteria on a carrier, contact time, temperature, test organisms and interfering substances, i.e. conditions which may influence the action of chemical disinfectants in practical situations.

The obligatory conditions are intended to cover general purposes and to allow reference between laboratories and product types. Each utilization concentration of the chemical disinfectant found by this test corresponds to 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 European Standard specifies a test method and the minimum requirements for mycobactericidal or tuberculocidal activity of chemical disinfectant products that form a homogeneous, physically stable preparation when diluted with hard water, or – in the case of ready-to-use products – with water.

This European Standard applies to products that are used in the medical area for disinfecting instruments by immersion – even if they are not covered by the EEC/93/42 Directive on Medical Devices.

This European Standard applies to areas and situations where disinfection is medically indicated. Such indications occur in patient care, for example:

— in hospitals, in community medical facilities and in dental institutions;

— in clinics of schools, kindergartens and nursing homes;

and may occur in the workplace and in the home. It may also include services such as laundries and kitchens supplying products directly for the patients.

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.

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 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 European Standard, the terms and definitions given in EN 14885 apply.

4 Requirements

The product, when tested in accordance with Clause 5 under simulated clean conditions (0,3 g/l bovine albumin solution) or simulated dirty conditions (3,0 g/l bovine albumin solution, plus 3,0 ml/l washed sheep erythrocytes) according to its practical applications and under the obligatory test conditions, (one or two selected test organisms, 20 °C, 60 min), shall demonstrate at least a decimal log (lg) reduction in counts of 4.

The mycobactericidal activity shall be evaluated using the following two test organisms: *Mycobacterium avium* and *Mycobacterium terrae*.

The tuberculocidal activity shall be evaluated using the following test organism: *Mycobacterium terrae*.

Where indicated, additional specific mycobactericidal or tuberculocidal activity shall be determined applying other contact times, temperatures and interfering substances in accordance with 5.5.1.1, in order to take into account intended specific use conditions.

NOTE For these additional conditions, the concentration defined as a result can be lower than the one obtained under the obligatory test conditions.

5 Test method

5.1 Principle

5.1.1 A test suspension of mycobacteria in a solution of interfering substances is spread on a glass carrier. After drying the carrier is immersed into a sample of the product as delivered and/or diluted with hard water (for ready to use products: water). The carrier is maintained at 20 °C ± 1 °C for 60 min ± 10 s (obligatory test conditions). At the end of this contact time, the carrier is transferred into a neutralizer containing glass beads. The mycobacteria are to be severed from the surface by shaking. The numbers of surviving mycobacteria in each sample are determined and the reduction is calculated.

5.1.2 The test is performed using *Mycobacterium avium* and *Mycobacterium terrae* or only *Mycobacterium terrae* as test organisms (obligatory test conditions).

5.1.3 Additional and optional contact times and temperatures are specified. Additional interfering substances may be used.

5.2 Materials and reagents

5.2.1 Test organisms

The mycobactericidal activity shall be evaluated using the following two test-organisms[1]:

— *Mycobacterium avium* ATCC 15769

— *Mycobacterium terrae* ATCC 15755

The tuberculocidal activity shall be evaluated using only *Mycobacterium terrae*.

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

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.

1) The ATCC numbers are the collection numbers of strains supplied by the American Type Culture Collections (ATCC). 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.

NOTE 1 To improve 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.

NOTE 2 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 water 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 (5.3.1). Sterilization is not necessary if the water is used – e.g. for preparation of culture media – and subsequently sterilized.

NOTE See 5.2.2.7 for the procedure to prepare hard water.

5.2.2.3 Middlebrook and Cohn 7H10 medium enriched by 10 % OADC (MCO)

Middlebrook 7H10 agar – powder	19,0 g
Glycerol ($C_3H_8O_3$) (see bibliographic reference [2])	5,0 ml
Water (5.2.2.2)	to 900,0 ml

Heat to boiling to dissolve completely. Sterilize in the autoclave (5.3.1) and cool to 50 °C to 55 °C. Add 100 ml Middlebrook OADC enrichment under aseptic conditions. Fill 18 – 20 ml per plate (5.3.2.10). The pH of the medium shall be equivalent to 6,6 ± 0,2 when measured at 25 °C.

NOTE In special circumstances (problems with neutralization – see 5.5.1.2 and 5.5.1.3) it may be necessary to add neutralizer to MCO (see Annex B). It is not recommended to use neutralizer that causes opalescence in the agar.

5.2.2.4 Diluent

Tryptone Sodium Chloride Solution:

Tryptone, pancreatic digest of casein	1,0 g
Sodium chloride (NaCl)	8,5 g
Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave (5.3.1). 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 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 Sterile defibrinated sheep blood

The sterile defibrinated sheep blood can be acquired from a commercial supplier or prepared according to EN 14820.

5.2.2.7 Hard water for dilution of products

Prepare:

— Solution A: Dissolve 19,84 g anhydrous magnesium chloride ($MgCl_2$) or an equivalent of hydrated magnesium chloride and 46,24 g anhydrous calcium chloride ($CaCl_2$) or an equivalent of hydrated calcium chloride in water (5.2.2.2) and dilute to 1 000 ml. Sterilize in the autoclave (5.3.1). Store the solution in a refrigerator (5.3.2.8) for no longer than one month.

