SIST

SLOVENSKI STANDARD
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Kemična razkužila in antiseptiki - Kvantitativna preskusna metoda za vrednotenje baktericidnega delovanja ali delovanja na kvasovke na neporoznih površinah z odvzemom brisa v medicini (4-področni preskus) - Preskusna metoda in zahteve (faza 2, stopnja 2)

Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test) - Test method and requirements (phase 2, step 2)

Chemische Desinfektion und Antiseptika - Quantitativer Prüfversuch zur Bestimmung der bakteriziden und levuroziden Wirkung auf nicht-porösen Oberflächen mit mechanischer Einwirkung mit Hilfe von Tüchern oder Mops im humanmedizinischen Bereich (4-Felder-Test) - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

Antiseptiques et désinfectants chimiques - Méthode d’essai quantitative pour l’évaluation de l’activité bactéricide et levuricide sur des surfaces non poreuses, avec action mécanique à l’aide de lingettes et de lavettes dans le domaine médical (essai à 4 zones) - Méthode d'essai et prescriptions (phase 2, étape 2)

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EUROPEAN STANDARD

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Chemical disinfectants and antiseptics - Quantitative test method for the evaluation of bactericidal and yeasticidal activity on non-porous surfaces with mechanical action employing wipes in the medical area (4- field test) - Test method and requirements (phase 2, step 2)

Antiseptiques et désinfectants chimiques - Méthode d'essai quantitative pour l'évaluation de l'activité bactéricide et levuricide sur des surfaces non poreuses, avec action mécanique à l'aide de lingettes dans le domaine médical (essai à 4 zones) - Méthode d'essai et prescriptions (phase 2, étape 2)

Chemische Desinfektionsmittel und Antiseptika - Quantitatives Prüfverfahren zur Bestimmung der bakteriziden und levuroziden Wirkung auf nicht-porösen Oberflächen mit mechanischer Einwirkung mit Hilfe von Tüchern im humanmedizinischen Bereich (4-Felder-Test) - Prüfverfahren und Anforderungen (Phase 2, Stufe 2)

This European Standard was approved by CEN on 3 January 2015.

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Foreword

This document (EN 16615:2015) 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 October 2015 and conflicting national standards shall be withdrawn at the latest by October 2015.

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.

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

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Introduction

This European Standard specifies a carrier test for establishing whether a chemical disinfectant for use on surfaces administered with wipes has a bactericidal and yeasticidal activity in the fields described in the scope.

The laboratory test closely simulates practical conditions of application such as contact time, temperature and interfering substances, including pre-drying specified test organisms on a test-surface as carrier and wiping the product on the test-surface with a wipe. The conditions are intended to cover general purposes. However, if for some applications the recommendations of use of a product differ additional test conditions may be or need to be used.

Each utilization concentration of the product found by this test corresponds to defined experimental conditions.

1 Scope

This European Standard specifies a test method and the minimum requirements for bactericidal and yeasticidal 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 non-porous surfaces including surfaces of medical devices by wiping – regardless if they are covered by the 93/42/EEC Directive on Medical Devices or not.

This European Standard includes ‘ready-to-use wipes‘ which are impregnated with a microbicidal solution.

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 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 This method corresponds to a phase 2, step 2. test.

EN 14885 specifies in detail the relationship of the various tests to one another and to “use recommendations”.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. 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 13624, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of fungicidal or yeasticidal activity in the medical area — Test method and requirements (phase 2, step 1)*

EN 13727, *Chemical disinfectants and antiseptics — Quantitative suspension test for the evaluation of bactericidal activity in the medical area — Test method and requirements (phase 2, step 1)*

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.

