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Utgåva 1

Kemiska desinfektionsmedel och antiseptiska medel – Kvantitativt suspensionstest för utvärdering av den mykobaktericida effekten av kemiska desinfektionsmedel för instrument inom hälso- och sjukvården – Provningsmetod och krav (Fas 2/Steg 1)

Chemical disinfectants and antiseptics – Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test methods and requirements (phase 2, step 1)

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English version

Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of mycobactericidal activity of chemical disinfectants in the medical area including instrument disinfectants - Test methods and requirements (phase 2, step 1)

Désinfectants chimiques - Essai quantitatif de suspension pour l'evaluation de l'activité mycobactéricide des désinfectants chimiques utilisés en médecine y compris les désinfectants pour instruments - Méthode d'essai et prescriptions (phase 2, étape 1)

Chemische Desinfektionsmittel und Antiseptika - Quantitativer Suspensionsversuch zur Bestimmung der mykobakteriziden Wirkung chemischer Desinfektionsmittel im humanmedizinischen Bereich einschließlich der Instrumentendesinfektionsmittel - Prüfverfahren und Anforderungen (Phase 2, Stufe 1)

This European Standard was approved by CEN on 22 November 2004.

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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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EUROPÄISCHES KOMITEE FÜR NORMUNG

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Contents

Foreword

This document (EN 14348:2005) 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of an identical text or by endorsement, at the latest by July 2005, and conflicting national standards shall be withdrawn at the latest by July 2005.

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 EU Medical Devices Directive 93/42.

For relationship with EU Directive, see informative Annex ZA, which is an integral part of this document.

A collaborative trial has been undertaken and will be evaluated to provide a precision annex to this document.

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

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

Introduction

This document describes a suspension test for establishing whether a chemical disinfectant has or does not have a mycobactericidal activity in the area defined 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 concentration of the chemical disinfectant 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 document 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. 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 document applies to products that are used in the medical area including those that are covered by the EEC/93/42 Directive on Medical Devices.

This document 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, of kindergartens and of 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.

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 (see Annex E).

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 microbial strains used for the determination of bactericidal and fungicidal activity.*

EN 14820, *Single-use containers for human venous blood specimen collection.*

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

3.1
product
chemical agent or formulation used as a chemical disinfectant or antiseptic

3.2
mycobactericide
product which 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 test organisms under defined conditions

3.4
mycobacteriostatic activity
capability of a product to inhibit the growth of mycobacteria under defined conditions

3.5
tuberculocide
product which kills *Mycobacterium tuberculosis* under defined conditions

NOTE The adjective derived from “tuberculocide” is “tuberculocidal”.

3.6
tuberculocidal activity
capability of a product to kill *Mycobacterium tuberculosis*, demonstrated by the capability to produce a reduction in the number of viable cells of *Mycobacterium terrae* under defined conditions

3.7
clean conditions
conditions representative of surfaces which have received a satisfactory cleaning programme and/or are known to contain minimal levels of organic and/or inorganic substances

3.8
dirty conditions
conditions representative of surfaces which are known to or may contain organic and/or inorganic substances

4 Requirements

The product, when diluted with hard water or - in the case of ready-to-use products - with water and tested in accordance with clause 5 under simulated clean conditions (0,3 g/l bovine albumin solution) or simulated dirty conditions (3 g/l bovine albumin solution, plus 3 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. It is possible to test also the product as delivered (highest test concentration is 80 %).

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 (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 methods

5.1 Principle

5.1.1 A test suspension of mycobacteria in a solution of an interfering substance is added to a sample of the product as delivered and/or diluted with hard water (for ready to use products: water). The mixture is maintained at 20 °C ± 1 °C for 60 min ± 10 s (obligatory test conditions). At the end of this contact time, an aliquot is taken; the mycobactericidal and/or the mycobacteriostatic activity in this portion is immediately neutralized or suppressed by a validated method.

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 can 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 corresponding 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 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 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 limitation for use should be fixed.

5.2.2.2 Water

The water shall be freshly glass distilled water and not demineralized water.

Sterilize in the autoclave (5.3.1).

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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 standard and does not constitute an endorsement by CEN of the product named.

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]) can be used.

5.2.2.3 Middlebrook and Cohn 7 H 10 medium with 10 % OADC enrichment (MCO)

Middlebrook 7 H 10 agar-powder	19,0 g
Glycerol ($C_3H_8O_3$) (see [1])	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 ºC to 55 ºC. Add 100 ml Middlebrook OADC enrichment under aseptic conditions. Fill 18 ml to 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 (5.3.2.4).

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.3). Do not 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 (see 5.3.1). After sterilization the pH of the diluent shall be equivalent to 7,0 ± 0,2 when measured at 20 °C.

NOTE There is scientific experience that sodium chloride (NaCl) may inhibit the growth of mycobacteria. But the presence of NaCl in products and in the interfering substance (5.2.2.8) is unavoidable and therefore diluent might be used for the preparation of neutralizer (5.5.1.2).

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 B.2.

5.2.2.6 Middlebrook 7 H 9 broth with 10 % ADC enrichment (MADC-broth)

MADC-broth will be used for deep freeze storage

Middlebrook 7H9 broth-powder	4,7 g
Glycerol ($C_3H_8O_3$) (see [1])	100,0 ml
Water (5.2.2.2)	750,0 ml

Sterilize in the autoclave (5.3.1) and cool to 45 ºC. Add under aseptic conditions 100 ml Middlebrook ADC enrichment and sterilized water (5.2.2.2) to 1 000,0 ml. The pH of the medium shall be equivalent to 6,6 ± 0,2 when measured at 25 ºC (5.3.2.4).

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 at 2 °C to 8 °C for no longer than one month.

— Solution B: Dissolve 35,02 g sodium hydrogencarbonate ($NaHCO_3$) in water (5.2.2.2) and dilute to 1 000 ml. Sterilize by membrane filtration (5.3.2.7). Store the solution at 2 °C to 8 °C for no longer than one week.

Hard water: For the preparation of 1 litre, place 600 ml to 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. 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 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 (at 2 °C to 8 °C) 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)

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 (5.2.2.9) or purchase such blood from a commercial supplier (5.2.2.9). Centrifuge the erythrocytes at 800 g 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 later 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 (5.5) shall be 3 g/l and 3 ml/l respectively.