— 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.

— Hard water: For the preparation of 1 l hard water, place 600 ml – 700 ml 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 (see 5.4.2) the addition of the product to this 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 substance

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, detergents) shall be defined.

NOTE In the following, the term “interfering substance” is used even if it contains more than one substance.

5.2.2.8.2 Clean conditions (bovine albumin solution – low concentration)

— Dissolve 0,30 g of bovine albumin fraction V (suitable for microbiological purposes) in 100 ml of diluent (5.2.2.4).

— Sterilize by membrane filtration (5.3.2.7), keep in a refrigerator (5.3.2.8) and use within 1 month.

— The final concentration of the bovine albumin in the test procedure (see 5.5) is 0,3 g/l.

5.2.2.8.3 Dirty conditions (Mixture of bovine albumin solutions – high concentration with sheep erythrocytes (see 5.2.2.6))

Dissolve 3,00 g of bovine albumin fraction V (suitable for microbiological purposes) in 97 ml of diluent (5.2.2.4).

Sterilize by membrane filtration (5.3.2.7).

Prepare at least 8,0 ml fresh sterile defibrinated sheep blood (5.2.2.6). Centrifuge the sheep blood at 800 g_N for 10 min. After discarding the supernatant, resuspend erythrocytes in diluent (5.2.2.4). Repeat this procedure at least 3 times, until the supernatant is colourless. Resuspend 3 ml of the packed sheep

erythrocytes in the 97 ml of sterilized bovine albumin solution (see above). To avoid contamination this mixture should be split in portions probably needed per day and kept in separate containers for a maximum of 7 days in a refrigerator at 2 °C to 8 °C.

The final concentration of bovine albumin and sheep erythrocytes in the test procedure (see 5.5) shall be 3 g/l and 3 ml/l respectively.

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:

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 20 °C ± 1 °C, at 50 °C to 55 °C (to prepare the MCO – see 5.2.2.3) and at additional test temperatures ± 1 °C (5.5.1).

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

NOTE 1 The same temperature should be used for all incubations performed during a test and its controls and validation.

NOTE 2 A CO_2 incubator and a temperature of 36 °C ± 1 °C are better suited for the test organisms. If a CO_2 incubator is not used, the inoculated plates should be protected from drying by sealing with insulating tape or packing them into polyethylene bags.

5.3.2.4 pH-meter, having an inaccuracy of calibration of not more than ± 0,1 pH units at (20 ± 1) °C.

NOTE For measuring the pH of the agar-media (5.2.2.3) a puncture electrode or a flat membrane electrode should be used.

5.3.2.5 Stopwatch.

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

5.3.2.6 Shakers

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

b) Mechanical shaker

5.3.2.7 Membrane filtration apparatus, constructed of a material compatible with the substances to be filtered, with a filter holder of at least 50 ml volume, and suitable for use of filters of diameter 47 mm to 50 mm and 0,45 µm pore size for sterilization of hard water (5.2.2.7) and bovine albumin (5.2.2.8).

The vacuum source used shall give an even filtration flow rate.

5.3.2.8 Refrigerator, capable of being controlled at 2 °C to 8 °C.

5.3.2.9 Graduated pipettes of nominal capacities 10 ml and 1,0 ml and 0,1 ml. Calibrated automatic pipettes may be used.

5.3.2.10 Petri dishes (plates) of size 90 mm to 100 mm.

5.3.2.11 Glass beads (Diameter: 3 mm to 4 mm).

5.3.2.12 Volumetric flasks.

5.3.2.13 Glass beads (Diameter: 0,25 mm to 0,5 mm).

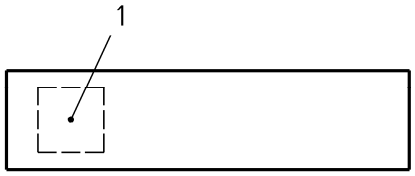
5.3.2.14 Centrifuge (800 g_N and 2 000 g_N).

5.3.2.15 Cylindrical plastic screw cap tubes, contents of about 15 ml, diameter about 18 mm (for the carrier).

5.3.2.16 Coned bottom screw cap tubes, contents of 50 ml (diameter about 28 mm).

5.3.2.17 Frosted glass carriers, 15 mm x 60 mm x 1 mm, one surface sandblasted.

Glass carriers shall be used once only. For preparation the glass carrier is boiled 10 min in a suitable detergent, cleaned minimum 3 times with water (5.2.2.2) and at the end once with ethanol (70 Vol.%). Mark a 10 mm square at one end of the dried carrier on its sandblasted surface, about 2 mm off the three edges. Sterilize in the heat oven [5.3.1b)]. After the sterilization process the markings of the “inoculation square” shall be clearly visible.



Key

1 Inoculation square

Figure 1 — Frosted glass carrier with markings

3) Vortex® in an example of a suitable product available commercially. This information is given for the convenience of users of this European Standard and does not constitute an endorsement by CEN of this product.