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) or simulated dirty conditions (3,0 g/l bovine albumin + 3,0 ml/l sheep erythrocytes) according to its practical applications and under the following test conditions: four selected test organisms, temperature between 4 °C and 30 °C,

contact time min. 1 min and max. either 5 min or 60 min[1)] shall demonstrate at least a decimal log (lg) reduction in counts of 5 (*Staphylococcus aureus*, *Enterococcus hirae, Pseudomonas aeruginosa),*or 4 (*Candida albicans*) on test field 1. The mean of the cfus on the test fields 2 to 4 shall be equal or less than 50, the mean of the cfus of the water control shall be equal or more than 10.

The bactericidal activity shall be evaluated using the following test organisms: *Staphylococcus aureus*, *Enterococcus hirae, Pseudomonas aeruginosa.* The yeasticidal activity shall be evaluated using the following test organism: *Candida albicans.*

Where indicated, additional specific bactericidal and yeasticidal activity shall be determined applying other contact times and test organisms 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 minimum test conditions.

5 Test methods

5.1 Principle

5.1.1 A test-surface is marked with 4 squares of 5 × 5 cm, the "test fields", in a row. Test field 1 on the test-surface is inoculated with a test suspension of bacteria or yeasts in a solution of interfering substances. The inoculum is dried. A wipe is soaked with a sample of the product as delivered and/or diluted with hard water (for ready to use products: water). The test-surface is wiped with the soaked wipe across the four marked test fields, starting in front of test field 1, turning immediately after test field 4 and wiped back to the starting point. In parallel a water control is performed: a wipe is soaked with water (5.5.2.2 e)) instead of the product.

Temperature, soiling and contact time are employed as recommended by the manufacturer. At the end of the contact time, the test organisms are recovered from each test field with moistened cotton swabs. The swabs are brought into a tube containing broth and neutralizer and the test organisms are to be severed from the swab by shaking. The numbers of surviving test organisms in each sample are determined, and the reduction is calculated by comparing the results of the drying control D_{Ct} and the results obtained with the product. In parallel to the test with the product water is applied in the same way to ensure that the test organisms are spread on the 4 fields and their number reaches a certain level. The test is performed using *P. aeruginosa*, *S. aureus*, *E. hirae* and *C. albicans* as test organisms (minimum test conditions).

5.1.2 Additional test organisms (only bacterial or fungal strains), contact times and interfering substances can be used.

5.2 Materials and reagents

5.2.1 Test organism

The bactericidal activity shall be evaluated using the following strains as test organisms[2)]:

— *Staphylococcus aureus* ATCC 6538;

— *Pseudomonas aeruginosa,* ATCC 15442;

— *Enterococcus hirae* ATCC 10541.

1) See 5.5.1.1 b).

2) 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.

The yeasticidal activity shall be evaluated using the following strain as test organism[3)]:

— *Candida albicans* ATCC 10231.

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

If additional test organisms are used, they shall be incubated under optimum growth conditions (temperature, time, atmosphere and 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.

The required incubation temperature for these test bacteria is 36 °C ± 1 °C or 37 °C ± 1 °C (5.3.2.3). The same temperature (36 °C or 37 °C) shall be used for all incubations performed during its control and validation. The required incubation temperature for *Candida albicans* is 30 °C ± 1 °C (5.3.2.3).

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 organism.

To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media if it complies with the formulas given below. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

For each culture medium and reagent a limitation for use should be fixed.

All specified pH values are measured at 20 °C ± 1°C (5.3.2.4).

5.2.2.2 Water

The water shall be freshly glass-distilled or deionized and demineralized water. If distilled water or deionized and demineralized water of adequate quality is not available, water for injections (see [1]) may be used.

Sterilize in the autoclave [5.3.2.1 a)]. Sterilization is not necessary if the water is used, e.g. for preparation of culture media and subsequently sterilized.

See 5.2.2.7 for the procedure to prepare hard water.

3) 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.

5.2.2.3 Medium

a) 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 (5.3.2.4) of the medium shall be equivalent to 7,2 ± 0,2.

