

Kemiska desinfektionsmedel och antiseptiska medel – Kvantitativt carriertest för utvärdering av den baktericida effekten av kemiska desinfektionsmedel för instrument inom hälso- och sjukvården – Provningsmetod och krav (fas 2, steg 2)

Chemical disinfectants and antiseptics – Quantitative carrier test for the evaluation of bactericidal activity for instruments used in the medical area – Test method and requirements (phase 2, step 2)

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**Chemical disinfectants and antiseptics - Quantitative carrier test
for the evaluation of bactericidal activity for instruments used 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 bactéricide
pour instruments utilisés en médecine humaine - Méthode
d'essai et prescriptions (phase 2, étape 2)

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

This European Standard was approved by CEN on 29 August 2005.

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 European Standard (EN 14561:2006) 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of an identical text or by endorsement, at the latest by November 2006, and conflicting national standards shall be withdrawn at the latest by November 2006.

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.

This European Standard 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 Directive(s).

For relationship with EU Directive(s), see informative Annex ZA, which is an integral part of 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, 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.

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 bactericidal activity in the fields described in the scope.

The laboratory test closely simulates practical conditions of application including pre-drying bacteria 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 bactericidal 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, of kindergardens 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 This method corresponds to a phase 2, step 2 test (see Annex E).

2 Normative references

The following referenced documents are indispensable for the application of this European Standard. 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, sporicidal 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

3.2

bactericide

product that kills vegetative bacteria under defined conditions

NOTE The adjective derived from "bactericide" is "bactericidal".

3.3

bactericidal activity

capability of a product to produce a reduction in the number of viable bacterial cells of relevant test organisms under defined conditions

3.4

clean conditions

conditions representative of surfaces which have been cleaned satisfactorily and/or are known to contain minimal levels of organic and/or inorganic substances

EN 14561:2006 (E)**3.5****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 (three selected test organisms, 20 °C, 60 min), shall demonstrate at least a decimal log (lg) reduction in counts of 5.

The bactericidal activity shall be evaluated using the following three test organisms: *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus hirae*.

Where indicated, additional specific bactericidal activity shall be determined applying other contact times, temperatures, test organisms 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 method**5.1 Principle**

5.1.1 A test suspension of bacteria 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 bacteria are to be severed from the surface by shaking. The numbers of surviving bacteria in each sample are determined and the reduction is calculated.

5.1.2 The test is performed using *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Enterococcus hirae* 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 bactericidal activity shall be evaluated using the following strains as test organisms¹⁾:

— <i>Pseudomonas aeruginosa</i>	ATCC 15442
— <i>Staphylococcus aureus</i>	ATCC 6538
— <i>Enterococcus hirae</i>	ATCC 10541

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 See Annex A for strain references in some other culture collections.

The required incubation temperature for these test organisms is $36\text{ °} \pm 1\text{ °C}$ or $37\text{ °C} \pm 1\text{ °}$ (5.3.2.3). The same temperature (either 36 °C or 37 °) 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.

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).

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.

NOTE 3 See 5.2.2.7 for the procedure to prepare hard water.

5.2.2.3 Tryptone Soya Agar (TSA)

— Tryptone, pancreatic digest of casein	15,0 g
— Soya peptone, papaic digest of Soybean meal	5,0 g
— Sodium Chloride (NaCl)	5,0 g
— Agar	15,0 g
— Water (5.2.2.2)	to 1 000,0 ml

Sterilize in the autoclave (5.3.1). After sterilization the pH of the medium shall be equivalent to $7,2 \pm 0,2$ when measured at $(20 \pm 1)\text{ °C}$ (5.3.2.4).

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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 TSA (see B.3).

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 $7,0 \pm 0,2$ when measured at $(20 \pm 1)^\circ\text{C}$.

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 B.2.

5.2.2.6 Sterile defibrinated sheep blood

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, 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 $7,0 \pm 0,2$ when measured at $(20 \pm 1)^\circ\text{C}$. 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 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 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, 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 (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 \bar{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 (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 equipment²⁾

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;

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