In case of encountering problems with neutralization (5.5.1.2) it may be necessary to add neutralizer to TSA. Annex B gives guidance on the neutralizers that may be used. It is recommended not to use a neutralizer that causes opalescence in the agar.

b) Malt Extract Agar (MEA)

Malt extract agar, consisting of:

—	Malt extract	30,0 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 5,6 ± 0,2 when measured at (20 ± 1) °C (5.3.2.4).

In case of an encountering problems with neutralization (5.5.1.2) it may be necessary to add neutralizer to MEA. Annex B gives guidance on the neutralizers that may be used. It is recommended not to use neutralizer that causes opalescence in the agar.

5.2.2.4 Diluent

a) General 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 (5.3.2.4) of the general diluent shall be equivalent to 7,0 ± 0,2.

b) Glycerol Diluent (for *Pseudomonas aeruginosa* only)

Tryptone Sodium Chloride Glycerol Solution:

—	Tryptone, pancreatic digest of casein	1,0 g
—	Sodium chloride (NaCl)	8,5 g

— Glycerol [bibliographic reference 1] 2,0 g

— Water (5.2.2.2) to 1 000,0 ml

Sterilize in the autoclave (5.3.1). After sterilization the pH (5.3.2.4) of the diluent shall be equivalent to 7,0 ± 0,2.

This modified diluent [5.2.2.4 b)] should be only used for the preparation of the test suspension of *Pseudomonas aeruginosa* (5.4.1.4). All further dilutions should be done with the general diluent [5.2.2.4 a)].

5.2.2.5 Neutralizer

The neutralizer shall be validated for the product being tested in accordance with 5.5.1.2 and 5.5.2. It shall be sterile.

Information on neutralizer that has 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.

5.2.2.7 Hard water for dilution of products

a) Hard water general

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 1 000 ml. Sterilize by membrane filtration (5.3.2.7) or in the autoclave [5.3.2.1 a)]. Autoclaving – if used - may 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 a refrigerator (5.3.2.8) for no longer than one month.

— Prepare solution B: dissolve 35,02 g sodium bicarbonate ($NaHCO_3$) in water 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 water (5.2.2.2) in a 1 000 ml volumetric flask (5.3.2.12) and add 6,0 ml (5.3.2.9) 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 (5.3.2.4) of the hard water shall be 7,0 ± 0,2 (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 h.

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 expressed as calcium carbonate ($CaCO_3$) is in the test tube lower than 375 mg/l.

b) Hard water with the addition of polysorbate 80

Use the procedure described in 5.2.2.7 a). At the end add 1 ml of polysorbate 80 per litre. Sterilized by membrane filtration. The hard water with the addition of polysorbate 80 shall be freshly prepared under aseptic conditions and used within 12 h.

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 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 general diluent [5.2.2.4 a)]

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)

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

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 general diluent [5.2.2.4 a)]. 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 equipment [4)]

and in particular, the following:

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

5.3.2.1 Apparatus for sterilization:

a) For moist heat sterilization, an autoclave capable of being maintained at $\left(121^{+3}_{0}\right)$°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 and at 45 °C ± 1 °C [to maintain melted TSA in case of pour plate technique 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 (bacteria) or 30 °C ± 1 °C (yeasts). The same temperature shall be used for all incubations of the bacteria performed during a test and its controls and validation.

5.3.2.4 pH-meter, having an inaccuracy of calibration of no more than ± 0,1 pH units at 20 °C ± 1 °C. A puncture electrode or a flat membrane electrode should be used for measuring the pH of the agar-media (5.2.2.3).

5.3.2.5 Stopwatch.

5.3.2.6 Shakers

a) **Electromechanical agitator**, e.g. Vortex® mixer[5].

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 (5.2.2.8.2 and 5.2.2.8.3).

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 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 Centrifuge (800 g_N).

5.3.2.14 Rectangular glass spatula (4 cm edge length).

5.3.2.15 Loop (metal or plastic).

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5) 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.